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GENERALIZED SPIKE-AND-WAVE PATTERN MECHANISM AND THE RETICULAR AROUSAL SYSTEM

COMMENT ON A CASE

By

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(Received February 22, 1967)

A case of atypical status epilepticus characterized by alternating patterns of generalized spike-and-wave and desynchronization seizures, has been reported. The possible relationships between generalized spike-and-wave pattern and the reticular arousal system have been discussed. On the grounds of experimental data and of the clinical observations as well as in the reported case it is claimed that cortical inhibition of facilitatory reticular influence of the reticular arousal system plays an important role in the spike-and-wave mechanism.

Investigations into the structural factors underlying the regulation of susceptibility to epileptic seizures have brought into prominence the role of the diffuse non-specific projection systems. The discovery of the reticular system and the recognition of its influence on overall electric activity of the brain have inevitably suggested that this system must be closely involved in the seizure mechanism of generalized spike-and-wave activity. Assumption of the existence of a centrencephalic integrating system gave rise to the theory that the impulses to synchronous bilateral spike-and-wave activity were generated in thalamic, non-specific projection systems [1]. This theory presumes that

(i) the cortical reflexions of this subcortical process are merely passive; and

(ii) the pattern is produced by an excitation of the non-specific thalamic structures.

The theory thus places the epileptic "focus" into the subcortical thalamic, non-specific, system.

Attempts at laying a solid experimental foundation to this hypothesis may be traced back to the investigations of JASPER and DROOGLEEVER-FORTUYN [2] who were able to elicit a generalized cortical spike-and-wave pattern in cats from the thalamic structures adjacent to the massa intermedia. Later investigators had difficulties in reproducing these results; in fact, although INGVAR [3] succeeded in achieving it in a slight minority of his experiments, he asserts that, quite as JASPER and DROOGLEEVER-FORTUYN, he failed to elicit a "self-sustaining" spike-and-wave pattern persisting after stimulation had been discontinued. In other words, we can regard the pattern as an expression of frequency dependent evoked potentials rather than as being epileptig in

character. On the evidence of his findings the recruiting spike responses elicited from the anterior part of the thalamus are converted to a spike-and-wave pattern in the case of a simultaneous excitation of the mesencephalic reticular structures.

Recently, a reproducible spike-and-wave pattern could be elicited in young cats in superficial barbiturate sleep, by the excitation of the non-specific thalamic structures [4]. At the same time it has again been confirmed that the pattern may also be evoked by the excitation of the mesencephalic arousal system [5].

Recent investigations have lent little support to the concept of a presumed "pacemaker", "driving" the passive cortex. It has been demonstrated, on the contrary, that the role of the cortex in the spike-and-wave mechanism is by no means a passive one. The pattern is not only reproducible when the cortex has been isolated, i.e. deprived from subcortical influences, but even easier to reproduce, and what is more, elimination of subcortical influences by itself may evoke various types of cortical convulsive activity [6]. It has been shown by means of microelectrode techniques that the pattern is due to the direct effect of cortical processes; for instance, the slow-wave component represents a superficial reflexion of hyperpolarization, i.e. of an inhibitory mechanism originating in deeper cortical layers [7].

There is increasing evidence, that the spike-and-wave mechanism may be triggered by cortical foci, particularly by that of the mesial areas of the frontal lobe [8, 9].

On the other hand, the part played by the reticular system in the mechanism of convulsions, though by no means cleared, appears in a different light by the recent finding that the susceptibility to synchronization and to seizures is greatly increased by the elimination (destruction) of either the non-specific thalamic system or of the mesencephalic arousal system [10]. There is evidence to suggest that it is the injury of the reticular formation rather than a presumed focus of excitation within this system which is responsible for the increase in susceptibility to convulsions, the role of the reticular formation being presumably of an inhibitory character [11].

Whether the results of these animal experiments are applicable to human subjects is a different question. On the evidence of stereoencephalographic studies, excitation of the non-specific thalamic nuclei fails to produce a spike-and-wave pattern and desynchronization can be elicited from the non-specific thalamic nuclei also [12]. In a case of petit-mal epilepsy associated with a 3 c/s spike-and-wave activity the thalamus failed to reveal any morphologic abnormality [13]. The findings of ALEMA et al. [14] clearly indicate that our knowledge concerning the reticular system, derived chiefly from cat experiments, are not valid for humans. It has been revealed by means of intravertebral barbiturate injections that the human arousal system is situated higher along the

neuraxis than the pontine-mesencephalic area, in all probability in the diencephalon.

Thus, it seemed interesting to reassess the relationships between spike-and-wave mechanism and the reticular arousal system in the light of that recent evidence.

The following clinical observations seem to be relevant.

1. The 3 c/s spike-and-wave seizure is accompanied by loss of consciousness.

2. The human spike-and-wave mechanism can be activated in drug-induced sleep during the phase of slumber [15], similarly, production of experimental cortical spike-and-wave pattern by thalamic excitation is facilitated by superficial barbiturate sleep.

3. Arousal stimuli inhibit the spike-and-wave seizure and have a desynchronizing effect.

4. Adrenergic drugs such as amphetamine which presumably stimulate the arousal system, are inhibiting the spike-and-wave-mechanism.

5. The clinical features of petit-mal seizures are prevalently those of minus symptoms, i.e. inhibitory in character.

6. Petit-mal seizures and the 3 c/s spike-and-wave mechanism are often associated with functional disturbances of the diencephalon.

Points 1 to 4 suggest that the spike-and-wave activity and excitation of the reticular arousal system represent contrasted (probably incompatible) mechanisms.

In the following we shall describe a case of status epilepticus characterized by alternating seizures of generalized spike-and-wave activity and of generalized desynchronization.

Report of a case

The patient, a 15-year-old girl, was admitted because of seizures appearing in ceaseless succession. Delivery, somatic and mental development had been normal until the age of 6 when she experienced her first seizure with loss of consciousness, generalized tonic motor phenomena and enuresis. Despite consequent antiepileptic drug treatment, similar seizures had appeared with variable frequency in the waking state as well as during sleep. She was mentally backward, she did poorly at school and had to stay in both the first and the second form two years. She had become unmanageable and had been educated since at an institution for backward children. On repeated hospital investigations no PEG and CSF abnormalities were found. EEG recording made two years before admission revealed bursts of generalized, high-voltage slow and sharp-wave activity with left temporal preponderance. At 13 years of age her I. Q. corresponded to that of a 9 or 10-year-old child. She had been regularly

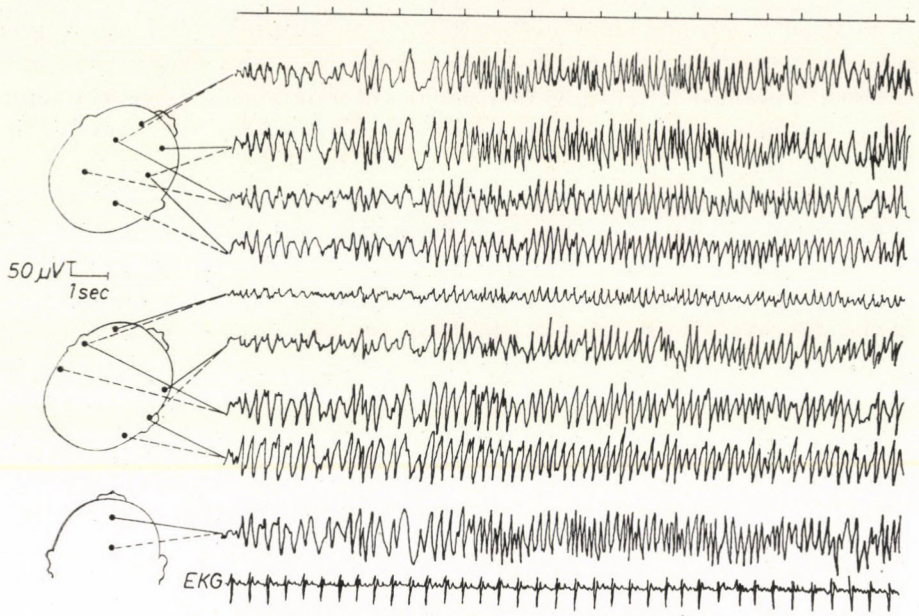




Fig. 1. a) Spontaneous oculo-clonic spike-and-wave seizure

treated with hydantoin, pyrimidin and barbiturates. Until the time of admission no status epilepticus had occurred.

Despite massive antiepileptic treatment including the above drugs, oxazolydin and succinimide, the seizures still increased in frequency. Between the seizures she was obnubilated. Two different types of seizure were witnessed. The main features of the first, the prevalent one, included a tonic stare or conjugated deviation to either side, eyelid flutter and loss of consciousness without other motor phenomena. The rarer type, entirely different from the first, was marked by wide, staring eyes, pupillar dilatation, facial flushing, tonic adversion to the right side, lifting of the head, tonic extension and abduction of the right arm, less frequently of both arms. The entire seizure impressed one as a tonic caricature of orientation. The inter-ictal EEG pattern

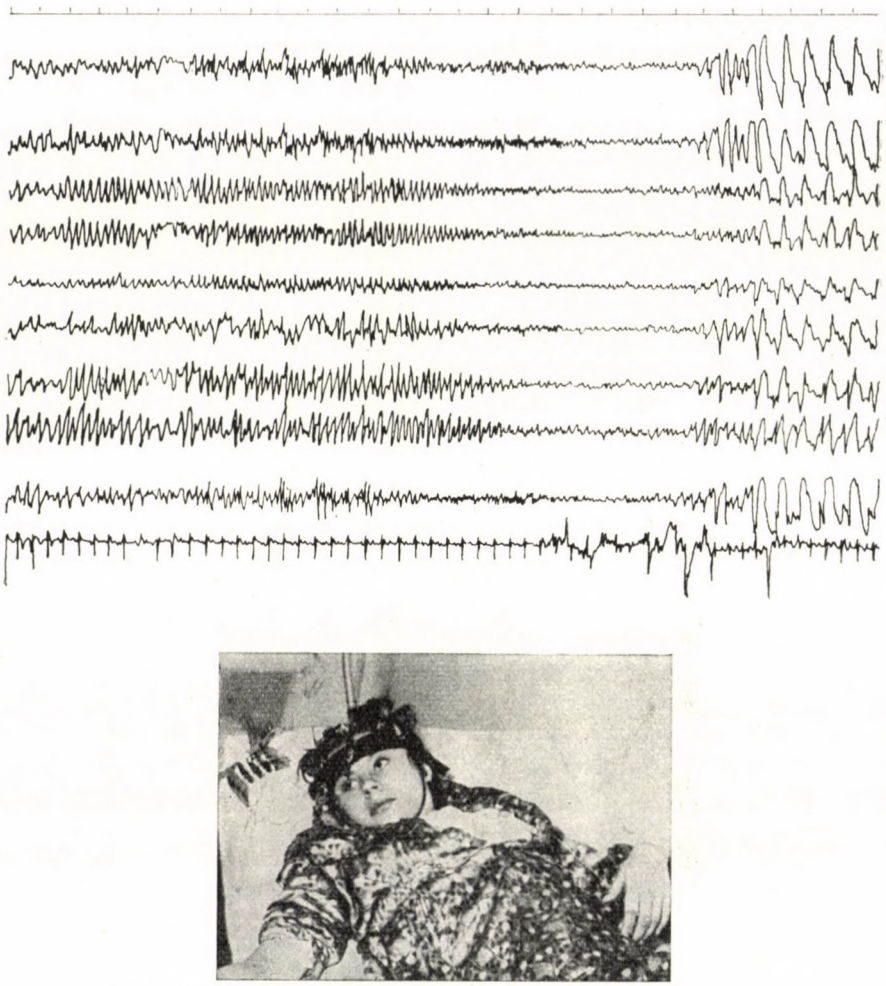
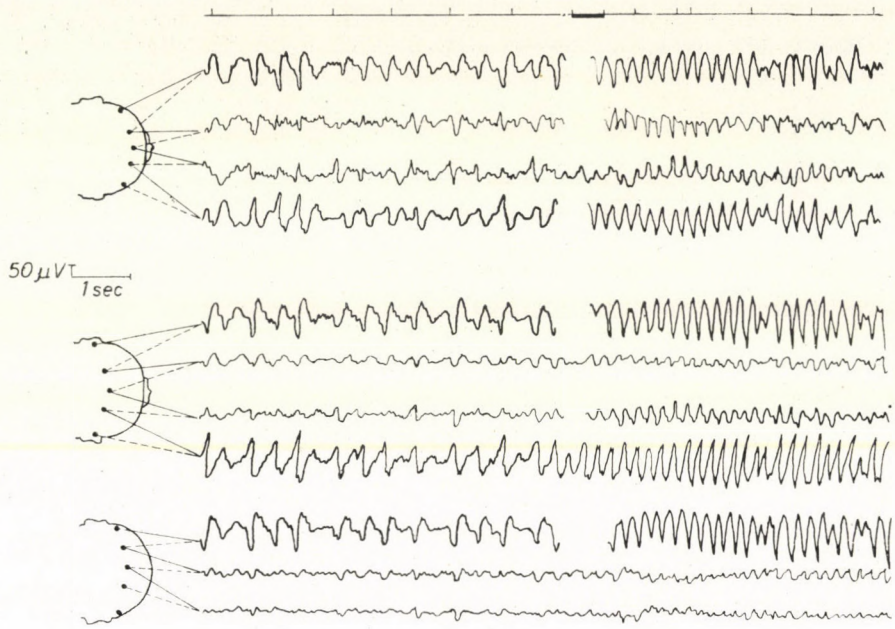


Fig. 1. b) Spontaneous tonic adversive desynchronization seizure. (Paper speed 1.5 cm/sec., on the other tracings 3 cm/sec.)

was marked by an almost continuous, slow spike-and-wave activity without hemispheric difference or focal character. The EEG counterpart of the oculoclonic seizure was a paroxystic intensification of the spike-and-wave pattern and a tendency to rhythmicity of the discharges (Fig. 1a).

In the case of the rarer type of seizure marked by tonic adversive features, a generalized 4 to 6 c/s sinusoid rhythmic slow-wave discharge appeared before the seizure had become manifest, and at its peak, a generalized desynchronization (reduction of the amplitudes and prevalence of beta-frequencies) developed. The electric seizure ceased by 1 to 2 c/s spike-and-wave activity (Fig. 1b).



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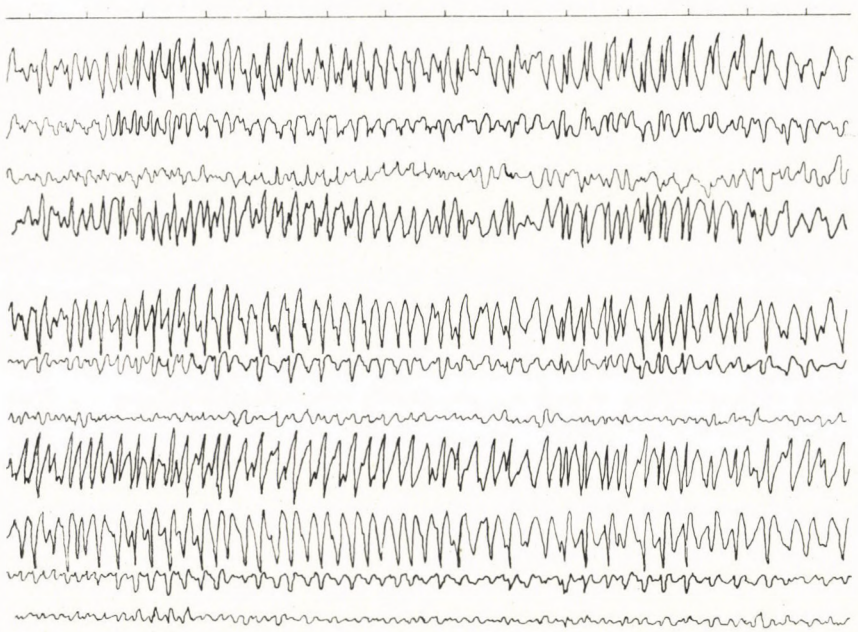


b)

Fig. 2. *(a—b:* **continuous recording). Paradoxical arousal reaction (theta-synchronization) and spike-and-wave seizure evoked by pinpricks (heavy line)**

Sensory stimuly, such as scintillating light, calling by name, nociceptive stimuli evoked a generalized high-voltage sinusoid theta-synchronization (Fig. 2) associated with signs of awakening, opening of eyes or turning to the side of the stimulus.

If the stimulus was strong enough it evoked oculo-clonic seizures (Fig. 2). If very intensive, it resulted in the activation of tonic adversive desynchronization seizures (Fig. 3). The evoked seizures were identical with the spontaneous

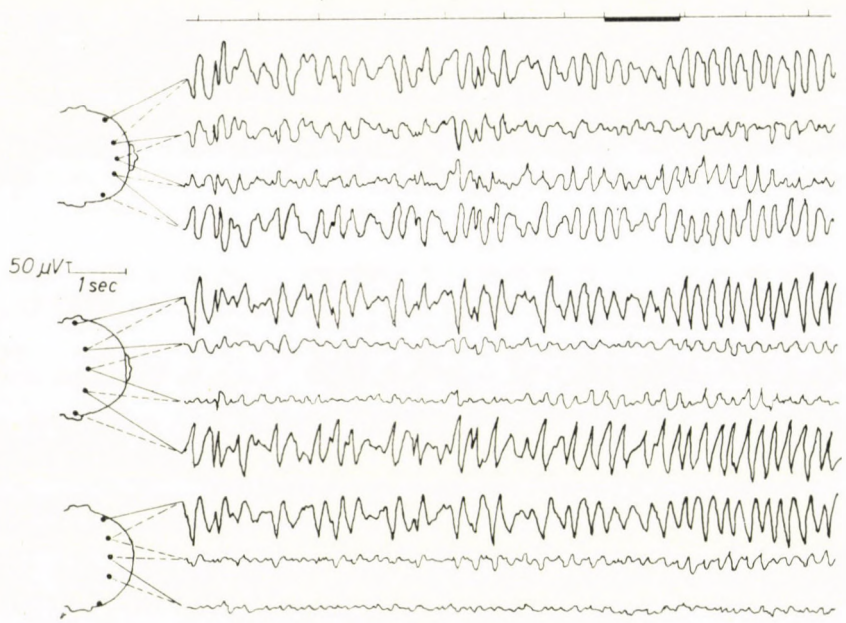
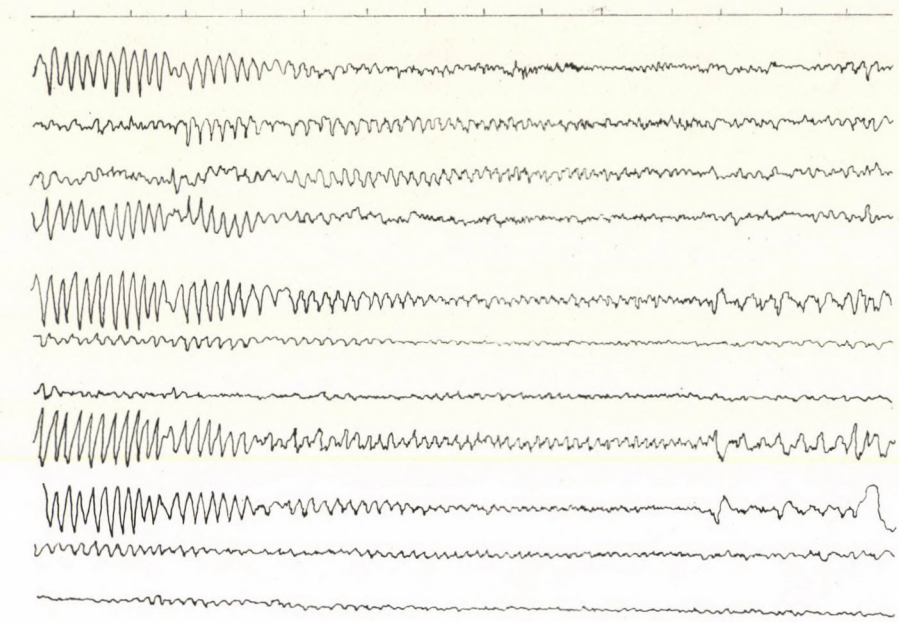


Fig. 3 a)

ones in both their clinical and electric manifestations. Occasionally, the evoked oculo-clonic spike-and-wave seizures were followed by a tonic desynchronization seizure. The reverse sequence was never observed.

The status epilepticus proved uncontrollable by the usual antiepileptic drugs. Chlorpromazine was efficient as far as it reduced or averted the seizures for the period of the neuroleptic phase; during its effect a low-voltage beta-activity appeared. Sensory stimuli applied during this period either failed to elicit any reaction or evoked the paradoxical arousal response described earlier. The status epilepticus persisted for three months and ceased only when pyrimidin and oxazolydin had been combined with diazepam in doses of 5 to 20 mg administered intravenously or intramuscularly depending on the clinical state and the EEG. Pyrimidin and oxazolydin then proved sufficient for preventing the seizures. Though electric activity was eventually normalized, nevertheless in barbiturate sleep a generalized spike-and-wave-like activity became manifest



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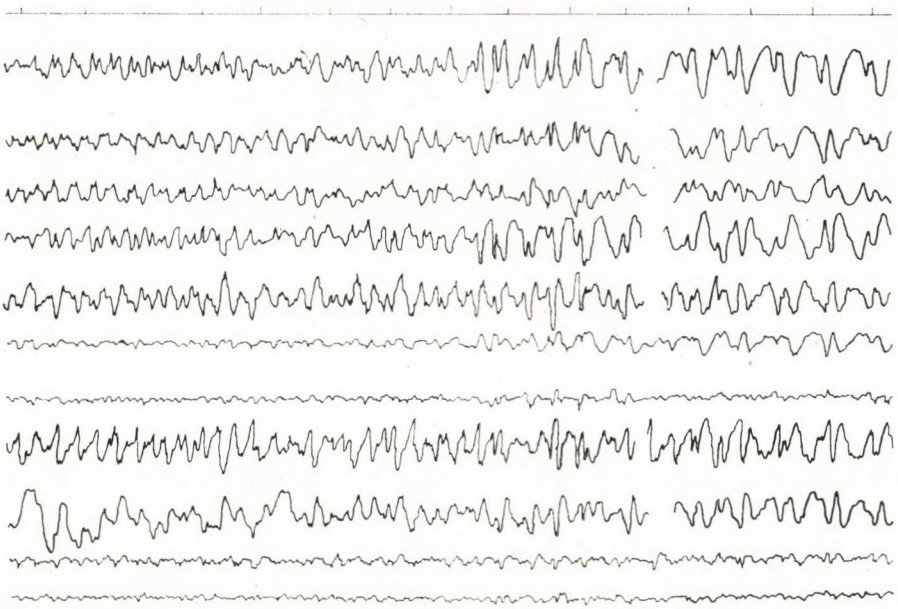
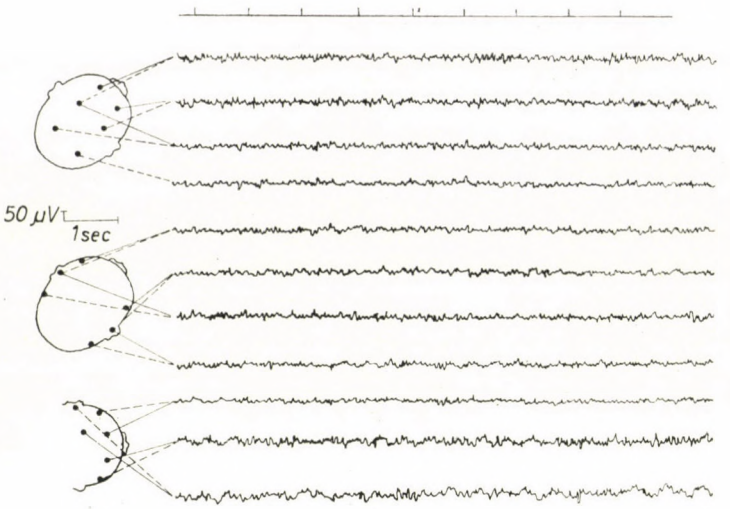


c)

Fig. 3. (*a—b—c:* continuous recording). Paradoxical arousal reaction (theta-synchronization) and desynchronization seizure evoked by strong pinpricks (heavy line)



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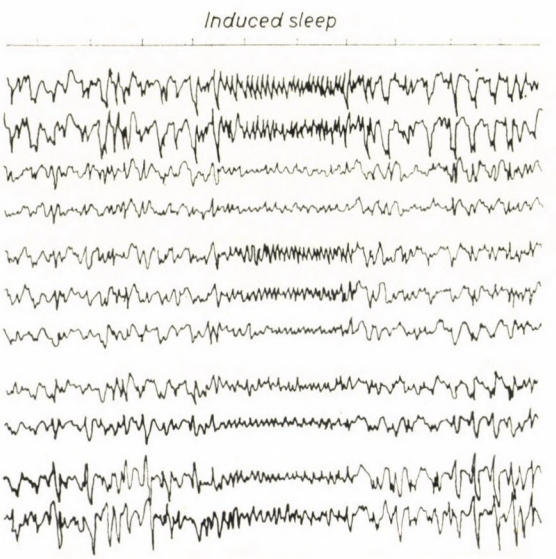

b)

Fig. 4. Resting activity in seizure-free state after successful diazepam therapy: *a)* in the waking state, *b)* in hexobarbital sleep

and on beginning to awake, characteristic generalized repetitive rhythmic fast (9 to 12 c/s) discharge with rostral predominance, reminiscent of the pattern termed "grand-mal discharge" by GIBBS, were observed (Fig. 4).

Discussion

In the status epilepticus of the present patient, two types of seizure were alternating. One was accompanied by an EEG pattern indicative of excitement of the reticular arousal system, and clinically reminiscent of a tonic caricature of orientation. The other was marked by oculo-clonic phenomena and a generalized spike-and-wave pattern. In other words, two types of cerebral activity which had seemed incompatible on the grounds of the earlier discussion were alternating in the same case.

Which mechanism may possibly account for the incompatibility or a competitive antagonism of the two functional states?

From the circumstances that the petit-mal seizure affects consciousness and that it is precipitated by drug-induced sleep it may be inferred that during its production the arousal system is affected in its functions or that it is under the predominance of inhibitory influence. This is consistent with the fact that the wave component of the spike-and-wave pattern results from intracortical inhibition, though it is by no means certain whether it meets at all the pathways of the cortical neurons activated by the arousal system. The facts that spike-and-wave seizures are desynchronized by arousal stimuli and that drugs stimulating the arousal system interrupt the spike-and-wave mechanism suggest that inhibition of the arousal system can be overcome or that it is accessible to a competitive antagonism.

A competitive inhibition of the cortical neurons may therefore well account for the incompatibility of the two conditions, the arousal system thus being inhibited during the spike-and-wave activity. It is known, on the other hand, that a suppression of non-specific subcortical influences favours the manifestation of cortical seizures activity.

The present case raises the question why the arousal stimuli have failed to inhibit the spike-and-wave mechanism, though indicated by the desynchronization seizures the arousal system had obviously been in a state of epileptic overexcitability. Yet the arousal reaction had the character of synchronization rather than of desynchronization. Paradoxical arousal reactions i.e. synchronization may be likewise observed in sleep and in certain comatose states. Thus, the occurrence of paradoxical arousal reactions is suggestive of a lesioned arousal system. On the other hand, intensive arousal stimuli were capable of overcoming the inhibition of the reticular system, probably by breaking through the spike-and-wave mechanism holding the entire system under its dominance, leading thus to an epileptic arousal reaction, to a desynchronization seizure.

There is a further possibility, namely that epileptic inhibition and epileptic excitation of the very same system, i.e. the reticular arousal system including its cortical projections, might have been responsible for both spike-and-wave-

as well as for the desynchronization seizure, since many of the data quoted earlier indicate that electric excitation or a lesion of the reticular arousal system may result in a cortical spike-and-wave pattern.

The mechanism of spike-and-wave activity is still far from being completely understood. It seems, however, certain that cortical structures as well as the non-specific systems are both involved in its production.

The literary data quoted earlier as well as the observations in the reported case strongly suggest that suppression of the function of the arousal system, in all likelihood by an inhibitory mechanism, is an important factor in the production of both clinical and electric phenomena of petit-mal seizures.

REFERENCES

1. Jasper, H., Kershman, J.: Arch. Neurol. Psychiat. (Chic.) **45,** 903 (1941).
2. Jasper, H., Droogleever-Fortuyn, J.: Res. Publ. Ass. nerv. ment. Dis. **26,** 272 (1947).
3. Ingvar, D. H.: Acta physiol. scand. **33,** 1 (1955).
4. Pollen, D. A., Perot, P., Reid, K. H.: Electroenceph. clin. Neurophysiol. **15,** 1017 (1963).
5. Weir, B., Sie, P. G.: Epilepsia **7,** 30 (1966).
6. Echlin, F. A.: Electroenceph. clin. Neurophysiol. **11,** 697 (1957).
7. Pollen, D. A.: Electroenceph. clin. Neurophysiol. **17,** 398 (1964).
8. Bancaud, J., Talairach, J., Bonis, A., Shaub, C., Szikla, G., Morel, P. and Bordas-Ferer, M.: La stéréoencéphalographie dans l'épilepsie. Masson, Paris 1965.
9. Mazars, Y., Mazars, G., Gottuso, C., Merienne, L.: Rev. neurol. **114,** 225 (1966),
10. Milhorat, T. H., Baldwin, M., Hantman, D. A.: J. Neurosurg. **24,** 595 (1966).
11. Walker, A. E., Serrano, H. R.: Arch. Neurol. (Chic.) **8,** 248 (1963).
12. Jung, R., Riechert, T.: Electroenceph. clin. Neurophysiol. **6,** 517 (1954).
13. Janz, D., Neimanis, G.: Epilepsia **2,** 251 (1961).
14. Alema, G., Perria, L., Rosadini, G., Rossi, G. F., Zattoni, J.: J. Neurosurg. **24,** 629 (1966).

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CARBON DIOXIDE CONTENT OF BRAIN TISSUE AND ACID-BASE BALANCE IN HAEMORRHAGIC SHOCK AFTER PRETREATMENT WITH DIBENZYLINE

By

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The pH, bicarbonate, and pCO_2 in carotid and sagittal venous blood have been examined at various stages of haemorrhagic shock in phenoxybenzamine-pretreated dogs and in control animals. Parallel estimations of the carbon dioxide content of frontal and occipital cortex, thalamus, hypothalamus, pons and medulla oblongata have been carried out.

The control animals developed metabolic acidosis during the bleeding period. The marked acidosis and hypercapnia found in sagittal venous blood as contrasted with that of the carotid artery were suggestive of central nervous factors being involved in the mechanism of haemorrhagic shock. Retransfusion brought about no significant change. The considerable cerabral hypercapnia at the end of the bleeding period and in the state of shock is held responsible for the elevation of pCO_2 in sagittal venous blood. Hypercapnia did not affect uniformly the regions of the brain; it was excessive in the hypothalamic and cortical areas, whereas the thalamus was less affected. This suggests that the individual regions of the brain are not uniformly involved in the pathomechanism of haemorrhagic shock.

In phenoxybenzamine-pretreated dogs a slight compensated metabolic acidosis was only found, with the pH values constant during the whole course of the experiment. Hypercapnia failed to appear in sagittal venous blood and in brain tissue.

The preventive use of phenoxybenzamine seems to reduce significantly the adverse effects of impaired circulation and of hypoxia on tissue metabolism; in this mechanism, central nervous factor seem to be involved.

In haemorrhagic shock, profound metabolic changes occur. There is metabolic acidosis, with a significant reduction of bicarbonate and arterial pCO_2 [1, 2, 3]. Blood lactic acid is greatly increased; this is probably one of the factors responsible for the acidosis [4]. Very little is however known about the relationship between acidosis and the irreversibility of shock.

In our earlier crossed circulation studies, isolated perfusion of the head with normal blood was found to prolong survival of animals in shock [5]. Cerebral metabolism in shock reveals various abnormalities [6], such as an increase in hexokinase activity [7] as well as in acetylcholine content [8] and a shift in the pattern of free amino acids. The increased O_2 difference between femoral artery and sagittal vein blood indicated that the O_2 requirement of brain tissue is higher than under normal conditions [9].

Phenoxybenzamine has been found to prolong survival in haemorrhagic shock [10, 11, 12] and to normalize in three or four hours the augmented arterio-venous O_2 difference and the veno-arterial CO_2 differences [13]. Given in the

advanced hypotensive stage of haemorrhagic shock, the drug had no influence on acidosis [2, 14].

Phenoxybenzamine was found favourably to influence the metabolic changes produced by haemorrhagic shock in brain tissue; O_2 consumption failed to increase in pretreated animals [15].

The aim of the present study was to examine the individual factors of acid-base balance in relation to the changes in the carbon dioxide content of brain tissue in haemorrhagic shock. In a series of experiments, the effect of phenoxybenzamine administered before bleeding on acid-base balance and brain tissue CO_2 content was examined.

Method

A total of 95 dogs of both sexes under 100 mg/kg chloralose anaesthesia was used. Into one carotid artery, a T cannula was inserted for blood sampling. A mercury manometer and a cannula for blood withdrawal were inserted in the femoral arteries. The head of the animal was fixed by means of a stereotaxic apparatus, and through a hole 1 cm in diameter bored in the skull a cannula was introduced into the sagittal vein. Respiration was registered by means of a Marey-capsule connected to an intratracheal cannula.

Induction of haemorrhagic shock was done according to WIGGERS [16]. Blood pressure was maintained between 50 and 60 mm Hg for 90 min. and between 30 and 40 mm Hg for another 90 min., then the withdrawn amount of blood was reinfused. In the untreated animals, this procedure invariably resulted in irreversible shock.

40 dogs received an intravenous dose of 4 mg/kg of phenoxybenzamine 18 hrs. before bleeding.

Blood samples were taken from the carotid artery and from the sagittal vein in the pretreated animals as well as in the controls, before bleeding, at the end of the second period, one hour after retransfusion and in the terminal phase of shock. Since in the untreated animals the average interval between retransfusion and the terminal stage was 150 min., this time was adopted for the last blood sampling in the pretreated animals, though these displayed no severe shock. The pH was determined with a Metrohm 353-type apparatus, the total CO_2 and standard bicarbonate by the method of VAN SLYKE [17]. The pCO_2 and actual bicarbonate were determined from nomograms derived from the HENDERSON—HASSEIBALCH-equation, on the basis of the pH and the total plasma CO_2 values [18]. O_2 saturation was estimated by means of an Elema oxymeter and the Hb, by the cyanmethaemoglobin method [19].

After each blood sampling, 10 animals were decapitated by a single blow. The skull was opened immediately, the brain was immersed in paraffin; specimens were excised from the frontal and occipital cortex, thalamus, hypothalamus, pons and medulla oblongata; the samples were pulverized in liquid nitrogen and placed in NaOH. CO_2 content of the tissues was estimated by the method of ANREP [20].

Results

I. *Acid-base balance in haemorrhagic shock*

(a) The figures derived from the control group have been summarized in Table I. By the end of the bleeding period, carotid plasma CO_2 content had fallen from the original level of 20·6 mM/l to 6·1 mM/l, the standard bicarbonate from 18·1 mM/l to 6.8 mM/l. As expected, the actual bicarbonate showed a similar fall, the pCO_2 decreased from 40 mm Hg to 20 mm Hg, and the pH

from 7·32 to 7·03, indicating severe acidosis. The changes were highly significant statistically. The values were scarcely affected by retransfusion. There was a slight elevation of pH, largely as a consequence of an increase in the bicarbonate level. In the further course even the development of shock brought about no significant change.

In the blood of the sagittal vein, total CO_2 and bicarbonates were slightly less reduced than in arterial blood. On the other hand, pCO_2 showed a rise from 56 mm Hg to 67 mm Hg ($p < 0.01$). The V—A pCO_2 difference had thus grown to thrice its normal value by the end of the second stage of bleeding (Fig. 1). This shift in the bicarbonate/CO_2 ratio must have been associated with still lower pH figures than in arterial blood. Here, too, retransfusion did not affect the CO_2- and bicarbonate values significantly. It caused a reduction in pCO_2 but the V—A pCO_2 difference still remained twice as high as it had been before bleeding. The values were practically identical in the state of shock, pH showed a slight tendency to increase but it remained far below its pre-haemorrhage level ($p < 0{\cdot}001$).

Fig. 2 shows the respiratory rate at the four predetermined times of blood sampling. During bleeding the respiratory rate rose from 12/min. to 27/min., to show a moderate reduction after retransfusion, but remaining significantly higher than prior to bleeding ($p < 0{\cdot}001$) and rising significantly again during the further course.

Table I

Total CO_2, *bicarbonate*, pCO_2 *and* pH *during haemorrhagic shock in control animals*
(p referred to values prior to bleeding)

		Total CO_2 mM/l	Standard bicarbonate mM/l	Actual bicarbonate mM/l	pCO_2 mm Hg	pH
carotid artery	Before bleeding	20·6±0·71	18·1±0·42	19·1±0·57	40±2·19	7·32±0·22
	At the end of 2nd phase of bleeding	6·1±0·76 $p \ll 0{\cdot}001$	6·8±1·76 $p \ll 0{\cdot}001$	5·7±0·67 $p \ll 0{\cdot}001$	20±3·84 $p < 0{\cdot}001$	7·03±0·03 $p < 0{\cdot}001$
	1 hr. after retransfusion	8·3±0·56 $p \ll 0{\cdot}001$	8·2±0·87 $p \ll 0{\cdot}001$	6·6±0·61 $p \ll 0{\cdot}001$	22±1·59 $p \ll 0{\cdot}001$	7·12±0·17 $p < 0{\cdot}001$
	Grave shock, 2·5 hrs. after retransfusion	9·9±0·67 $p \ll 0{\cdot}001$	10·5±0·75 $p < 0{\cdot}001$	9·2±0·56 $p < 0{\cdot}02$	26±5·36 $p < 0{\cdot}01$	7·21±0·08 $p < 0{\cdot}001$
sagittal vein	Before bleeding	24·6±1·31	19·0±0·73	22·4±0·50	56±2·00	7·24±0·06
	At the end of 2nd phase of bleeding	14·6±1·06 $p \ll 0{\cdot}001$	8·9±1·26 $p \ll 0{\cdot}001$	11·9±0·87 $p \ll 0{\cdot}001$	67±5·43 $p < 0{\cdot}01$	6·88±0·23 $p < 0{\cdot}01$
	1 hr. after retransfusion	14·7±1·30 $p \ll 0{\cdot}001$	9·5±1·11 $p \ll 0{\cdot}001$	13·1±1·27 $p \ll 0{\cdot}001$	53±4·00 $p = 0{\cdot}2$	7·02±0·02 $p < 0{\cdot}001$
	Grave shock, 2·5 hrs. after retransfusion	16·9±0·90 $p \ll 0{\cdot}001$	11·9±1·17 $p < 0{\cdot}001$	15·2±0·87 $p \ll 0{\cdot}001$	56±5·73 $p > 0{\cdot}9$	7·08±0·20 $p < 0{\cdot}001$

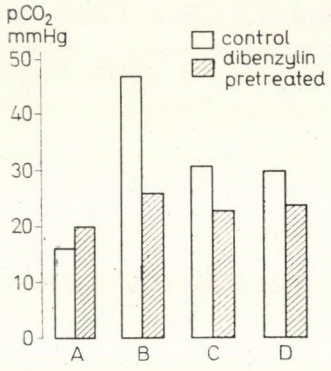

Fig. 1. V—A (sagittal vein—carotid artery) pCO_2 difference during haemorrhagic shock in control dogs and dogs pretreated with phenoxybenzamine. A = before bleeding; B = at the end of the 2nd phase of bleeding; C = 1 hr. after retransfusion; D = 2.5 hrs. after retransfusion (grave shock in the controls)

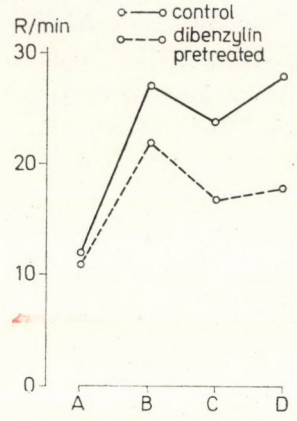

Fig. 2. Respiratory rate during haemorrhagic shock in control dogs and dogs pretreated with phenoxybenzamine. A = before bleeding; B = at the end of the 2nd phase of bleeding; C = 1 hr. after retransfusion; D = 2.5 hrs. after retransfusion (grave shock in the controls)

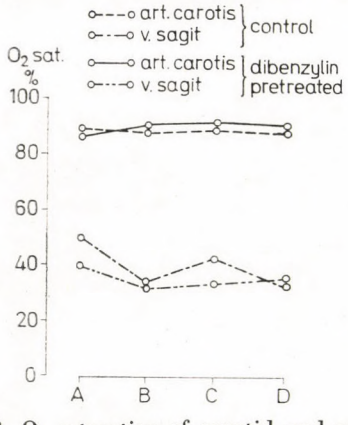

Fig. 3. O_2 saturation of carotid and sagittal venous blood during haemorrhagic shock in control dogs and dogs pretreated with phenoxybenzamine. A = before bleeding; B = at the end of the 2nd phase of bleeding; C = 1 hr. after retransfusion; D = 2.5 hrs. after retransfusion (grave shock in the controls)

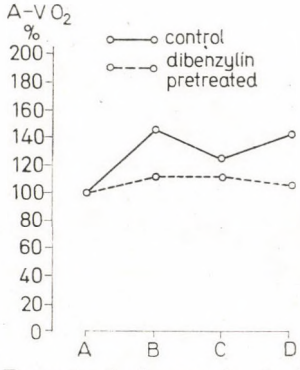

Fig. 4. Percentual changes in A—V (carotid artery—sagittal vein) O_2 difference during haemorrhagic shock in control dogs and dogs pretreated with phenoxybenzamine. A = before bleeding; B = at the end of the 2nd phase of bleeding; C = 1 hr. after retransfusion; D = 2.5 hrs. after retransfusion (grave shock in the controls)

Arterial O_2 saturation displayed no change during the whole course of the experiment (Fig. 3), whereas in the sagittal vein it fell considerably during hypotension, to show a slight transient rise upon the effect of retransfusion. This involved a nearly 50% increase in A—V O_2 difference during bleeding which diminished transiently after retransfusion (Fig. 4).

(b) In the phenoxybenzamin pretreated dogs the values prior to bleeding were similar to those found in the controls (Table II), while those obtained at the end of bleeding period diverged from the figures registered in the controls. Total CO_2 and bicarbonate levels in arterial as well as in venous blood declined to a far lesser extent than in the controls. pCO_2 in carotid and sagittal venous blood showed a nearly parallel fall, in other words, no rise as evidenced in the sagittal venous blood of the controls occurred. In the phenoxybenzamine-pretreated animals the V—A pCO_2 difference was scarcely affected at the end of bleeding (Fig. 1) and showed no significant change ($p > 0{\cdot}2$) in the later course either. Retransfusion did not modify any of these factors. pH remained unchanged during the entire course of the experiment. Respiratory rate (Fig. 2) increased in the pretreated animals during haemorrhage, but the rise from 11 to 22 per min. was significantly less ($p > 0{\cdot}01$) than that registered in the controls, and it declined still more after retransfusion, to remain at practically the same level in the subsequent course of the experiment.

In the pretreated animals, as contrasted with the controls, neither bleeding nor retransfusion had any effect on O_2 saturation either in carotid or in sagittal venous blood (Fig. 3). In agreement with our previous findings, the increase in A—V O_2 difference during bleeding was insignificant and became practically normal after retransfusion (Fig. 4).

Table II

Total CO_2, *bicarbonate*, pCO_2 *and* pH *during haemorrhagic shock in dogs pretreated with phenoxybenzamine*

		Total CO_2 mM/l	Standard bicarbonate mM/l	Actual bicarbonate mM/l	pCO_2 mm Hg	pH
carotid artery	Before bleeding	$19{\cdot}9 \pm 1{\cdot}10$	$17{\cdot}0 \pm 0{\cdot}59$	$17{\cdot}8 \pm 0{\cdot}94$	$41 \pm 3{\cdot}78$	$7{\cdot}28 \pm 0{\cdot}08$
	At the end of 2nd phase of bleeding	$12{\cdot}5 \pm 0{\cdot}73$ $p \leqslant 0{\cdot}001$	$13{\cdot}4 \pm 0{\cdot}67$ $p < 0{\cdot}02$	$11{\cdot}9 \pm 0{\cdot}66$ $p < 0{\cdot}01$	$25 \pm 2{\cdot}62$ $p < 0{\cdot}001$	$7{\cdot}30 \pm 0{\cdot}05$ $p > 0{\cdot}1$
	1 hr. after retransfusion	$13{\cdot}6 \pm 0{\cdot}63$ $p < 0{\cdot}001$	$12{\cdot}6 \pm 0{\cdot}80$ $p < 0{\cdot}01$	$12{\cdot}7 \pm 1{\cdot}14$ $p < 0{\cdot}01$	$27 \pm 2{\cdot}47$ $p < 0{\cdot}001$	$7{\cdot}27 \pm 0{\cdot}05$ $p > 0{\cdot}3$
	Grave shock 2·5 hrs. after retransfusion	$14{\cdot}1 \pm 0{\cdot}96$ $p < 0{\cdot}001$	$14{\cdot}9 \pm 1{\cdot}15$ $p < 0{\cdot}05$	$13{\cdot}5 \pm 0{\cdot}88$ $p < 0{\cdot}001$	$23 \pm 1{\cdot}98$ $p < 0{\cdot}001$	$7{\cdot}36 \pm 0{\cdot}04$ $p > 0{\cdot}02$
sagittal vein	Before bleeding	$23{\cdot}5 \pm 0{\cdot}77$	$18{\cdot}9 \pm 0{\cdot}81$	$21{\cdot}3 \pm 0{\cdot}75$	$61 \pm 3{\cdot}67$	$7{\cdot}19 \pm 0{\cdot}04$
	At the end of 2nd phase of bleeding	$19{\cdot}2 \pm 0{\cdot}83$ $p < 0{\cdot}01$	$15{\cdot}2 \pm 0{\cdot}61$ $p < 0{\cdot}01$	$17{\cdot}4 \pm 0{\cdot}71$ $p < 0{\cdot}01$	$51 \pm 4{\cdot}57$ $p < 0{\cdot}001$	$7{\cdot}18 \pm 0{\cdot}07$ $p > 0{\cdot}4$
	1 hr. after retransfusion	$19{\cdot}2 \pm 0{\cdot}71$ $p < 0{\cdot}01$	$15{\cdot}4 \pm 1{\cdot}75$ $p < 0{\cdot}01$	$17{\cdot}3 \pm 0{\cdot}76$ $p < 0{\cdot}01$	$50 \pm 3{\cdot}00$ $p < 0{\cdot}001$	$7{\cdot}18 \pm 0{\cdot}08$ $p > 0{\cdot}4$
	Grave shock 2·5 hrs. after retransfusion	$20{\cdot}1 \pm 1{\cdot}08$ $p < 0{\cdot}01$	$16{\cdot}5 \pm 1{\cdot}80$ $p < 0{\cdot}02$	$18{\cdot}9 \pm 0{\cdot}93$ $p < 0{\cdot}02$	$47 \pm 2{\cdot}80$ $p < 0{\cdot}001$	$7{\cdot}24 \pm 0{\cdot}03$ $p > 0{\cdot}05$

II. *CO_2 content of individual brain regions*

(a) CO_2 content of cerebral tissue obtained from six different parts of the brain ranged between 15 and 20 mM/kg prior to bleeding (Table III). By the end of the bleeding period the figures had risen to 40 mM/kg in the hypothalamus, to 30·5 mM/kg in the frontal cortex whereas the value found in the thalamus (20·4 mM/kg) was not significantly higher. Retransfusion was followed by a reduction of hypercapnia in the hypothalamus and the frontal cortex, and by its complete normalization in pons and medulla oblongata whereas in the thalamic sample the values remained unchanged. With increasing severity of shock, the CO_2 content of cerebral tissue rose throughout and the increase found in severe shock was highly significant, the values being 35·9 mM/kg in the frontal cortex, 36·9 mM/kg in the occipital cortex, 26·3 mM/kg in the thalamus, 40·0 mM/kg in the hypothalamus, 22·8 mM/kg in the pons and 25·2 mM/kg in the medulla oblongata. The percentual relations to the pre-haemorrhagic values are presented in Fig. 5 which clearly shows the extent of hypercapnia affecting the individual regions.

(b) CO_2 values recorded before bleeding were higher in the pretreated animals than in the controls. Levels over 20 mM/kg were, however, confined to the frontal cortex (23.1 mM/kg; $p < 0{\cdot}1$) and to the hypothalamus (26·3 mM/kg;

Table III

Tissue CO_2 content of cerebral areas during haemorrhagic shock in control dogs and in dogs pretreated with phenoxybenzamine

	Controls				Pretreated			
	Before bleeding	At the end of 2nd phase of bleeding	1 hr. after retransfusion	Grave shock	Before bleeding	At the end of 2nd phase of bleeding	1 hr. after retransfusion	2.5 hrs. after retransfusion
Frontal cortex	18·8	30·5 $p < 0{\cdot}01$	24·8 $p < 0{\cdot}05$	36·9 $p < 0{\cdot}01$	23·1	15·0 $p < 0{\cdot}02$	21·0 $p > 0{\cdot}2$	18·6 $p > 0{\cdot}2$
Occipital cortex	19·1	24·8 $p = 0{\cdot}05$	25·9 $p < 0{\cdot}05$	36·9 $p < 0{\cdot}01$	19·9	13·2 $p < 0{\cdot}02$	18·9 $p > 0{\cdot}5$	16·6 $p > 0{\cdot}2$
Thalamus	17·3	20·4 $p > 0{\cdot}01$	20·8 $p > 0{\cdot}02$	26·3 $p < 0{\cdot}01$	17·7	14·7 $p > 0{\cdot}1$	16·3 $p > 0{\cdot}02$	13·1 $p > 0{\cdot}2$
Hypothalamus	14·9	40·0 $p < 0{\cdot}01$	25·3 $p < 0{\cdot}02$	40·0 $p < 0{\cdot}01$	26·3	34·5 $p > 0{\cdot}1$	18·8 $p < 0{\cdot}02$	23·1 $p > 0{\cdot}2$
Pons	15·9	23·8 $p = 0{\cdot}05$	14·4 $p > 0{\cdot}5$	22·8 $p = 0{\cdot}05$	17·9	17·9	16·5 $p > 0{\cdot}5$	10·9 $p < 0{\cdot}02$
Medulla oblongata	14·9	18·7 $p < 0{\cdot}01$	15·7 $p > 0{\cdot}5$	25·2 $p < 0{\cdot}01$	15·7	14·2 $p > 0{\cdot}5$	19·0 $p > 0{\cdot}1$	10·9 $p < 0{\cdot}05$

$p < 0{\cdot}01$). In opposition to the controls, in these animals the CO_2 content of cerebral tissue diminished, except for the hypothalamus, but apart from the cortical portions, the reduction was not significant statistically. No tissue hypercapnia was found after retransfusion either, and the absolute values of tissue CO_2 ranged between 10 and 21 mM/kg in the further course of the experiment (Table III). Fig 5 shows this difference still more clearly.

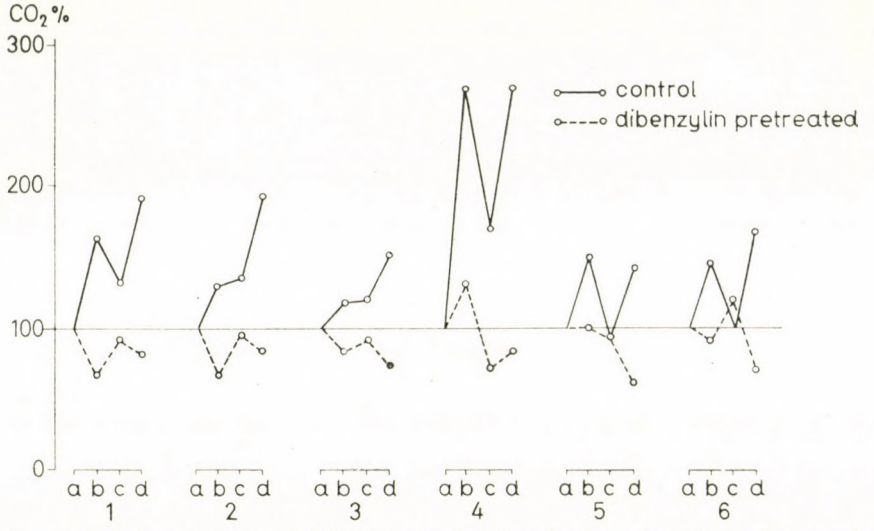

Fig. 5. Percentual changes in tissue CO_2 content of various brain regions during haemorrhagic shock in control dogs and dogs pretreated with phenoxybenzamine. 1 = frontal cortex; 2 = occipital cortex; 3 = thalamus; 4 = hypothalamus; 5 = pons; 6 = medulla oblongata. *a* = before bleeding; *b* = at the end of the 2nd phase of bleeding; *c* = 1 hr. after retransfusion; *d* = 2.5 hrs. after retransfusion (grave shock in the controls)

Discussion

The acid-base disturbance characteristic of haemorrhagic shock [1, 2, 3] has been reproduced in the present study. By the end of the bleeding period there was a substantial reduction in pH and the bicarbonate level had dropped to one third, pCO_2 to half of the values found prior to haemorrhage. These are signs of metabolic acidosis. Examining the time relations of the changes of acid-base balance in hypovolaemia, BAUBKUS and KIRCHHEIM observed that during the early phase of bleeding there is a sudden drop in standard bicarbonate which the animal compensates by hyperventilation, i.e. by decreasing the pCO_2. Consequently, pH remains unaffected for a time. Later, however, it drops suddenly; this fall has been found to coincide with the retransfusion of blood [3]. In the present study the second withdrawal of blood took place at the end of the bleeding period when reflow of blood from the tank was already in progress. The values found in the respective samples therefore reflect the conditions subsequent to retransfusion.

As shown by the present results, acidosis is not substantially reduced by retransfusion. A slight rise in the bicarbonate level at unchanged pCO_2 produces an insignificant increase of the pH. In the further course of the experiment there was no noteworthy change.

The part played by acidosis in the pathomechanism of shock is not clear. According to LILLEHEI et al. [21] correction of the pH alone does not affect the outcome. This is in agreement with the findings of MEHMEL et al. [22] who failed to demonstrate any relationship between the changes in pH and the prognosis of shock. DARBY and WATTS [2] have shown that correction of acidosis by means of Tham or Na-bicarbonate during bleeding results in an improvement of cardiac performance, in a reduction of the respiratory rate as well as of the blood epinephrine level and in an increase of arterial pO_2. SCHWEIZER et al. [4] advocate the use of Na-bicarbonate for the prevention of surgical shock. We are, however, still ignorant of the changes in intracellular pH accompanying the acidosis.

Comparison of the pH, bicarbonate and pCO_2 in carotid and sagittal venous blood suggests that the central nervous system must be involved in the disturbance of acid-base balance. It is striking to find a significant increase in pCO_2 in the blood of the sagittal vein during bleeding, while the bicarbonate level declines in the same manner as in arterial blood. This causes a substantial fall in pH, largely in consequence of the increase in pCO_2. Acidity thus increases in the blood perfusing the brain. This goes hand in hand with an increase in the A—V O_2 difference and a fall of tissue pO_2, interpreted by KOVÁCH et al. [9] as a sign of increased cerebral O_2 consumption.

The hypercapnia revealed in the cerebral areas at the end of bleeding was not uniform. The site of the most significant changes was the hypothalamus, next in order the cortex; the thalamus was less affected. Hypercapnia of the medulla oblongata was confined to severe shock. From this it may be inferred that haemorrhage and a shock affect the individual parts of the central nervous system to a different extent and not simultaneously. Similar differences in cerebral blood distribution have been found in earlier haemodynamic studies in this laboratory. During hypotension and in advanced shock, blood flow in the hypothalamus and cortex was significantly reduced, whereas in the pons and medulla oblongata it was more or less adequate. The pO_2 in these brain areas also points to a greater vulnerability of the hypothalamic and cortical parts. During bleeding pO_2 in the hypothalamus fall to 20% of its original level and even subsequent retransfusion failed to restore it [15]. This applied to the cortical portions as well. The fall in pO_2 was much too great to be ascribed to a poor regional blood flow alone.

Our results seem to suggest that the reduction in blood flow and the increase in O_2 requirement during the hypotensive phase lead not only to hypoxia but also to metabolic changes in the cerebral tissue, as reflected by tissular

hypercapnia and by increasing acidosis. It also emerges from our studies that the various parts of the brain are not uniformly affected. The hypothalamus and the cortex suffer the severest damage. This is presumably involved in the development of shock.

None of the pretreated dogs presented any of the signs of severe acidosis found in the controls during haemorrhage. The pH remained constant throughout. The presence of a moderate and compensated metabolic acidosis was indicated by a slight reduction in the bicarbonate. The metabolic imbalance was well within the compensatory capacity of the animals as it had indeed been overcome by hyperventilation, i.e. the reduction of pCO_2. In the blood of the sagittal vein pH remained constant and no increase in pCO_2 occurred during haemorrhage as it did in the controls; there was, on the contrary, a slight reduction in pCO_2 parallel with a decline in the bicarbonate level. In short, these factors were not significantly affected in the blood perfusing the central nervous system of the pretreated animals. In agreement with our earlier findings [15], O_2 consumption was likewise within normal limits in the pretreated animals and no cerebral hypercapnia could be observed. The contrast between the pretreated and the control animals is emphasized by the fact that, as has been shown in this laboratory, the slowing down of circulation during the bleeding period was practically of the same extent in the pretreated animals as in the controls.

Phenoxybenzamine is assumed to exert a direct influence on tissue metabolism, to reduce central nervous injury, and to enhance tissue flow. Its protective influence in haemorrhagic shock is probably connected with these factors.

REFERENCES

1. Root, W. S., Allison, J. P., Holmes, J. H., Walkott, W. W., Gregersen, M. I.: Amer. J. Physiol. **149,** 52—63 (1947).
2. Darby, T. D., Watts, D. T.: Amer. J. Physiol. **206,** 1281—1284 (1964).
3. Baubkus, H., Kirchheim, H.: Acta physiol. Acad. Sci. hung. **29,** 342 (1966).
4. Schweizer, O., Howland, W. S.: Anesth. Analg. Curs. Res. **43,** 420—432 (1964).
5. Kovách, A. G. B., Roheim, P., Irányi, M., Kiss, S., Antal, J.: Acta physiol. Acad. Sci. hung. **14,** 231—238 (1958).
6. Kovách, A. G. B., Fonyó, A.: Metabolic Response to Injury in Cerebral Tissue. Ed.: Stoner, H. B. Blackwell, Oxford 1960, Pp. 129—161.
7. Kovách, A. G. B., Fonyó, A., Vittay, T., Pogátsa, G.: Acta physiol. Acad. Sci. hung. **11,** 173—180 (1957).
8. Kovách, A. G. B., Fonyó, A., Halmágyi, M.: Acta physiol. Acad. Sci. hung. **13,** 1—4 (1958).
9. Kovách, A. G. B., Róheim, P., Irányi, M., Cserháti, E., Gosztonyi, G., Kovách. E.: Acta physiol. Acad. Sci. hung. **15,** 217—229 (1959).
10. Lotz, F., Beck, L., Stevenson, J. A. F.: Canad. J. Biochem. **33,** 741—752 (1955).
11. Berger, R. L., Healey, P. J. M., Byrne, J. J.: Proc. Soc. exp. Biol. (N. Y.) **110,** 225—227 (1962).
12. Nickerson, M.: Amer. J. Cardiol. **12,** 619—623 (1963).
13. Vick, J. A., Ciuchta, H. P., Merickel, J. H., Lindseth, E. O.: Circulat. Res. **16,** 58—64 (1965).

14. BAVE, A. E., JOHNSON, D. G., PARKINS, W. M.: Amer. J. Physiol. **211,** 354—360 (1960).
15. KOVÁCH, A. G. B., MITSÁNYI, A., STEKIEL, W.: Acta physiol. Acad. Sci. hung. Suppl. **26,** 35 (1965).
16. WERLE, J. M., COSBY, R. S., WIGGERS, C. J.: Amer. J. Physiol. **136,** 401—420 (1942).
17. PETERS, J. P., VAN SLYKE, D.: Quantitative Clinical Chemistry. II. Williams & Wilkins, Baltimore 1932. P. 981.
18. VAN SLYKE, D., SENDROY, J. jr.: J. biol. Chem. **79,** 781—798 (1928).
19. BAKOS, GY.: Orv. Hetil. **104,** 2474—2476 (1963).
20. ANREP, G. V., AYADI, M. S., TALAAT, M.: J. Physiol. (Lond.) **86,** 153—161 (1936).
21. LILLEHEI, R. C., LONGERBEAM, J. K., BLOCH, J. H., MANAX, W. G.: Ann. Surg. **160,** 682—710 (1964).
22. MEHMEL, H., VAN ACKERN, K., SCHMIDT, H. D., SCHMIER, J.: Pflügers Arch. ges. Physiol. **34,** 32 (1966).

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INDUCED REGENERATION OF THE RAT LIVER DURING EXPERIMENTAL CIRRHOSIS

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Liver regeneration preferentially affects parenchymal cells. An attempt has been made to make use of this peculiar character of regeneration for restoring the cellular balance of hepatic tissue disturbed by the cirrhotic process.

Liver regeneration following partial hepatectomy was found to be essentially unaltered in moderate cirrhosis. The overall picture of the mitotic response elicited by partial hepatectomy resembled that of the normal group. As a result of the induced regeneration, cirrhotic hypertrophy (high L/B ratio) changed to eutrophy (normal range of L/B ratio).

Conversely, an almost complete loss of the regeneration potency was observed in severe cirrhosis. No mitotic response could be elicited by partial hepatectomy at this stage. This explained why atrophy in severe cirrhosis invariably remained unaffected by partial hepatectomy.

The significance of a preserved mitotic response — and the consequence of its loss — in restoring cellular proportionality in the cirrhotic liver, and the possible mechanism of the beneficial influence of induced regeneration in cases of moderate cirrhosis have been discussed.

Parenchymal cells constitute normally 90 to 95% of the total hepatic cellular volume, but merely 60 to 65% of the total cell number. Therefore, approximately 5 to 10% of the total hepatic cellular mass and 35 to 40% of the cellular population is composed of non-parenchymal elements [4, 6].

In cirrhosis, due to the destruction of parenchymal cells and the growing number of fibrotic components, the characteristic balance of cellular composition is considerably disturbed. To suspend or eventually to reverse this undesirable structural consequence of cirrhosis, the number of parenchymal cells ought to be increased at the expense of the fibrotic elements.

The idea therefore that in cirrhosis the tissue proliferation stimulus should be counteracted by some kind of a parenchymal regeneration stimulus seemed only natural.

While searching for such a stimulus it has been found that the restoration process following partial hepatectomy was preferentially affecting parenchymal cells [6] and their restoration proceeded much more rapidly than that of connective tissue, while collagen formation started late at a low rate when parenchymal restoration had been complete [6].

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The few attempts carried out so far with the aim of using partial hepatectomy for restoring the disturbed balance of cellular composition in advanced cirrhosis, provided controversial results. Several investigators failed to induce regeneration in this way [3, 8], while others [10, 11] were fairly successful in correcting cirrhotic damage by partial hepatectomy.

In the present experiments an attempt was made to find quantitative means for evaluating the real value of partial hepatectomy in abolishing, or at least diminishing, the severity of cirrhotic alterations in liver tissue. An additional aim was to establish the proportion of regeneration potency escaping destruction in cirrhosis of different severity.

Materials and methods

A total of 176 male rats, weighing between 120—160 g was used for the study. The animals were divided into four groups.

55 intact rats were used as controls.

In 73 rats moderate cirrhosis was induced by the subcutaneous administration of 0·15 ml/100 g of CCl_4 on every second day for 8 weeks. One week after the termination of CCl_4 administration, of the 73 rats 49 were subjected to about 65% partial hepatectomy, using the method of HIGGINS and ANDERSON [7]. The remaining 24 rats were not subjected to surgery and served as controls.

In 48 rats severe cirrhosis was induced by identical treatment. In this case CCl_4 administration was carried out for 18 weeks. Partial hepatectomy was perfomed one week after CCl_4 treatment had been terminated. A number of animals was not subjected to surgery and served for comparative purposes.

The degree of liver regeneration was estimated at different intervals postoperatively, indicated in the figures. Calculation was based upon measurement of dry weight of the newly formed liver tissue removed at surgery.

The efficacy of regeneration in restoring the original state was judged by serial determination of the ratios of liver weight per body weight (L/B ratio) at regular intervals.

For estimating the fraction of regeneration capacity spared by cirrhotic destruction, mitotic responses of individual livers were regularly followed. Mitotic counts were done at different postoperative intervals, as indicated in the figures. In every case, 2000 cells were counted in HE stained sections. Values were expressed in per cent, using the formula

$$\frac{\text{mitotic figures found among 2000 cells counted}}{20}$$

For statistical analysis, Student's *t*-test was used.

Results

A) *Regeneration* (Fig. 1)

1. *Control* (Curve No. 1). Regeneration of normal livers following partial hepatectomy was almost complete on the 10th postoperative day. Values indicating more than 100% "regeneration" between the 30th and 180th day (Fig. 1) were not considered a result of a true regeneration process. Instead, they presumably indicated "developmental growth" of livers, which kept pace with the growing body-size of the animals.

2. *Moderate cirrhosis* (Curve No. 2). The course of tissue restoration, if at all, was not altered substantially in this group. The curve agreed well with that of the control group.

3. *Severe cirrhosis* (Curve No. 3). Regeneration following partial hepatectomy was seriously impaired, if not completely blocked, in severe cirrhosis. Hardly any restoration of liver parenchyma could be detected even on the 30th postoperative day.

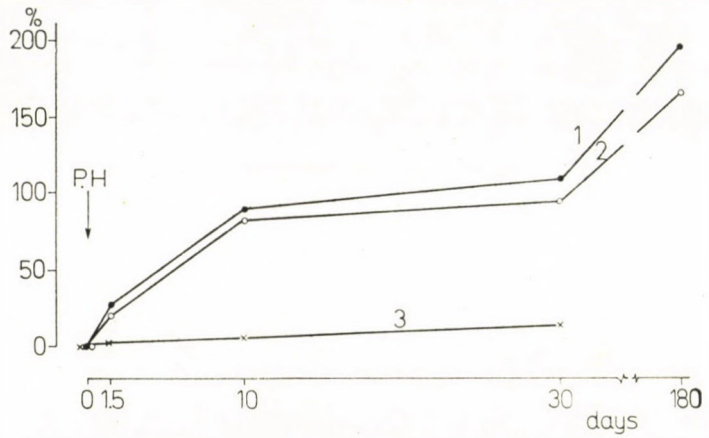

Fig. 1. Course of tissue restoration following partial hepatectomy, observed in normal (Curve No. 1), moderately cirrhotic (Curve No. 2) and in severely cirrhotic (Curve No. 3) livers. *Ordinate:* regeneration in per cent. *Abscissa:* time in days

B) *L/B ratio.* (Fig. 2)

Information obtained from L/B ratios was, in some respects, even more conclusive. The preoperative value scattered widely in the different experimental groups: it was 3.65 in the normal group; 5.70 in moderate, and 2.57 in severe cirrhosis. The differences were statistically significant at the five per cent level (normal vs. moderate cirrhosis $p < 0.005$; normal vs. severe cirrhosis $p < 0.05$). L/B ratios seen preoperatively in moderate cirrhosis indicated a liver hypertrophy. On the other hand, low values, found mostly in severe cirrhosis, pointed to liver atrophy.

As a result of partial hepatectomy, the L/B ratio diminished irrespective of the state of the liver. Striking differences between individual groups were however found in the postoperative course of the ratios.

1. *Control* (Curve No. 1). In intact animals, complete normalization of L/B ratio occurred by the 10th postoperative day.

2. *Moderate cirrhosis* (Curve No. 2). Characteristic were the high L/B ratios in the postoperative interval. Loss in body weight due to the CCl_4-treatment, contributed about 30% to the elevation while for the rest the

active increment in liver weight was responsible. The preoperatively elevated resting L/B ratio normalized after partial hepatectomy. After the highest value had been reached around the 10th day, a characteristic drop of the ratio followed. As a result, values approached the control range at the end of the observation period.

Conversely, merely a moderate decrease in the ratio could be demonstrated in the similarly cirrhotic group left without surgery (Curve No. 3).

3) *Severe cirrhosis* (Curve No. 4). Characteristically low preoperative atios were found in this group. This was all the more remarkable, because this

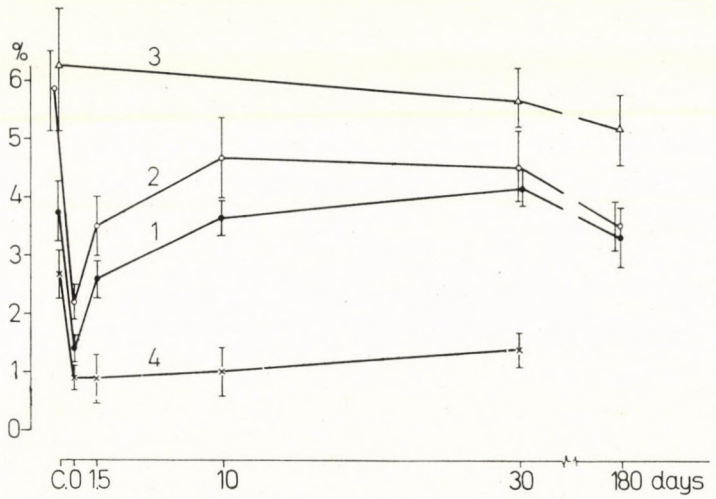

Fig. 2. L/B ratios. Curve No. 1, control. Curve No. 2, moderate cirrhosis. Curve No. 3, moderate cirrhosis without surgery. Curve No. 4, severe cirrhosis. *Ordinate:* L/B ratios in per cent. *Abscissa:* time in days

phase of cirrhosis was dominated by a radical loss in body weight. Following a sharp drop in ratios due to partial hepatectomy, not even the slightest tendency towards restoration could be detected. Values continued to remain low even on the 30th postoperative day. These animals showed a poor tolerance to the surgical manipulation. Both the partial hepatectomy and the convalescence period were characterized by an unusually high mortality. As a result values of three animals could only be registered on the 30th day; no animal survived longer.

C) *Mitotic incidence* (Fig. 3)

The proportion of regeneration potency spared by cirrhotic destruction was determined by evaluating the pattern of mitotic responses elicited by partial hepatectomy.

1. *Control* (Curve No. 1). The curve started from a low level. The stimulus of partial hepatectomy increased the mitotic incidence considerably until

a peak had been reached at around the 30th postoperative hour. The downward slope of the curve was equally steep and the whole mitotic response was essentially over by the 10th postoperative day.

2. *Moderate cirrhosis* (Curve No. 2). The course of mitotic responses started from a level much higher than the control. It appeared as if a phase shift would have occurred; postoperative mitotic frequency lagged considerably behind the normal, both in time and numerical value. At the 30th postoperative hour, when the peak had already been reached in the normal group, the

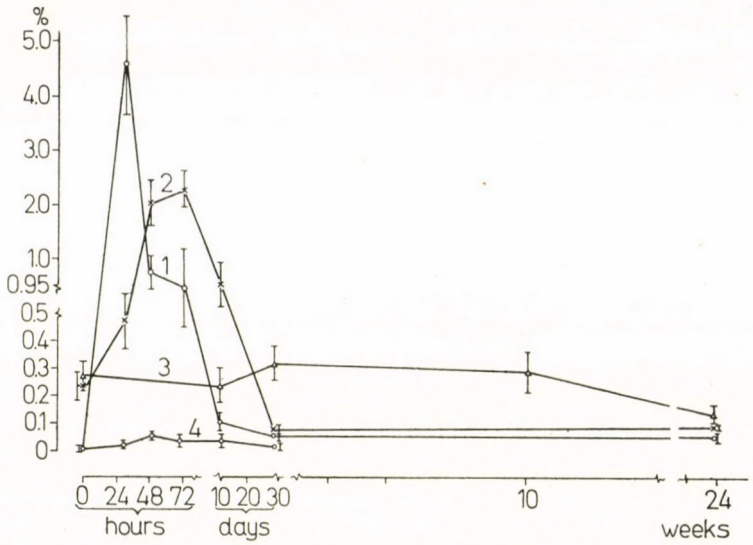

Fig. 3. Mitotic response in livers following partial hepatectomy. Curve No. 1, control. Curve No. 2, moderate cirrhosis. Curve No. 3, moderate cirrhosis without surgery. Curve No. 4, severe cirrhosis. *Ordinate:* mitotic incidence in per cent. *Abscissa:* time in hours, days and weeks

cirrhotic values attained a mere 10% of the control value. However, in contrast to the control, these values continued to increase till the highest values had been reached around the 72nd postoperative hour, but even this peak amounted to merely 50% of the control.

The downward slope of the curve was less steep. While the normal curve reached its preoperative level around the 10th postoperative day, the curve of moderate cirrhosis took considerably longer to decrease, and continued to diminish progressively below the initial values. When around the 30th day the curve had established the new level, it was identical with that of the control group.

The mitotic curve of the group which undervent identical CCl_4 treatment but was left without surgery, also exhibited a tendency to normalization (Curve No. 3). This tendency was however significantly weaker than in the operated group. Accordingly, while mitotic values of the operated group reached the

control value in the 4th week, the non-operated group achieved the same between the 10th and 24th week.

3. *Severe cirrhosis* (Curve No. 4). Equally characteristic was the curve of severe cirrhosis. Here, like in the intact cases, preoperative mitotic incidence was scarce. Partial hepatectomy failed to elicit a mitotic response. The number of mitotic figures continued staying near zero throughout the entire observation period, whether or not the proliferative stimulus was present.

Discussion

Cirrhotic alterations of the hepatic parenchyma involve both morphological and functional deviations from normal. They are reflected in distortion of liver structure as well as in gradual deterioration of several differentiated organ functions, in addition to a considerable shift in the haemodynamics of the organ [1, 9]. All these changes are obviously interrelated and, at the final analysis, they are interfering with, or even are based upon alterations of, cellular metabolism in one way or other.

The most significant event of regeneration is the drastically elevated rate of cellular multiplication. The normal course of cellular division requires a subtle coordination of a number of processes, including synthesis of nucleic acids, building up of the mitotic apparatus, new formation of cellular proteins, etc. It is equally obvious that both resting mitotic frequency and patterns of mitotic responses obtained after an appropriate stimulus, are equally dependent on the physiological state of the tissue in question. Thus e.g. the failure of mitotic machinery to be activated by partial hepatectomy, could be looked upon as a consequence of the impairment in cellular metabolism.

In our experiments, CCl_4 cirrhosis developed through two, well defined stages. One was characterized by tissue hypertrophy (high L/B ratio), while the other by tissue atrophy (low L/B ratio). They corresponded to the moderate and the severe variety of cirrhosis respectively.

Liver hypertrophy by itself has to be considered a manifestation of a satisfactorily maintained proliferation potency. The process of hypertrophization namely must be based upon a preserved capacity of cells to divide when stimulated by the destruction of liver tissue due to the cirrhogenic injury. The preserved regeneration potency was convincingly illustrated by the brisk proliferation response regularly obtained following partial hepatectomy in the case of hypertrophic livers. A similar response could not be elicited in atrophic livers.

Thus, it is reasonable to suppose that, maintenance or loss of the mitotic machinery in a state of responsiveness will occasionally decide whether hypertrophy or atrophy will prevail in a given cirrhotic process. On the other hand,

this is exactly what determines whether the process will belong to the moderate or severe type of cirrhosis; whether or not it will be reversible.

Resting mitotic frequency is negligible under normal circumstances [6] and the L/B ratio is low. This state corresponds to the "eutrophic" condition. In such cases, the mitotic machinery is easily set into motion by an appropriate stimulus. The response is quick and extensive.

On the other hand, resting mitotic frequency was found to be elevated during moderate cirrhosis. L/B ratios were likewise higher than normal, indicating the hypertrophic state of the liver. Simultaneously, mobilization of the mitotic machinery encountered some difficulty. The augmentation in the number of mitotic figures was sluggish after partial hepatectomy, the peak value was also lower than normally. The time course of the mitotic response was substantially prolonged. All these events might be regarded as early indications of a mild disturbance in metabolic processes underlying mitotic cellular events.

In severe cirrhosis, resting mitotic incidence, resembling the normal, was very scarce, L/B ratios were unusually low. This latter fact pointed to tissue atrophy. In these livers, however, unlike in normal ones, the low number of mitosis was presumably due not to a lack of stimulus, but rather to the cells having lost their capacity to respond to stimuli. This supposition has been confirmed by the fact that, partial hepatectomy — otherwise one of the most powerful proliferation stimuli — failed to elicit a mitotic response of any extent. This was indicative of a severe damage of the metabolic processes responsible for cellular division.

The tissue hypertrophy (high L/B ratio) observed in moderate cirrhosis, changed to eutrophy due to the induced liver regeneration. Concomitantly, a significant normalization of the cellular composition of the distorted liver parenchyma could be observed. This was associated in turn by a considerable improvement of hepatic functions partially destroyed during the cirrhotic process (unpublished data). The striking reorganization of the cirrhotic liver parenchyma, described also in the pertaining literature [3, 10], was based presumably on the peculiar character of tissue restoration induced by partial hepatectomy.

Partial hepatectomy is a powerful stimulus of liver regeneration in the rat [2, 7]. Removal of about 65% of the organ is followed as a rule by quick proliferation, resulting in complete restoration of the original liver mass in a surprisingly short interval [5, 13]. A characteristic feature of this proliferation is that the different cellular components of the parenchyma are represented heterogeneously in the process. It appears as if parenchymal cells were the primary targets of the proliferation stimulus, while the non-parenchymal elements were much more reluctant in obeying regenerative command [5, 6]. Accordingly, parenchymal cells appear to be more sensitive to the stimulus [5].

Owing to the ensuing and disproportional activation of different cellular components by partial hepatectomy, the relative proportion of parenchymal cells is successively increasing. By this means, restoration of the original pattern of hepatic cellular composition is efficiently supported in the cirrhotic liver.

The efficacy of evoked regeneration in restoring cellular proportionality may be further supplemented by other mechanisms. CAMERON assumed that, although liver restoration during induced regeneration is mainly based upon proliferation of the liver cells, nevertheless the active disappearance of fibrotic tissue bands may be a concomitant factor in the process [3].

Thus, as a result of the regeneration, replacement of cirrhotic liver structure by a nearly normal one could be reached in two ways, viz. 1. by increasing the relative proportion of parenchymal cells; and 2. by actively decreasing the amount of fibrotic tissue elements.

A similar set of events could be followed also in our experiments. It was shown earlier how in moderate cirrhosis the high L/B ratio decreased due to the regeneration process. It was also demonstrated that, following partial hepatectomy the high mitotic incidence characteristic of moderate cirrhosis failed to stabilise at the preoperative level but diminished further until it had reached the normal range. It seemed therefore likely that, due to the peculiar character of the regeneration process elicited by partial hepatectomy — acting predominantly on parenchymal cells [3] and allegedly destroying fibrotic elements [5, 6] — the desorganizing effect of cirrhosis was successfully counteracted. As a result, the cirrhotic liver parenchyma was gradually replaced by a nearly normal one. This gave rise to the eutrophy of the originally hypertrophic liver tissue. In contrast to the earlier state, the recomposed liver parenchyma presumably ceases to act as a mitotic stimulus. Therefore, after the near-normal balance of cellular composition had been reached, mitotic frequency decreased to normal.

It must however be stressed that, all the assumed beneficial influences of partial hepatectomy were strictly confined to the moderate phase of cirrhosis. In the severe form, partial hepatectomy failed to elicit either a regeneration or a favourable change of the L/B ratio, or a mitotic response. In this way, the efficacy of evoked regeneration in repairing cirrhotic damage appeared to be determined by the phase of the cirrhotic process. The borderline seems to be between the hypertrophic (reversible or moderate) and atrophic (irreversible or severe) stages. While in the first case, the tissue still possesses all the biological means required for the restoration and reorganization of mass and structure, respectively, this ability is gradually lost in the second case. Therefore, our expectation towards eliminating a cirrhotic damage by induced liver regeneration, should always be based upon the consideration of the phase of cirrhosis.

On the grounds of the present data, RABINOVICI and WIENER's optimistic

view, attributing an unambiguos curative effect of induced regeneration [10], cannot be accepted without serious restrictions.

During the development of cirrhosis — since the liver cells are becoming less and less capable of proliferation and of repairing any damage — the liver as a whole is less capable of normalization. Eventually a stage is reached when the liver shows little or no tendency to recover, because the proliferative capability of its cells is inhibited or blocked. At this stage of cirrhosis, neither the "natural" stimulus of tissue destruction activated by the cirrhogenic agent, nor the "artificial" stimulus of partial hepatectomy could provoke tissue regeneration.

The statement seems therefore justified that, under appropriate conditions, induced liver regeneration might present suitable means for reorganizing the cirrhotic liver parenchyma and improving its functional capacity. However, for these beneficial influences to be efficient, a certain level of functional integrity of cellular metabolism must be preserved. The latter requirement is apparently covered in the case of moderate cirrhosis, but it is far from being so in severe cirrhosis.

REFERENCES

1. Benacerraf, B., Bilbey, D., Biozzi, G., Halpern, B. N., Stiffel, C.: The measurement of liver blood flow in partially hepatectomized rats. J. Physiol. (Lond.) **136,** 287 (1957).
2. Brues, A. M., Drury, D. R., Brues, M. C.: Quantitative study of cell growth in regenerating liver. Arch. Path. **22,** 658 (1936).
3. Cameron, G. R., Karunaratne, W. A. E.: Carbontetrachloride cirrhosis in relation to liver regeneration. J. Path. Bact. **92,** 1 (1936).
4. Gates, G. A.: Cell population of human liver. Gastroenterology **38,** 739 (1960).
5. Grisham, J. W.: Deoxyribose nucleic acid synthesis and cell renewal in regenerating rat liver. Histochem. and Cytochem. **8,** 330 (1960).
6. Harkness, R. D.: Regeneration of the liver. Brit. med. Bull. **13,** 87 (1957).
7. Higgins, G. M. and Anderson, R. M.: Experimental pathology of the liver. I. Restoration of the liver of the white rat following partial surgical removal. Arch. Path. **12,** 182 (1931).
8. Mann, F. C., Fishback, F. C., Gay, J. G., Green, G. T.: Experimental pathology of the liver. Arch. Path. **12,** 787 (1931).
9. Menyhárt, J., Simon L.: Circulatory events accompanying liver regeneration following partial hepatectomy. Acta physiol. Acad. Sci. hung. **30,** 169 (1966).
10. Rabinovici, N., Wiener, E.: Partial hepatectomy as a stimulus to regeneration of the liver in experimental cirrhosis. Israel med. J. **17,** 169 (1958).
11. Solopaev, B. P., Butnev, Y. P., and Kuznetsova, G. G.: Reparative regeneration of liver in experimental cirrhosis. Bull. Eksper. Biol. Med. (Moscow), **51,** 74 (1961) (in Russian).
12. Stevens, C. E., Daoust, R., Leblond, C. P.: Rate of synthesis of DNA and mitotic rate in liver and intestine. J. biol. Chem. **202,** 177 (1953).
13. Weinbren, K.: Regeneration of the liver. Gastroenterology **37,** 657 (1959).

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ERYTHROCYTE ENZYMES IN HEPATOGENIC ANAEMIA

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AcPh, ALD, GOT, LDH, G-6-PDH and PE activity in erythrocytes has been studied in subjects with chronic hepatitis and model experiments have been designed to clarify the influence of experimental hepatic injury on reactive haemopoiesis, on the reticulocytic response and on the connections between maturation and enzyme activity of erythrocytes. Clinical and experimental evidence allowed to differentiate between potential hepatogenic anaemia where the erythrocyte count is normal from its manifest form with reduced erythrocyte count. The significance of enzymatic studies lies in the early detection of the latent stage and in the assessment of erythropoietic responsiveness in cases of manifest anaemia.

A number of diseases of enzymopathic origin may be identified by enzymatic analysis of the erythrocytes [14, 19, 20, 21]. This gives particular interest to the study of the erythrocytic enzyme systems from the aspects of diagnosis. Their importance in acquired diseases has yet to be established. The aim of the present investigations was to clarify the behaviour of erythrocytic enzymes in hepatogenic anaemia.

Investigating the erythrocytic enzymes in 25 cases of hepatic cirrhosis, 6 of acute hepatitis and 7 of obstructive jaundice, BOCK et al. [3] found a transitory rise in erythrocytic LDH and Hb-reductase concentrations in acute hepatitis. Though not more than 10% of the patients with hepatic cirrhosis had anaemia, ALD, PGADH and Hb-reductase were significantly increased in the eryrhrocytes. HUBER [10] observed an increase of G-6-PDH in 16 cases of hepatic cirrhosis and assumed that macrocytosis and reduction of the lifespan of the erythrocyte population may be connected with this finding. This was, however, at variance with the results of MARKS et al. [18] who failed to

Abbreviations, enzyme standard units and methods:

AcPh = acid phosphatase [1,11]; U = mg phenolphthalein (37° C) 60 min/10^9 erythrocyte
AlPh = alkaline phosphatase [1,11]; U = mg phenolphthalein (37° C) 60 min/100 ml serum
ALD = fructose-1,6-diphosphate aldolase [1,33]; U = microliter FDP (37° C) 60 min/10^9 erythrocyte
GOT = glutaminic acid-oxalacetic acid transaminase [1,25]; Wroblewski-U/10^9 erythrocyte
G-6-PDH = glucose-6-phosphate dehydrogenase [1]; Wroblewski-U/10^9 erythrocyte
PE = peptonase [5]; U = mg amino-N (37° C) 24 hrs/10^9 erythrocyte, or 100 ml serum
LDH = lactate-dehydrogenase [1]; Wroblewski-U/10^9 erythrocyte
PGADH = 3-phospho-glyceraldehyde dehydrogenase

The procedure of washing and haemolysis has been described earlier [29].

demonstrate any close relationship between reticulocyte count and erythrocytic G-6-PDH level. We have compared the concentrations of ALD, PE, G-6-PDH, LDH, GOT and AcPh in the erythrocytes of 25 patients with chronic hepatitis with those of 10 healthy subjects. The results, as shown in Table I and Figs 1, 2 and 3, were as follows.

Table I

AcPh, ALD, GOT, LDH, G-6-PDH *and* PE *activity of erythrocyte population in chronic hepatitis*

Patient No.	AcPh (0.085—0.24 U)	ALD (20—60 U)	GOT (45—120 U)	LDH (3800—5800 U)	G-6-PDH (160—315 U)	PE (0.28—0.56 U)	I	Mill. Ery II	III
							> 4	4—3	3—1
1							0		
2		+						1	
3					+		1		
4					+		1		
5					+		1		
6	+				+		2		
7	+				+		2		
8		+		+			2		
9		+				+		2	
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11				+		+		2	
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19			+	+	+	+			4
20			+	+	+	+			4
21	+	+	+		+	+			5
22	+	+		+	+	+		5	
23		+	+	+	+	+			5
24		+	+	+	+	+			5
25		+	+	+	+	+			5

The upper row shows the normal range of the respective values found in healthy individuals. Va ues of the patients exceeding the normal range are marked by + . The three columns at the right side represent the groups into which the patients fall on the basis of their erythrocyte counts. The figures in the columns represent the number of enzymes with increased activity

1. In a significant number of subjects with chronic hepatitis, the concentrations of GOT, ALD, G-6-PDH, LDH, AcPh and PE per 10^9 erythrocytes were higher than in the healthy controls.

2. In the majority of cases, more than one enzyme is present in increased amounts. All that may be inferred from the various combinations of the simultaneously increased enzyme levels is that

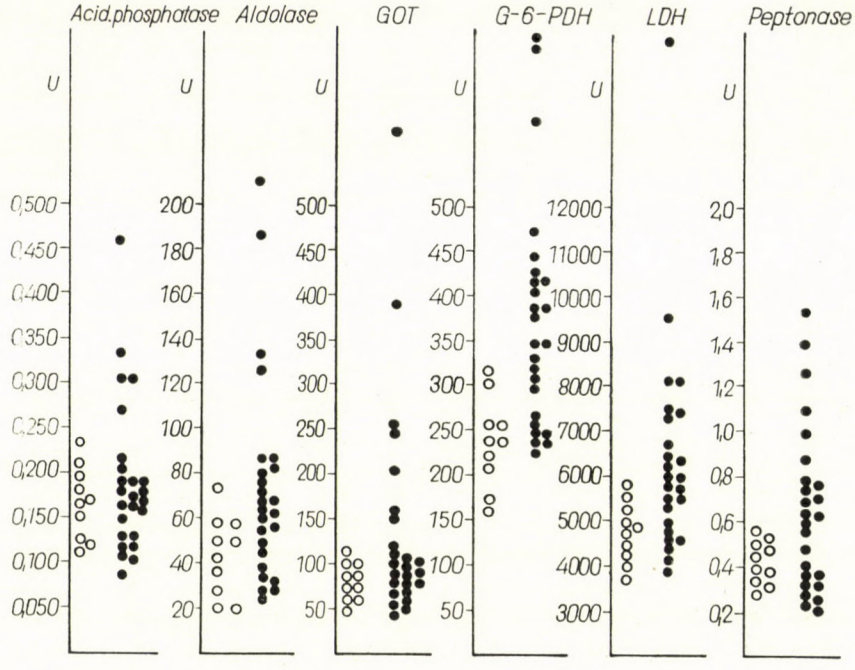

Fig. 1. AcPh, ALD, GOT, G-6-PDH, LDH and PE activity of erythrocyte population in 10 healthy individuals (○) and in 25 patients with chronic hepatitis (●)

(a) increased enzymatic contents of the erythrocyte population may occur in anaemia and in its absence alike;

(b) on the other hand, the incidence of simultaneous increase in the concentrations of several enzymes is related to the severity of anaemia (Table I). In Group I (5—4 million) comprising 10 cases, 4 individual enzymes showed increased concentrations in 2 cases, and 3 enzymes in one case only. In Group III (definite anaemia) with 6 cases, 4 enzymes had increased concentrations in 5 cases, and 3 in one.

3. No correlation has been found between the enzymatic contents and the results of liver function tests. This is illustrated by the evidence of the thymol turbidity test in Fig. 3, as also by the serum enzyme values and the electrophoretic serum protein pattern. To see the significance of the data shown in Figs

1 to 3 and in Table I in their proper light, it should be borne in mind that the enzymatic contents of the erythrocyte change during the successive phases of its existence (birth, maturity, ageing). Certain correlations reflecting this biological necessity are displayed in Fig. 4, on the grounds of the literature [15, 16, 17, 24] and our earlier results [29].

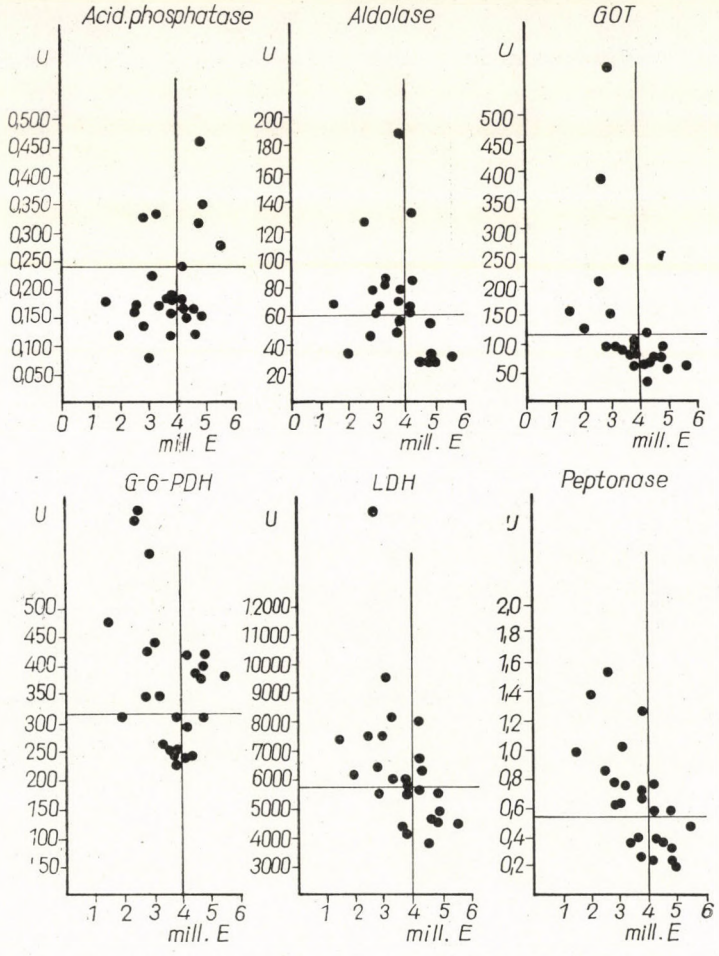

Fig. 2. Enzyme activity of eryhtrocyte population plotted against erythrocyte count. The horizontal line dividing the diagram represents the upper limit of normal enzyme activity, and the intersecting perpendicular line the lowest normal erythrocyte count (E = erythrocyte)

Fig. 4 shows the following relationships.

1. The enzymatic contents of the erythrocyte population (the blood sample) depend on the ratio of young, maturing, mature and ageing elements. A high incidence of young elements may naturally account for increased enzymatic activity.

2. During the successive life phases of the erythrocyte, the activity of the individual enzymes changes to a variable extent. As regards the quantitative changes, the enzymes fall into three groups.

Type a). The maximal activity of these enzymes is confined to the reticulocytes, to diminish gradually during the process of maturation to values characterizing the mature elements (G-6-PDH, ALD).

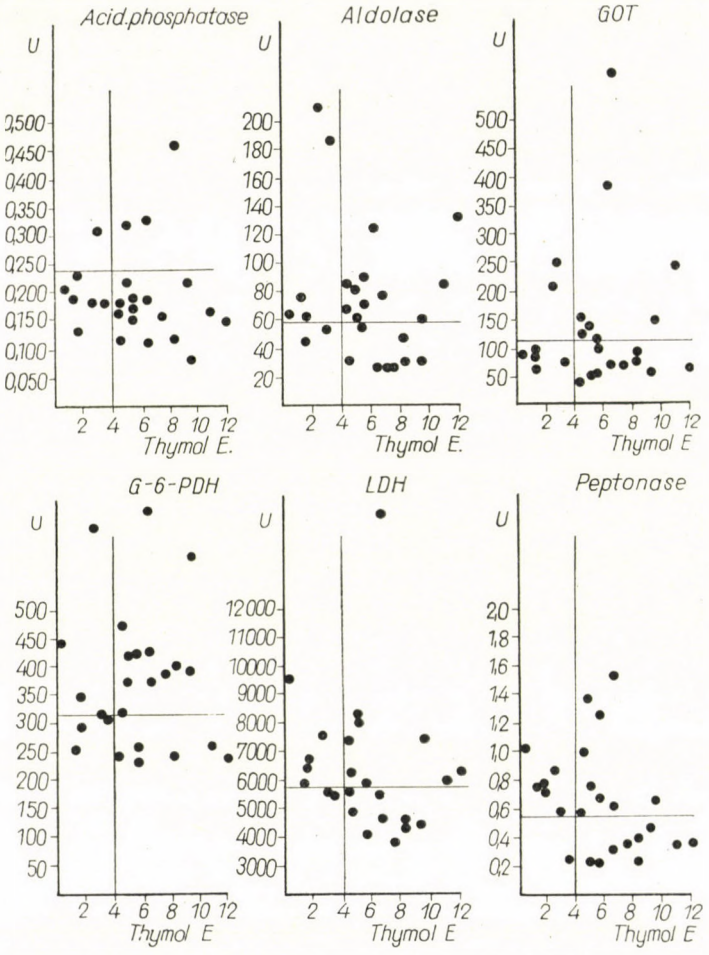

Fig. 3. Enzyme activity of erythrocyte population plotted against the values of the thymol turbidity test. The horizontal line dividing the field represents the upper limit of normal enzyme activity, the intersecting perpendicular line the upper limit of normal thymol turbidity values (expressed in McLagan units) (E = erythrocyte)

Type b). These enzymes do not change their activity significantly during the process of maturation. The values per 10^9 reticulocyte and per 10^9 mature erythrocyte are nearly equal (Diaphorase).

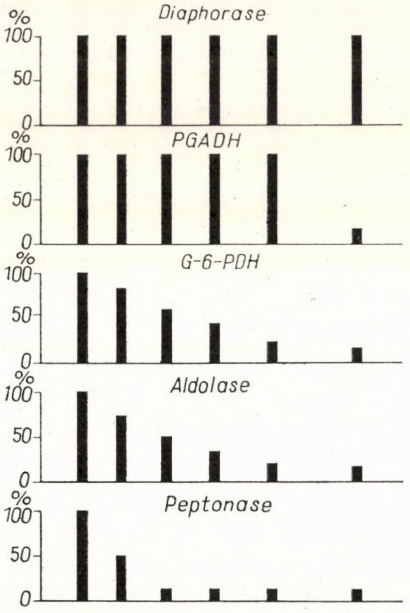

Fig. 4. Individual enzyme activities during the various life periods of erythrocytes. Changes in enzyme activities in the course of the maturation of erythrocytes is expressed by the height of the column, in a manner that the reticulocytic enzyme activities are given for each individual enzyme as 100%. The lowest part of the diagram illustrates that liver disease may be associated with a short erythrocyte life-span but it is unknown at which phase of its development the injury responsible for the change occurs

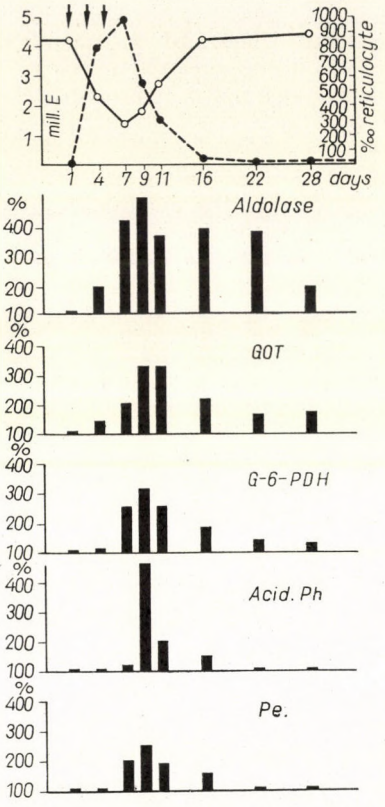

Fig. 5. Changes of ALD, GOT, G-6-PDH, AcPh and PE activity in rabbit erythrocytes, parallel with the production and recovery of phenylhydrazine-anaemia. The height of the columns expresses the percentual changes of the individual enzymes against the initial values assumed to be 100%. The changes in the activities may be correlated with those of the erythrocyte and reticulocyte counts also in their time relations. The times of the phenylhydrazine injections are marked by arrows

Type c). These enzymes preserve their original activity throughout, except for a slight decline parallel with ageing of the erythrocyte (PGADH).

3. The time required by the individual enzymes to attain the concentrations characterizing the mature erythrocytes is individual, as shown by the present findings (Figs 5, 6).

4. It is well-known that the process of maturation and ageing of the erythrocytes implicates metabolic changes affecting the uptake of O_2, sugar and amino acids, fat exchange, electrolyte, ADP and ATP contents, etc. It need not be emphasized that enzymatic activity is intimately involved in these changes.

To understand the pathophysiologic involvement of the enzymatic processes in liver disease and to derive diagnostic indications from these changes, we must know in which particular phase of erythrocytic development the enzymes assume pathologic levels. More exactly, we would have to clarify the following questions.

(a) Are the erythrocytes affected by liver disease while still within the bone marrow? This would imply that the very reticulocytes entering the blood stream have an abnormal metabolism, abnormal enzymatic activities;

(b) or is it in the phases of maturation, maturity or ageing of the circulating erythrocytes that their metabolism is damaged?

(c) does it bear any relationship to the nature or severity of liver disease whether it affects the erythrocytes during their intramedullary or extramedullary life or during both?

Haematologic studies point partly to intramedullary [7, 35, 37], partly to extramedullary damage [3, 8, 26, 30] of the erythrocytes. This still leaves many questions open which might be clarified by model experiments on animals suited for a comprehensive evaluation of the hepatogenic and haematogenic factors including the phases of erythrocytic maturation as well as the nature and severity of hepatic damage. The method described earlier [29] has been found to suit the purpose. It consists in inducing phenylhydrazine anaemia in rabbits and to analyse the subsequent changes in the blood counts and in the erythrocytic enzymes, according to Fig. 5.

The changes induced in healthy rabbits presented the following pattern.

1. 24 hrs. after the first injection of phenylhydrazine, significant anaemia and reticulocytosis appear. Seven days after the third phenylhydrazine injection, reticulocytosis attains 70 to 80% and the enzymatic activities of the red blood cell population are at their peak.

2. Discontinuation of phenylhydrazine treatment is followed by a successive increase in the erythrocyte count and by a parallel decline in the reticulocyte count. The enzyme contents of the red blood cell population shows a simultaneous decline, but the time in which the activity characteristic of mature elements is attained varies with the individual enzymes.

(a) The PE and AcPh activities decline together with the reticulocyte count. Of all the enzymes examined these showed the earliest return to the values characteristic of mature erythrocytes.

(b) On the 28th day when the reticulocyte count has returned to normal, the GOT, ALD, and G-6-PDH activities in the young erythrocyte population are still higher than in the mature population.

3. If haemopoiesis is within the physiologic range, in other words, if it has to provide for the replacement of the physiologic breakdown of erythrocytes, then the enzyme concentrations of the erythrocyte population are within normal limits.

4. Enzyme activity of the erythrocytes thus defines the extent of reactive erythropoiesis by which the normal bone marrow responds to anaemization (by blood loss, phenylhydrazine, etc.). The enzyme pattern of haemopoiesis associated with definite reticulocytosis is characterized by an overall increase in the GOT, ALD, G-6-PDH, LDH, AcPh and PE activities of the erythrocytes.

5. A simultaneous increase in the GOT, ALD, and G-6-PDH activities of the erythrocyte population is suggestive of active erythropoiesis, i.e. of the prevalence of juvenile maturing elements, even in the absence of reticulocytosis.

Owing to their regularity, these responses are thus suited for model experiments in the study of hepatic lesions. The wide range of metabolic changes associated with chronic hepatitis makes it necessary to study the reactive erythropoietic capacity of the bone marrow in hepatic injury of various character and severity, released by acute, chronic, CCl_4, ethionine, thioacetamide, alcohol, immune and nutritive factors.

The aim of the studies to be reported was to establish how in rabbits after CCl_4 liver injury enzyme erythrocytic activity is affected by

(a) phenylhydrazine anaemia;
(b) the subsequent reactive reticulocytosis;
(c) the process of maturation of fresh erythrocytes.

Since CCl_4 may cause injury to the bone marrow also, we had to find out the dose definitely injurious to the liver without affecting the bone marrow. The data of this experiment are presented in Table II.

The figures of Table II reveal the following:

1. In rabbits, CCl_4 induces hepatic damage the severity of which depends on the dose. The erythrocyte count is unaltered even after the second dose. On the fourth day there is a slight reticulocytosis which may be ascribed to the blood loss connected with the blood samplings since it was manifest in the test animals as well as in the intact controls. This discreet reactive reticulosis is one of the very signs that the dose of CCl_4 employed in the study has not affected the bone marrow.

2. After the second injection of CCl_4, on the eighth day of the experiment, the hepatic parenchyma is still severely affected. This must be stressed because of being coincident with the birth of the new erythrocyte population. The presence of liver damage was reflected by increased serum ALD and GOT levels. Its criterion, however, was the microscopic appearance of the liver, since the levels of ALD and GOT showed no consistent relationships either with the morphologic picture or with the administered dose of CCl_4. In other words, the increase in enzyme activity merely reveals the presence of liver damage without being correlated with its severity.

Table II

Effects of various doses of CCl_4 *on reticulocyte count, serum* GOT, *and on the morphology of the liver in rabbits*

Number of animal		CCl_4 ml/kg on 1st and 3rd day of study		Reticulocyte per mil on				Serum GOT U on				Microscopic appearance of liver on the 8th day of experiment. (Haematoxilin-eosin staining)
		single dose	total dose	1st	2nd	3rd	4th	1st	2nd	3rd	4th	
				day of experiment				day of experiment				
Controls	1	—	—	6	12	10	22	18	20	17	21	—
	2	—	—	7	11	11	20	16	19	23	17	—
CCl_4	3	0.2	0.4	4	10	8	22	20	650	2800	1400	Prevalently centrilobular round cell infiltration; cloudy swelling, vacuolation, amitotic cell divisions, binuclear cells.
	4	0.2	0.4	8	9	10	23	16	450	2100	1350	
CCl_4	5	0.4	0.8	7	13	12	29	23	540	2740	860	The foregoing changes with a greater number of amitoses.
	6	0.4	0.8	7	10	13	27	30	500	1450	720	
CCl_4	7	0.6	1.2	10	8	11	24	25	1200	3100	1140	Centrilobular necrosis, round cell infiltration, vacuolation. The number of amitotic divisions and of binuclear cells is still greater than in the former group.
	8	0.6	1.2	11	10	10	23	18	850	2050	720	
CCl_4	9	0.8	1.6	5	7	12	30	17	1150	3200	1250	Centrilobular necrosis is more marked than in the other groups. Cell divisions and binuclear cells are scarcer.
	10	0.8	1.6	8	8	14	31	21	940	3130	740	

After these preliminary experiments acute liver injury was induced with 0.2 ml per kg of CCl_4 administered by the oral route on three occasions. In the predetermined times marked in the diagrams, two controls received CCl_4 alone, four animals received CCl_4 and subsequently phenylhydrazine. Results are shown in Table III and in Fig. 6, which represents data of animal No. 5 and illustrates the changes in the erythrocyte and reticulocyte counts, as well as in the enzyme activities of the erythrocyte population.

I. Effect of CCl_4 on erythrocytic and serum enzyme activity

1. Serum GOT and ALD levels were increased on the very first day following the first dose of CCl_4.

2. The enzyme contents of the erythrocyte population remained unaffected even after the second dose of CCl_4, but serum GOT and ALD activity continued to increase. The reticulocytosis elicited by the blood samplings was too slight to be made recognizable owing to the measure of the present scale (Table III).

II. Effect of phenylhydrazine in CCl_4-pretreated rabbits

1. (a) Phenylhydrazine-induced anaemia, the accompanying reticulocytosis and the eventual normalization of the erythrocyte and reticulocyte counts followed the same pattern as in healthy rabbits.

(b) Enzyme activities per 10^9 erythrocyte in the new population increased parallel with the reticulocyte count.

(c) Reticulocytosis reached 80% in the poisoned rabbits as well.

(d) Erythrocytic PE and AcPh activities declined to values characteristic of mature elements parallel with the reticulocyte count.

(e) Erythrocytic GOT, ALD and G-6-PDH activities exceeded normal values even after normalization of the reticulocyte count.

2. It has thus been demonstrated that in CCl_4 induced liver injury whose severity is characterized by centrilobular round cell infiltration, cloudy swelling and vacuolization, a tendency to amitotic cell division and numerous binuclear cells:

(a) the medullary response pattern to anaemization is that of the healthy bone marrow;

(b) reticulocytosis likewise follows the normal pattern;

(c) as estimated by the enzyme activities, maturation of erythrocytes developed during liver injury also proceeds normally.

This evidence further suggests that the reticulocytic enzyme activities are also normal, a supposition which has yet to be confirmed.

Table III

Serum and eryhtrocytic enzymes in rabbits in the course of development and subsidence of phenylhydrazine anaemia released during CCl_4 induced liver injury

Day of experiment	E million	Reticulocyte per mil	Serum enzymes				Erythrocytie enzymes				
			AlPh	GOT	ALD	PE	AcPh	GOT	ALD	PE	G-6-PDH
					Rabbit No. 1						
1	4.7	14	0.6	30	4.8	49	0.36	90	12	0.67	286
4	4.5	9	1.8	235	27.0	108	0.25	108	11	0.40	273
8	4.6	23	6.5	312	32.0	69	0.32	112	11	0.73	290
12	4.0	18	3.0	46	6.0	82	0.39	115	12	0.80	250
15	4.2	28	0.9	56	4.4	54	0.29	114	13	0.73	287
20	4.2	46	0.7	14	4.8	66	0.26	105	10	0.47	295
26	4.7	20	0.7	30	3.7	53	0.30	110	12	0.67	290
33	4.6	24	0.6	32	4.2	54	0.31	100	12	0.67	292
					Rabbit No. 3						
1	4.4	19	0.6	18	4.8	65	0.40	100	12	0.83	271
4	4.0	32	4.2	100	12.0	82	0.48	126	15	0.97	264
8	4.2	23	8.4	205	21.0	63	0.47	97	15	0.67	290
12	2.4	632	3.6	57	8.2	66	0.44	420	37	1.60	415
15	1.2	785	0.9	60	12.0	83	0.65	418	44	0.93	754
20	3.4	198	1.0	76	8.8	82	0.65	290	27	0.77	680
26	3.5	22	0.7	80	5.2	60	0.45	330	24	0.90	540
33	4.3	8	0.7	36	4.1	51	0.38	130	18	0.87	392
					Rabbit No. 5						
1	4.6	25	1.0	16	7.3	79	0.37	102	15	0.75	256
4	4.6	24	7.2	76	11.0	140	0.38	85	16	0.68	274
8	4.2	25	16.0	185	30.0	170	0.36	110	14	0.77	272
12	2.6	242	14.0	52	11.0	100	0.53	310	25	1.36	527
15	1.2	895	5.0	48	15.0	61	0.77	460	39	1.42	842
20	2.9	196	1.4	62	12.0	76	0.53	310	31	1.33	626
26	3.9	22	1.2	61	3.0	70	0.31	240	27	0.87	493
33	4.5	16	0.8	26	5.6	63	0.33	156	23	0.73	401

Rabbits Nos. 1 and 2 received an oily solution of 0.2 ml/kg CCl_4 through a stomach tube on the 1st, 3rd and 5th days respectively of the study. Rabbits Nos 3 to 6 received 0.2 ml/kg CCl_4 through a stomach tube on the 1st, 3rd and 5th days respectively, and 15 mg/kg phenylhydrazine subcutaneously on the 9th, 11th and 13th days respectively.

Table III, continued

Day of experiment	E million	Reticulocyte, per mil	Serum enzymes				Erythrocytic enzymes				
			AlPh	GOT	ALD	PE	AcPh	GOT	ALD	PE	G-6-PDH
					Rabbit No. 2						
1	4.9	23	0.9	28	6.8	54	0.37	91	13	0.87	260
4	4.9	25	4.9	60	18.0	90	0.50	100	15	1.06	246
8	4.6	18	11.0	210	28.0	120	0.41	90	13	0.93	273
12	4.7	32	7.6	70	10.0	96	0.32	87	13	0.80	298
15	4.3	27	2.1	48	5.0	72	0.37	87	13	0.97	254
20	4.5	18	1.0	32	3.9	70	0.39	87	12	0.70	272
26	4.8	24	1.1	29	4.6	68	0.31	91	13	0.73	278
33	4.7	30	0.8	34	5.2	50	0.37	86	13	0.87	280
					Rabbit No. 4						
1	4.6	2	0.5	19	4.2	30	0.28	105	11	0.78	293
4	4.8	11	2.6	166	11.0	22	0.35	97	11	0.73	272
8	4.6	15	6.9	326	27.0	16	0.30	90	11	0.53	310
12	4.3	370	4.1	86	9.0	26	0.32	176	18	0.87	574
15	2.6	889	1.4	86	10.0	15	0.52	264	31	0.50	927
20	2.8	312	0.6	64	6.2	33	0.66	264	39	1.10	632
26	2.9	230	0.5	40	7.1	32	0.27	168	28	1.06	580
33	4.4	18	0.6	22	5.0	32	0.25	140	15	0.72	420
					Rabbit No. 6						
1	4.8	20	0.6	60	6.2	60	0.37	104	13	0.67	293
4	5.0	14	2.6	200	13.0	100	0.24	107	11	0.66	271
8	4.2	30	4.9	300	21.0	110	0.33	116	15	0.70	250
12	2.1	309	2.8	210	16.0	80	0.33	450	30	1.10	486
15	2.2	608	1.0	70	12.0	70	0.66	470	40	2.08	742
20	3.9	312	0.7	82	8.0	58	0.43	366	33	2.00	452
26	4.3	95	0.8	40	6.4	50	0.35	145	22	1.34	452
33	4.9	17	0.7	26	6.0	52	0.33	130	17	0.70	371

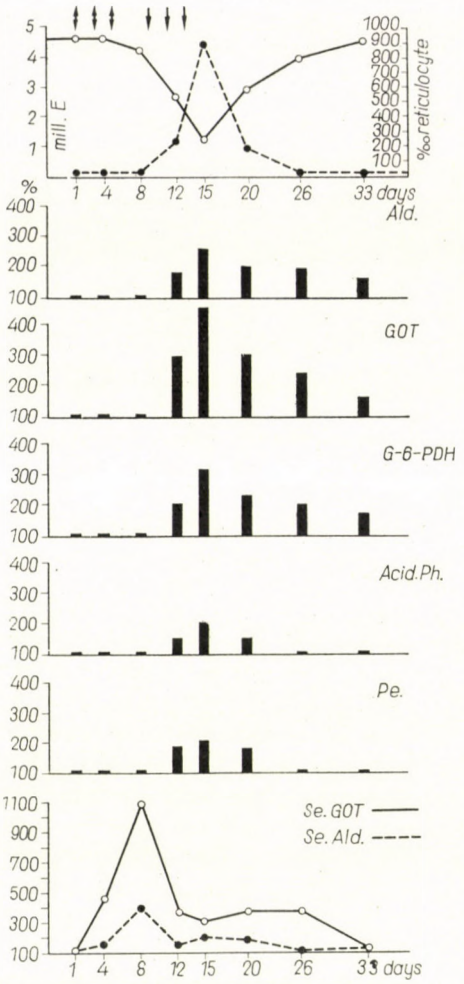

Fig. 6. Changes in the activities of erythrocytic ALD, GOT, G-6-PDH, AcPh, PE and of serum ALD and GOT in rabbit (No 5) in the course of development and recovery of phenylhydrazine-induced anaemia during hepatic injury caused by CCl_4. The height of the columns expresses the percentual changes of the individual enzymes against the initial values assumed to be 100%. The time relationship of these changes with those of the erythrocyte and reticulocyte counts emerge from the diagram. The times of administration of CCl_4 are marked by bidirectional, those of phenylhydrazine by unidirectional arrows

Discussion

Against the conclusive value of the enzymologic data presented in Table I and in Figs 1 to 3 we have to weigh the following inconsistencies:

(a) Enzyme activities in the erythrocyte population were unrelated to the evidence of liver function tests reflecting the severity of hepatic damage.

(b) The enzyme contents of the erythrocytes may be increased whether or not the subjects with liver disease were anaemic. The only finding worthy of note was that the severity of anaemia seemed to be related to the number of individual enzymes accounting for the high activities in the erythrocyte population.

A similar inconsistency emerges from the literature in respect to the incidence of haematologic changes suggestive of erythropoietic damage in liver disease and to the possible connections between these abnormalities. Comprehensive studies [2, 3, 6, 12, 22, 37] yield the following information concerning these points.

1. In liver cirrhosis the incidence of anaemia is 10, 48, 78%; increased susceptibility of erythrocytes to haemolysis 40; anomalies of folic acid metabolism 30; anomalies of vitamin B_{12} metabolism 30, 50; latent intestinal haemorrhages 30, 40; macrocytosis 10, 30%, respectively.

2. It also deserves mentioning that

(a) the above anomalies of haemopoiesis may occur in established liver cirrhosis even in the absence of anaemia;

(b) no consistent relationships are demonstrable between the individual parameters indicative of haemopoietic impairment. For instance, on the evidence of the erythrokinetic studies by TARTAROGLU et al. [36] in 25 cases of chronic hepatitis, a shorter life-span of erythrocytes may be found under normal conditions, as well as in the presence of a pattern of medullary hyperactivity. On the other hand, a normal erythrocyte life-span may be likewise associated with a pattern of medullary hyperactivity. The correlations between the life-span of erythrocytes and splenomegaly emerge from the literature as being similarly inconsistent. In 50% of the reported cases the life-span of erythrocytes was reduced and in the other 50%, normal. An insignificant enlargement of the spleen may be associated with a short erythrocyte life-span.

Two circumstances may be held responsible for the inconsistency in the incidence and in the mutual correlations of the various indicators of haemopoietic impairment, even in typical cirrhosis, *viz.*:

1. The complex pathogenesis of hepatogenic anaemia. Anaemia in liver disease is the result of multiple factors such as metabolic anomalies of various character, noxious metabolites, autoimmune mechanisms, etc., which may affect haemopoiesis at different phases and at different levels, including the sites of neurohormonal regulation.

2. The compensatory capacity of the erythropoietic system. Anaemia remains presumably absent in liver disease until reactive erythropoiesis is no longer sufficient for the compensation of the injury to erythrocytes.

In the light of the above it seems justified to differentiate (i) "*manifest*" from (ii) "*potential*" hepatogenic anaemia which latter term refers to the condition characterized by certain abnormal features pointing to erythropoietic

damage such as macroplanocytosis, reduced erythrocyte life-span, one or more of the above enzymatic abnormalities, though actual anaemia is absent.

Early detection of potential hepatogenic anaemia is of obvious importance. The practical issue of these investigations on the question whether the changes in erythrocytic enzyme activities in hepatic disease may be regarded as one of the early diagnostic signs of hepatogenic anaemia, and its theoretical issue involves the questions (i) whether acquired damage to the erythrocytes may be defined by the enzyme contents of the erythrocyte population, and (ii) whether it is the medullary function or the circulating erythrocytes which suffer damage.

I. The present experiments have led to the definition of the character and severity of a parenchymal diffuse lesion where

(a) the medullary function, expressed in terms of reticulocytosis, is still unaffected, i.e. capable of normal reticulocytic response, and

(b) where the process of erythrocyte maturation measured by the changes in the enzyme activity of the population also seems unaffected.

In hepatic damage of the described nature and severity, a simultaneous increase in the erythrocytic G-6-PDH, ALD and GOT activities is a sign of reactive erythropoiesis even in the absence of reticulocytosis.

As generally known, certain morphological and biochemical characters of the erythrocytes are common to all animal species, others are particular to the individual species [9, 34]. For instance, the activity of the individual enzymes per 10^9 erythrocyte varies according to the species. The erythrocytic GOT, ALD, G-6-PDH, LDH, AcPh and PE activities found in the present study apply to rabbits only. However, the enzymatic changes associated with erythrocyte maturation have a general validity from theoretical as well as from diagnostic aspects, the more so as the G-6-PDH, ALD and GOT activities of juvenile erythrocytes per 10^9 is high in humans, too.

II. 1. Many subjects with chronic hepatitis display increased concentrations per 10^9 erythrocyte of one or several enzymes. Increase in GOT, ALD, G-6-PDH is suggestive of increased reactive erythropoiesis, i.e. the proportion between juvenile and mature elements exceeds the physiological range by the prevalence of the former. Arising as a response to the increased breakdown of mature erythrocytes, this is an indirect sign of erythropoietic damage.

2. Some of the patients with liver disease studied in the present series showed increased LDH, AcPh and PE activity. The activity of other enzymes was increased or normal. The possible diagnostic value of this finding has yet to be established. Still, it may be assumed that the changes in these and possibly in other enzymes may well be due to damage to the mature rather than to the immature elements. This is what the results of our earlier experiments seem to suggest [28]: in severe chronic liver injury the high erythrocytic PE and AcPh activities were not invariably associated with reactive erythropoiesis.

3. Our claims are supported by the close relationship between the severity of hepatogenic anaemia and the prevalence of the enzyme pattern of reactive erythropoiesis, i.e. a simultaneous increase in the activity per 10^9 erythrocyte of several enzymes.

On the grounds of these considerations hepatogenic anaemia may be regarded as a process which falls into the following periods.

1. Erythrocyte count is normal and the GOT, ALD and G-6-PDH activity of the erythrocyte population is in the normal range. This still does not exclude the possibility that the bone marrow may have suffered damage of some kind, but if it has, it is certainly not of a degree to elicit a reactive erythropoiesis reflected by the erythrocytic GOT, ALD and G-6-PDH activities.

2. The erythrocyte count is normal. Their GOT, ALD and G-6-PDH activities are higher than normal, as a sign of increased reactive haemopoiesis. The normal erythrocyte count indicates that the organism still possesses sufficient means of compensation for the damage to the erythrocytes ("potential hepatogenic anaemia").

3. Anaemia is present and erythrocytic enzyme activity is increased.

This indicates that the compensatory capacity of the bone marrow, expressed in terms of erythrocyte count, is incomplete ("partial compensation of hepatogenic anaemia").

4. In the presence of anaemia, there is no increase in erythrocytic GOT, ALD and G-6-PDH activity; if there is any reactive erythropoiesis, it is inadequate. This is suggestive of major damage to haemopoiesis ("decompensation of erythropoiesis").

Distinction of three phases of hepatogenic anaemia is, moreover, justified on the following clinical grounds: (a) anaemia and the enzymatic changes in the erythrocyte population associated with acute hepatitis are transitory and cease parallel with recovery; (b) permanent anaemia occurs principally in chronic hepatitis when the injury to erythropoiesis may have exhausted the compensatory capacity to its utmost limits. The importance of enzymatic studies in chronic hepatitis lies in the possibility of detection of hepatogenic anaemia in its latent phase, it being apt to reveal the presence of reactive erythropoiesis arising in response to haemopoietic damage at a stage where anaemia is still absent.

III. The blood samples contain damaged ertyhrocytes which obviously affect the enzyme contents of the population. It is to be assumed that metabolic impairment of mature erythrocytes, for instance their early ageing, must have definite connections with changes in the enzyme activity of the erythrocyte population. That hepatic disease affects the metabolism of mature erythrocytes is a fact substantiated by extensive clinical investigations. There is evidence of a reduction in ATP content [38] and in the glutathion-capacity [23] of the erythrocyte population, further of a delay in the oxygen saturation

curve of haemoglobin [4]. Erythrocytes of healthy individuals transfused into subjects with hepatic cirrhosis may suffer morphologic changes characteristic of hepatic disease, including planocytosis as well as reduced life-span [30, 31, 32]. The questions whether injury to maturing erythrocytes is identifiable on the grounds of enzymologic evidence and if so which of the enzyme systems will reveal the injury must be decided by further studies. This will at the same time help to reconcile the opposing views voiced in the literature for more than eighty years whether hepatic damage to the erythrocytes asserts itself within or outside the bone marrow or possibly in both ways.

* * *

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REFERENCES

1. Bergmeyer, H. U.: Methods of Enzymatic Analysis. Academic Press, New York 1963.
2. Bingham, J. R.: Canad. med. Ass. J. **85,** 178 (1961).
3. Bock, H. E., Karges, O., Löhr, G. W., Waller, H. D.: Klin. Wschr. **36,** 56 (1958).
4. Caldwell, P. R. B., Fritts, H. W., Cournand, A.: J. appl. Physiol. **20,** 316 (1965).
5. Dán, S., Nagy, S., Tóth, J.: Acta med. Acad. Sci. hung. **12,** 177 (1958).
6. Fellinger, H., Klima, R.: Wien. klin. Wschr. **46,** 1191 (1933).
7. Freerksen, E.: Klin. Wschr. 1238 (1937).
8. Heilmeyer, L.: Handbuch der inneren Medizin. Herausg. Springer, Berlin 1951. Vol. II, p. 319.
9. Hinterberger, U., Rapoport, S., Gerischer-Mothes, W., Sukrow, D.: Folia haemat. (Lpz.) **78,** 471 (1962).
10. Huber, H., Ciresa, M., Shirakura, N.: *Z.* Wien. inn. Med. **44,** 26 (1963).
11. Huggins, C., Talalay, P.: J. biol. Chem. **159,** 399 (1945).
12. Hyman, G. A., Southworth, H.: Amer. J. med. Sci. **22,** 338 (1951).
13. Levy, L. M., Walter, H., Sass, M. D.: Nature (Lond.) **184,** 643 (1959).
14. Linneweh, F.: Klin. Wschr. **41,** 937 (1963).
15. Löhr, G. W., Waller, H. D., Karges, O., Schlegel, B., Müller, A. A.: Klin. Wschr. **36,** 1008 (1958).
16. Löhr, G. W., Waller, H. D.: Klin. Wschr. **37,** 843 (1959).
17. Löhr, G. W., Waller, H. D.: Folia haemat. (Lpz.) **78,** 396 (1962).
18. Marks, P. A.: Science **127,** 1338 (1958).
19. Marti, H. R.: Schweiz. med. Wschr. **94,** 341 (1964).
20. Marti, H. R.: Schweiz. med. Wschr. **92,** 1430 (1962).
21. Martin, H., Nowicki, L.: Internist **7,** 302 (1966).
22. Movitt, E. R.: Blood **5,** 468 (1950).
23. Pitcker, C. S., Williams, R.: Proceedings of the Eighth Congress of the European Society of Haematology. Vienna 1961. Vol. II, p. 326.
24. Rapaport, S.: Folia haemat. (Lpz.) **78,** 364 (1962).
25. Reitman, S., Frankel, S.: Amer. J. clin. Path. **28,** 56 (1957).
26. Rosenberg, D. H.: Amer. J. med. Sci. **192,** 86 (1936).
27. Sass, M. D., Levy, L. M., Walter, H.: Canad. J. Biochem. **41,** 2287 (1963).
28. Sári, B., Kirillina, Sz. V., Dán, S., Orosz, L.: Acta III. Conventus Medicinae Internae Hungarici. Budapest 1965, pp. 923—928.
29. Sári, B., Prékopa, Á., Deményi, P., Dán, S.: Haemat. hung. **5,** 91 (1965).
30. Schlegel, B.: Bibl. haemat. (Basel) **2,** 99 (1955).
31. Schlegel, B., Böttner, M.: Verh. dtsch. Ges. inn. Med. **58,** 732 (1952).
32. Schlegel, B., Böttner, M.: Verh. dtsch. Ges. inn. Med. **182,** 261 (1938).
33. Sibley, J. A., Lehninger, A. L.: J. biol. Chem. **177,** 859 (1949).
34. Smith, J., Barnes, J. K., Kaneko, J. J., Freedland, R. A.: Nature (Lond.) **205,** 298 (1965).

35. TAI, H. B.: Blut **10,** 67 (1964).
36. TARTAROGLU, N., EISENBURG, J., FREY, K. W., PFISTERER, H., STICK, W.: Acta hepatosplenol. (Stuttg.) **13,** 257 (1966).
37. TISCHENDORF, W.: Dtsch. Arch. klin. Med. **182,** 261 (1938).
38. VILLA, L., SECCHI, G. C., DIOGUARDI, N.: Minerva med. **53,** 41 (1962).

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PROBLEMS OF ERYSIPELAS. III

STREPTOCOCCAL VACCINE TREATMENT OF RELAPSING ERYSIPELAS

By

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A total of 69 patients with recurrent erysipelas (the number of recurrences ranging between 2 and 72) has been subjected to active immunization with a polyvalent A-type streptococcal vaccine prepared from 14 strains prevailing in Hungary, all possessing M antigen and being toxic to albino mice. The majority of the patients received 6 immunizing and 6 booster doses. 29 patients (42%) had no recurrence during a period from 9 to 27 months. Considering the individual pattern or earlier recurrences in these cases, this means that 4.5 recurrences remained absent in each case. In 22 cases (31.9%) relapses appeared as before. 18 patients (26.1%) had no relapse but were not expected to have any, the intervals between earlier relapses having been longer than the follow-up period. The skin reaction elicited by intradermal injection of the streptococcal vaccine was enhanced in 7.3% of the cases in the course of treatment in contrast to that produced by the intradermal injection of the thermostable factor which diminished in intensity or became normal in 20.4%. As regards the circulating antibodies, the titre of antistreptolysin-O increased in 20.3%, that of antistreptokinase in 11.6% of the cases.

The first study of this series (RAJKA [1966]) was concerned with the mechanism of sensitization involved by streptococcal infections, in the first line by erysipelas and other streptococcal skin affections. It has been shown that the conditions in question are characterized by a certain immune deficiency, particularly as regards the delayed type of sensitization essentially cellular in character, and that persistent recurrences of erysipelas are suggestive of a deficiency in circulating "protective" antibodies.

The second study (BÖSZÖRMÉNYI [1967]) dealt with the grouping and typing of streptococci and with questions of their virulence. The results offered an explanation for the deficiency of "protective" antibodies, isolated strains having been identified as avirulent or of slight virulence owing to the lack of M-protein-antigen.

The present study deals with problems posed by the treatment of recurrent erysipelas.

1. The primary task is to control the acute inflammatory process induced by the streptococcal infection. Penicillin is still generally held to be the drug of choice for this purpose (ADAM [1966] , WEBER [1966]), a view to which the present authors fully subscribe on the grounds of their own observations. Combined with sulphonamides, it may gain in efficiency (KOROSSY [1964], KORTING [1966]). Allergy to penicillin or to sulphonamides calls for erythromycin in uncomplicated cases (LIVINGOOD [1964]) and for tetracyclines in the case of venous involvement (MEYER-ROHN [1961]).

2. One of the prerequisites of efficient prevention is to deal with the portal of entry of the agent and to influence the regional factors favourable to the infective process (DOSTROVSKY [1955], BEREZHNOI [1959], MEYER-ROHN [1961], RÖCKL [1965], ADAM [1966], DIMITRESCU [1966], KORTING [1966], WEBER [1966]). It has been pointed out earlier (RAJKA [1966]) that the extensive spread of mycotic infections has greatly contributed to the incidence of recurrent erysipelas, since the interdigital erosions, macerations and fissures of mycotic origin generally harbour streptococci as demonstrated by several authors (BAZYKA [1963], MAIBACH [1965], EREMINA [1966], DIMITRESCU [1966]). Flare-up of the mycotic process facilitates the access to the regional lymphatics of streptococci and their spread to the affected part, thus giving rise to a relapse. Prolonged antimycotic treatment may in itself lead to complete cure (BEREZHNOI [1959], RÖCKL [1965]). The source of infection may be situated in any region (e.g. retroauricularly, or in the submammary folds). The nasal cavity of healthy carriers is another possible source of streptococcal contamination of clothing and skin (HAMBURGER [1946]). Interpretation of the pathogenesis of recurrent erysipelas in the light of an "infective streptococcal focus" gave rise to prolonged treatments with high doses of penicillin, e.g. 1 MU daily (MEYER-ROHN [1961], LIVINGOOD [1964], ADAM [1966], KORTING [1966]) continued for ten further days after the clinical manifestations had been brought under control (MEYER-ROHN [1961], LIVINGOOD [1964], ADAM [1966], KORTING [1966]). LIVINGOOD (1964) suggested monthly injections of 1.2 M benzathine-penicillin, or 400,000 U every other day by the oral route, to be continued over six months.

The terrain in which the process develops should be given due attention. The recurrent inflammations inevitably affect the skin's structure and functions. The high protein content of the inflammatory exudate enhances fibroblastic proliferation (PFLEGER [1964], TAPPEINER [1964]). The subsequent stages are marked by an indurative degeneration of mesenchymal tissue (KONOPIK [1965]), and obliteration (TAPPEINER [1964]), even saccular dilatations (PFLEGER [1964]) of the lymphatics as a result of proliferative, productive, fibrous inflammation. The process may affect regional capillary blood flow by a vegetative vascular mechanism (GOTTRON [1965]).

Various procedures have been suggested to reduce the effects of hyperproteinization and to increase tissue permeability. These procedures consist in repeated injections of penicillin with hyaluronidase around the affected area (KONOPIK [1965]), compression of varicous veins (LIVINGOOD [1964], ADAM [1966]), subaqual massage (MEYER-ROHN [1961]), combined application of antibiotics and steroids to the affected part (MUSSGNUG [1957], GALPERIN [1960]), radiotherapy (SZODORAY [1964], RÖCKL [1965], KORTING [1966]), "general stimulation" of the organism's "protective capacity" (artificial pyrexia, autohaemotransfusions combined with ultraviolet light, etc.) (MEYER-

ROHN [1961], ADAM [1966], KORTING [1966]). Though these procedures may be of a certain benefit, they are far from what may be regarded as efficient causal therapy.

3. The failure of the methods in use has prompted us to study the possibilities of active immunization despite the fact that, as it turned out later, the streptococcal strains responsible for recurrent erysipelas are prevalently avirulent, being devoid of M-antigen, moreover the relative immune defect in respect to delayed sensitization is combined with a poor production of certain circulating antibodies [antistreptolysin-O (ASL), antistreptokinase (ASK)] (BÖSZÖRMÉNYI [1967]). Since, according to the general view, microbial infections of the cellular type, are none the less inseparable from a humoral production of antibodies, the question arises whether immunization with streptococcal vaccine induces changes in the responsiveness of any of these immune mechanisms or of both.

The difficulty in clearing these questions lies in the fact that for the evaluation of cellular allergy we had to rely on skin tests, while the quantitative and qualitative changes in the circulating antibodies were assessed on the basis of the ASL and ASK titres. It was nevertheless judged desirable to undertake active immunization with a vaccine obtained from common streptococcal strains. Our purpose was not to attain basic immunity, but to enhance the production of "protective antibodies" in subjects who did presumbly have some degree of basic immunity owing to several past streptococcal infections, since in our view reinfections in patients susceptible to streptococcal infection are caused by a few persistent strains rather than by new types.

Our vaccine was therefore intended as a secondary stimulus capable of increasing the immune response by means of small doses. From November, 1964, onward we have been carrying out streptococcal vaccinations in recurrent erysipelas, provided there was no clinical contraindication for such treatment. Thus far, 69 patients have been treated by means of active immunization and followed up for 9 to 27 months.

Material and methods

Patient material. From November, 1964, onward 78 successive cases with 2 to 72 recurrences of erysipelas were subjected to streptococcal vaccination; of these, 69 reported for follow-up studies.

Composition of polyvalent streptococcal vaccine. Selection of the strains was prompted by the following considerations. 1. to use strains common in Hungary, and 2. to select virulent strains, possessing antigen-M. As a result of this selection, types 1, 2, 3, 4, 5, 6, 8, 11, 12, 13, 14, 19, 25, and 29 were cultured. The virulent strains were grown on KALBAK's medium during 16 hrs. and killed with 0.3% formalin, centrifuged under sterile conditions, washed twice and suspended in sterile physiologic saline containing merthiolate 1 : 10,000. Suspensions of equal density obtained from the 14 strains were mixed in equal parts. The dilution of this stock suspension was adjusted to a N-concentration of 0.15 mg/ml, corresponding to approximately 10^9 germs per ml. The vaccine was applied after the required tests of sterility and innocuity.

The schedule of active immunization comprised two stages, i. e. primary immunization and booster doses. During the first stage 48 patients received a total of 6 doses divided over two or three weeks, i. e. one dose every second or third day. 18 patients received one or two doses more, 3 patients one or two less. This course was followed by booster doses at monthly intervals. 41 patients had six, the others either one or two more, or one or two less, doses. The first dose was determined by the character and intensity of the cutaneous reaction elicited by the intradermal injection of the threshold dose of the vaccine diluted 1 : 800 (*vide* Part I). On these indications 0.02 to 0.1 ml were given of the stock suspension or of its 1 : 10 or 1 : 100 dilutions. Then the volumes as well as the concentrations were gradually raised according to the local reaction, to attain occasionally 0.4 ml of the undiluted vaccine. Doses up to 0.2 ml were given intradermally, while higher doses subcutaneously.

After completion of immunization the patients were *controlled* at intervals of three or six months. The overall period of follow-up covered 9 to 27 months. Accordingly, the patients were seen on one to four occasions. At follow-up clinical examination, routine laboratory investigations, a skin test and serologic studies were carried out.

Skin tests were done at the start and on completion of treatment and on each follow-up study, with 0.1 ml of a mixture of streptococcal vaccine and thermostable antigen (LYAMPERT [1964]) diluted to 1 : 800. Adjustment of the threshold value of these antigens has been described (RAJKA [1966]). The reaction was checked at 48 hrs. and according to the general routine followed in the evaluation of delayed type reactions, the result was regarded as positive if the wheal measured 8×8 mm. Differences had to exceed 3×3 mm to be evaluated in either direction.

From the *humoral antibodies*, ASL and ASK were studied. In accordance with serial tests performed in Hungary, 250 U was regarded as the highest limit of the normal range for both ASL and ASK (BÖSZÖRMÉNYI [1961, 1965]).

Results

I. Pre-vaccination data

Of the 78 patients 40% were male, 60% female. In both groups the condition was prevalent in the age group of 50 to 59. In younger age groups and beyond 70 years of age the incidence was lower.

Age grouping correlated to sex distribution

Years	Males	Females
10—19	—	1
20—29	4	3
30—39	5	3
40—49	3	9
50—59	10	19
60—69	7	8
70—79	2	4
	31	47
	(40%)	(60%)

Distribution of clinical types

Erysipelas simplex	58
Erysipelas vesiculo-bullosum	6
Erysipelas haemorrhagicum	11
Erysipelas haemorrh.-bullosum	3
	78

Distribution of site

Legs	69	(88.4%)
Face, ears, nose	6	(7.7%)
Chest, upper arms	3	(3.9%)
	78	(100.0%)

Presumable site of invasion

Mycosis of foot (interdigital erosions, fissures, vesicular inflammation)	58
Injuries to the legs (surgery for varicous veins; injuries; thrombosis)	11
Rhinitis, otitis (otitis media, mastoidectomy, rhinitis)	3
Unidentified	6
	78

Number of relapses

(in most cases, between 2 and 4)

2	18	46
3	16	
4	12	
5	3	
6	5	
7 to 72	24	
	78	

Interval between original infection and first relapse
(in most cases, between 1 and 4 years)

Within	1 year		15	50
Within	1 to	4 years	35	
Within	5 to	9 years	11	
Within	10 to	19 years	11	
Within	20 to	29 years	5	
Within	30 to	39 years	1	
			78	

Enlargement of regional lymph nodes during the relapse: 34 cases.

Leucocyte count and erythrocyte sedimentation rate were determined in 76 cases.

The leucocytes numbered less than 8000 in most cases:

3000—8000	46
8000—9000	12
9000—10,000	6
Over 10,000	12
	76

Erythrocyte sedimentation rate, though increased in most cases, was not regarded as excessive in view of the prevalence of erysipelas in higher age groups. The upper limit of the normal range below 50 years of age was 15 mm per hr. in males, and 25 mm in females; beyond this age, 20 mm in males and 30 mm in females (BÖTTINGER [1967]). Excessive values were unfrequent.

ESR	
5— 10 mm	6
11— 20 mm	8
21— 60 mm	49
61—120 mm	13
	76

The leucocyte count was found to be unrelated to the sedimentation rate. In some cases, leucocytosis was associated with a normal sedimentation rate and *vice versa*.

The urine revealed no abnormality except for occasional urobilinogenuria or one or two erythrocytes per high power field.

II. Active immunization

The results might have been evaluated in two ways, i.e. (*i*) by the observation and analysis of the number and frequency of recurrences, and (*ii*) by the demonstration of "protective" antibodies in serum after immunization. We had to rely on the first possibility, as the informative value of the second was made dubious by our failure to recover from patients with erysipelas streptococcal strains of any notable virulence, i.e. possessing antibody-M (see Part II), unless we presume the emergence of former virulent streptococcal strains having been responsible for infections of other kind. But even in that case, demonstration of the M-protein would have been made difficult by the residual antibodies originating in former relapses as well as by the diversity of types used for the vaccine, moreover by the individual character of the primary or secondary stimulus in each case.

The efficacy of vaccination was thus weighed on the basis whether it was followed by a reduction in the number of frequency of relapses established at follow-up in 69 cases. Thirthy-three of these were controlled on one occasion, 17 cases on two, 14 on three and 5 on four occasions. The material was divided into three groups according to the frequency of relapses to be expected on the grounds of their pre-vaccination pattern.

Group I included patients who during the follow-up period had none of their previously regular relapses. The 29 subjects (42%) falling into this group had 2 to 72 relapses (average 14.2) prior to immunization. This means that to 4.5 recurrences had been inhibited per patient (range, 1 to 22).

Group II included 22 patients (31.9%) who had 2 to 70 (average 10.3) relapses prior to vaccination. There was one subsequent relapse in 16 cases, two in 5, and 3 in one between the first and 22nd months, beyond 7.6 months, on the average.

The relapses invariably appeared at the original site of the condition. A second immunization after the failure of the first one was restricted to 7 cases.

Group III comprised 18 patients (26.1%) who though having had no relapse during the period of follow-up, were not expected to have any on the basis of their history. Here the number of relapses ranged between 2 and 25 (average 5.1) and the intervals, between one month and 39 years (average 32.4 months). Therefore the fact that the present period of observation was free from relapses had a limited value as supportive evidence in favour of the treatment.

Judged, however, on the grounds of the 51 cases of Groups I and II where evaluation was possible, immunization proved to be efficient in 29 cases (56.9%) and failed to prevent relapses in 22 (43.1%). Though, on the evidence of the early results, streptococcal vaccination offers protection from relapses in scarcely more than 50% of the cases, the fact that each patient had been saved 4.3 relapses fully justifies the continuation of specific vaccine therapy.

Cellular skin hypersensitization of the delayed type was likewise checked prior to immunization, as well as on its completion and at follow-up. The changes will be evaluated together owing to their congruency in all three groups.

The proportion of cases with a delayed skin reaction to the streptococcal vaccine prior to vaccination hardly exceeded that found in non-streptococcal skin disease (see Part I), but as a result of immunization this proportion increas-

Table I

Positive delayed skin reaction to streptococcal vaccination in erysipelas

Groups	Number of patients	Positive skin reaction to intradermal streptococcal vaccine		Number of patients	Positive skin reaction to intradermal thermostable factor	
Patients with erysipelas						
Pre-immunization	69	23	33.3	69	25	36.2
Post-immunization follow-up	69	28	40.6	69	11	15.8
*Control groups**						
Streptococcal skin affections	45	19	42.2	40	14	35.0
Non-streptococcal skin affections	253	77	30.4	202	59	29.2

* For comparison with pre-immunization values in erysipelas.

ed by 7.3% in the patients with erysipelas, approximating the positivity rate found in streptococcal affections of the skin.

The ratio of the cases with positive delayed reaction elicited by the thermostable factor, though greater than in the healthy controls, was similar to that found in a patient material with other cutaneous manifestations of streptococcal origin. As a result of immunization, it fell, however, by 20.4% to even less than the ratio found in the controls.

To gain information about the humoral antibodies, ASL and ASK were systematically controlled (Table II). The positivity rate of ASL in recurrent

Table II

Positive serologic tests after streptococcal vaccination in erysipelas

Groups	Number of patients	Titre in excess of 250 U			
		ASL		ASK	
		number	per cent	number	per cent
Patients with erysipelas					
Pre-immunization	69	47	68.1	34	49.2
Post-immunization follow-up	69	61	88.4	42	60.8
*Control group**					
Streptococcal skin affections	45	28	62.2	19	42.2
Non-streptococcal skin affections	253	108	42.7	47	18.5

* For comparison with pre-immunization values in erysipelas

erysipelas was slightly higher than in other streptococcal skin diseases and rose still further after immunization to exceed its initial titre by 20.3 per cent at the end of the follow-up period.

The positivity rate of ASK was likewise slightly higher than in other streptococcal skin diseases and rose by 11.6% after immunization.

To answer the question of a relationship between the responses of cellular and humoral sensitization to immunization, the ratio of cases with positive skin reaction and positive serological tests was examined. From this comparative study it emerged that there is no parallelity between cellular sensitization of the delayed type and the production of circulating antibodies.

Discussion

The attempt to influence susceptibility to recurrent streptococcal infection by means of vaccination raised the fundamental question whether vaccination had any advantage over natural infection. If natural infection fails to

confer sufficient protection to the host from reinfection with strains of identical type, the fault may lie in a deficiency of immune globulin or phagocytic host response. Vaccination has no protective value against relapses unless, phagocytic activity being adequate, it may be reasonably expected to elicit a more active immune globulin response than does natural infection, which is often less antigenic than is vaccination.

Immune treatment of recurrent erysipelas may be traced back to the studies of BIRKHAUG (1926) into the immuno-allergic mechanism of the condition and to his first attempts at active immunization by means of erysipelas toxin and killed streptococci, claimed to secure freedom from recurrences during two years, together with negative skin reactions, in certain cases. Apart from a few exceptions (HÉNOCQ [1964]), later results were less favourable. Combined schemes (streptococcal vaccine + anatoxin + immune serum (were devised in order to improve the results (WEBER [1966]). For active immunization in the present study, a polyvalent streptococcal vaccine has been prepared from 14 M-protein-carrying strains of type A. These have been selected from strains prevailing in Hungary. 69 patients with 2 to 72 earlier relapses were treated in this manner. In 29 of 51 cases suited for evaluation no relapse was noted during a period of 9 to 27 months. In other words, immunization had been successful in 56.9%. This implicates that immunization alone offers no complete protection from relapses, if for no other reason than because of the vaccine having been prepared from 14 strains, whereas from the patients 6 types have only been grown; 5 of these were included in the vaccine but isolation of the other 9 types from the patients was impossible. The importance of the latent infective focus as well as of the portal of entry has already been pointed out. On the other hand, no benefit seemed to be gained by increasing the number of doses (some patients had 9, 11, 12, or 18 doses). The intensity of the local reaction has no predictive value as regards the efficiency of treatment. Reactions of average or greater intensity (vesicular erythema eventually with central haemorrhage) were equally represented in both groups. Focal reactions were unfrequent, a systemic reaction did not occur.

It has been suggested that the streptococci may persist in a "latent", "attenuated" or "L" form at the affected site, similarly to herpesvirus, until being rendered invasive in consequence of stimulatory factors affecting the reactivity of the host (HEGLER, WEICHARDT [1960], RÖCKL [1965], ADAM [1966], DIMITRESCU [1966], KORTING [1966]).

It has been shown in earlier studies (RAJKA [1966], BÖSZÖRMÉNYI [1967]) that in erysipelas patients there is some deficiency of immune reactivity involved in the late type of sensitization. This immune deficiency accounts for the contrast between the clinical severity of the infection and the relatively slight virulence of the strains. A genetically predetermined relative cellular defect of the lymphatic system may well account for the inadequacy of im-

mune response even to streptovaccines carrying M-protein, and for the inefficiency of this treatment in 43.1 per cent of the cases.

The avirulent strains identified in erysipelas are not deficient in any other respect beside being devoid of M-protein which makes them incapable of eliciting an adequate production of type-specific protective antibodies. The other circulating antibodies, particularly those directed against the extracellular enzyme antigens, of which ASL and ASK have only been studied, increased during treatment. This is, however, irrelevant from the point of view of protection. The favourable results of vaccination in more than half of the cases raise the question to which mechanism this might have been due. It has been pointed out that the positivity rate of delayed reactions in recurrent erysipelas scarcely attained that found in the controls. From this it may be inferred that the factor responsible for cellular sensitization is not the erysipelas infection but earlier streptococcal infections of the most common type the usual causative agents of which are M-protein-carrying virulent strains capable of producing type-specific antibodies (BÖSZÖRMÉNYI [1967]). Since in the manner of an anamnestic reaction, latent sensitization may develop anew on the injection of specific or related strains, the favourable effect of immunization may well be due, at least in a number of cases, to a revival of immunity. This is supported by the fact that the vaccine was prepared from strains commonest in Hungary. The other possibility to be considered is connected with the fact that some of the strains isolated from the patients, far from being totally avirulent, were even lethal at dilutions of 1 : 100 to albino mice. These might have elicited the production of "protective" antibodies in a number of cases which remained undetected by the examinations confined to ASL and ASK.

The skin reaction elicited by the two different antigens, i.e. streptococcal vaccine and the thermostable factor, showed opposite changes in the course of treatment. The reaction to streptovaccine increased in intensity by the end of treatment, i.e. by the end of approximately three weeks comprising 6 injections. This is in agreement with the observation that in the course of specific bacterial vaccination the delayed skin reaction grows initially in intensity (unless being unchanged) and it is only a prolonged treatment which leads to its attenuation or negativity. But even this intensification of the delayed reaction which is in the order of 7.5% represents an intermediate pattern, because, as shown by our earlier observations (RAJKA [1966]) recurrences reduce the positivity rate associated with fresh cases of erysipelas by 23.5% to the level of the controls.

The positivity rate of the delayed skin reaction to the LYAMPERT thermostable factor was reduced from the very outset. Though ignoring the cause of this phenomenon, we may possibly connect it with a relatively poor antigenicity of the "cocto" antigen in consequence of its deficiency in M-protein and in enzyme antigens.

The rise in titre of the humoral antibodies (ASL and ASK), apart from being uninformative of the degree of active immunity, gives no indication concerning the time of infection (CAYEUX [1966]), whether the levels had been higher prior to erysipelas infection or rose subsequent to it. Persistent relapses are usually associated with higher titres than fresh infections.

REFERENCES

1. ADAM, W.: Dtsch. med. Wschr. **91,** 821 (1966); **91,** 2353 (1966).
2. BAZYKA, A. P., MESCANINOVA, E. A., BOGDANOVA, M. G.: Vestn. Derm. Vener. **37,** 3 (1963).
3. BEREZHNOI, Z. G.: Vestn. Derm. Vener. **33,** 40 (1959).
4. BIRKHAUG, K. E.: Ref. DELBANCO, E., CALLOMON, F.: Erysipel, in J. JADASSOHN (ed.) Handbuch der Hautkrankheiten. Springer, Berlin 1929, Vol. IX/2, Pp. 1—92.
5. BÖSZÖRMÉNYI, J.: Acta microbiol. Acad. Sci. hung. **8,** 243 (1961); **12,** 157 (1965).
6. BÖSZÖRMÉNYI, J., SZITA, J., RAJKA, Ö., KOROSSY, S., GÓZONY, M.: Magy. Tud. Akad. V. Orv. Oszt. Közl. **18,** 239 (1967); Acta med. Acad. Sci. hung. **23,** 337 (1967).

6a. BÖTTINGER, L. E., SVEDBERG, C. A.: Brit. med. J. **1,** 85 (1967).

7. CAYEUX, P.: Presse méd. **74,** 2679 (1966).
8. DIMITRESCU, A.: Derm.-Vener. (Buc.) **11,** 97 (1966).
9. DOSTROVSKY, A., RAUBITSCHEK, F.: Dermatologica (Basel) **111,** 14 (1955).
10. EREMINA, N. I.:-Zh. Mikrob. Epid. Immunobiol. **40/7,** 9 (1966).
11. GALPERIN, E. A.: Zh. Mikrob. Epid. Immunobiol. **34,** 125 (1960).
12. GOTTRON, H. A.: Cit. RÖCKL (27).
13. HAMBURGER, M. jr., GREEN, M. J.: J. infect. Dis. **79,** 33 (1946).
14. HEGLER: Cit. Röckl (27).
15. HÉNOCQ, E., BAZIN, J. C.: Presse méd. **72,** 1151 (1964).
16. KONOPIK, J.: Hautarzt **16,** 158 (1965).
17. KOROSSY, S.: Orv. Hetil. **105,** 1464 (1964).
18. KORTING, G. W.: Dtsch. med. Wschr. **91,** 1619 (1966).
19. LIVINGOOD, CL. S.: J. Amer. med. Ass. **188,** 335 (1964).
20. LONGHIN, SC., ANTONESCU, ST.: Excerpta med. **11,** Congr. Ser. No. **8,** 62 (1957).
21. LYAMPERT, I. M., SMIRNOVA, M. N., SJOMINA, N. A.: Zbl. Bakt. I. Abt. Ref. **196,** 665 (1964).
22. MAIBACH, H. I., HILDICK-SMITH, G.: Skin Bacteria and their Role in Infection. McGraw-Hill, New York 1965. P. 49.
23. MEYER-ROHN, J.: Derm. Wschr. **86,** 2293 (1961).
24. MUSSGNUG, G.: Dtsch. med. Wschr. **82,** 381 (1957).
25. PFLEGER, L.: Arch. klin. exp. Derm. **221,** 23 (1964).
26. RAJKA, Ö., GÓZONY, M., KOROSSY, S., BÖSZÖRMÉNYI, J.: Acta derm.-vener. Hellerström Memorial Issue, 1966. P. 128; Magy. Tud. Akad. V. Orv. Oszt. Közl. **17,** 397 (1966).
27. RÖCKL, H.: Erysipel. In: J. JADASSOHN (ed.): Handbuch der Hautkrankheiten. Ergänzungswerk. Springer, Berlin 1965. Vol. IV, Part 1A, P. 115.
28. SZODORAY, L.: Orvosképzés 446 (1964).
29. TAPPEINER, J., PFLEGER, L.: Hautarzt **15,** 218 (1964).
30. WEBER, G.: Dtsch. med. Wschr. **91,** 326 (1966).
31. WEICHARDT, H.: Pro Medico **19.** 439 (1950).

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EFFECT OF CARDIOVASOPATHOGENIC DIET ON CARBOHYDRATE METABOLISM

By

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The influence of the cardiovasopathogenic diet (S.65) on carbohydrate metabolism has been examined in the rat. It has been demonstrated, in conformity with earlier studies *in vitro*, that the diet affects the degradation of carbohydrates, as reflected by a significant increase in the extracellular pyruvic acid level. The lactic acid level increased slightly. The diet has been found to affect the carbohydrate metabolism of the liver as well as that of the heart.

Though the breakdown of the carbohydrates does not fully cover the energy demands of the heart muscle, the effect of a cardiovasopathogenic diet on carbohydrate metabolism is nevertheless worthy of study, particularly *in vivo*, from the aspect of the entire organism. In this Institute Hegyváry et al. [6] have demonstrated *in vitro* alterations in the carbohydrate metabolism of animals kept on a cardiovasopathogenic diet, namely a reduction in the release of lactic acid and an increase in the production of pyruvic acid.

In the present study the effects of the cardiovasopathogenic diet (S. 65), rich in cholesterol, vitamin D_2, sodium and calcium, while poor in potassium and magnesium, has been examined on the carbohydrate metabolism in rats.

Material and methods

A total of 60 male albino rats of our own stock, weighing between 130 and 150 g, were used. Two groups were set up. The daily ratio per control animal was 12 to 14 g standard rat food (Lati, Budapest). The test animals received the cardiovasopathogenic diet S. 65. Water was allowed to both groups ad libitum.

In the sixth week the animals were killed under ether anaesthesia by bleeding from the abdominal aorta. Blood samples were collected in centrifuge tubes containing 10 mg NaF and centrifuged. In the serum and in heart muscle specimens homogenized by grinding with purified sand in the cold, the following determinations were made: blood sugar [7], glycogen extractable with TCA in the cold [17], lactic acid [1] and pyruvic acid [5].

The left lobes of the liver were ground in the cold and O_2-consumption of aliquots was measured in Ringer's solution containing 0.20% glucose and 0.20% bicarbonate. The manometers were gassed with air and allowed to equilibrate at 37° C for 15 min. Oxygen uptake was measured for 15 min. [16].

The results were evaluated by Student's *t*-test.

Results

The blood sugar level was 112.9 $\pm$ 11 mg per 100 ml in the controls and 110.5 $\pm$ 11.8 mg per 100 ml in the animals kept on the cardiovasopathogenic diet. The glycogen content of the heart showed no difference either, these being

0.289 ± 0.044 g per 100 ml in the controls and 0.309 ± 0.054 g per 100 ml in the test animals (Fig. 1).

Serum lactic acid level was 20.1 ± 3.82 mg per 100 ml in the controls, and 28.4 ± 7.8 mg per 100 ml in the animals kept on the diet ($p < 0.05$). The lactic acid content of the heart was, however, similar in both groups, i.e. 1.92 ± 0.27 mg/g wet tissue in the controls and 1.97 ± 0.19 mg/g in the test animals (Fig. 2).

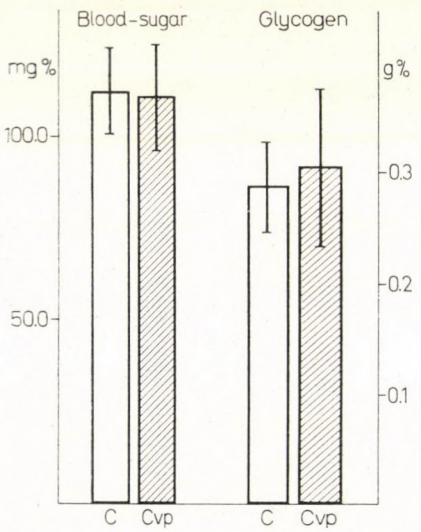


Fig. 1. Effect of cardiovasopathogenic diet on blood sugar level and on myocardial glycogen content. C=control; Cvp=dietary group

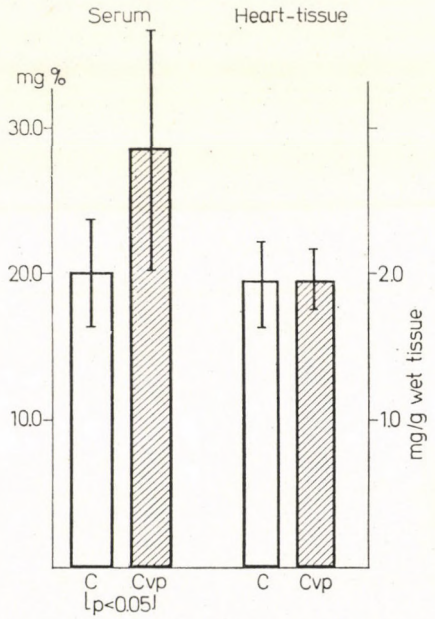


Fig. 2. Effect of cardiovasopathogenic diet on the lactic acid concentration in serum and myocardium. C=control; Cvp=dietary group

The serum pyruvic acid concentrations was 0.81 ± 0.17 mg per 100 ml in the controls, whereas 1.28 ± 0.31 mg per 100 ml ($p < 0.001$) in the dietary group (Fig. 3).

Hepatic O_2-consumption was significantly reduced, i.e. 125.4 ± 36.5 μl O_2/hr/100 mg dry weight in the dietary group, as against 188.4 ± 42.9 μl O_2/hr/100 mg dry weight ($p < 0.001$) in the controls (Fig. 4).

Discussion

Results obtained *in vivo* never reflect clearly the functional changes of an isolated organ, since they usually involve systemic effects.

The glycogen and lactic acid levels in the heart muscle were similar in both groups. Under the effect of 60 minutes hypoxia, the lactic acid content of the

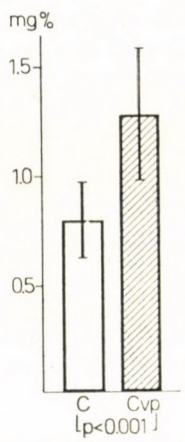
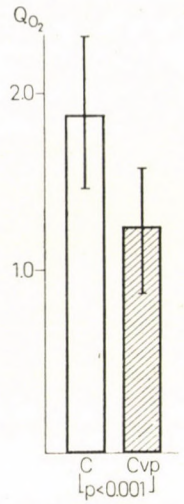


Fig. 3. Effect of cardiovasopathogenic diet on serum pyruvic acid level. C=control; Cvp=dietary group



4. Effect of cardiovasopathogenic diet on hepatic O_2 consumption. C= control; Cvp= dietary group

myocardium increased, indicating a shift of glycolysis into the anaerobic direction [2]. According to Sós [13] the cardiovasopathogenic diet brings about a narrowing of the coronaries and this may induce prolonged myocardial hypoxia. No increase was, however, found in myocardial lactic acid content. This may be attributed to a reduction of myocardial LDH-activity under the effect of the cardiovasopathogenic diet (SELMECI [11]) which supposedly leads to a reduction in lactic acid production and to a simultaneous increase in the pyruvate level. The high pyruvate concentrations are, in turn, inhibitory to the LDH_1 isoenzyme, predominant in heart muscle [3, 18].

Hypoxia due to coronary sclerosis is not the only effect of the cardiovasopathogenic diet. For the infarctoid lesions, not solely the vascular occlusion but some slowly developing metabolic changes are also responsible. The unchanged lactic acid and glycogen contents are consistent with our interpretation that cardiovasopathogenic diet (S. 65) has an additional direct effect on myocardial metabolism.

The increase in pyruvic acid production is, moreover, due to a reduction in the activity of the SZENT-GYÖRGYI—KREBS cycle, since myocardial succinyldehydrogenase activity is also affected in animals kept on a cardiovasopathogenic diet [10]. The marked reduction in myocardial Mg-concentration may be one of the possible causes of the high pyruvic acid levels, since adequate activity of the condensing enzyme requires the presence of magnesium [14]. Changes in succinyldehydrogenase, alkaline and acid phosphatase and esterase activity belongs to the pattern of the myocardial metabolic damage caused by the cardiovasopathogenic diet (PUCSOK et al. [9]).

The liver may be closely involved in the production of increased serum pyruvic and lactic acid levels. The slight increase in lactic acid content may be connected with an anaerobic shift of hepatic metabolism. Hepatic O_2 consumption is reduced by the cardiovasopathogenic diet. Hepatic succinyldhydrogenase activity is also inferior to that of the controls [15]; this fact, too, is suggestive of an increased anaerobic carbohydrate breakdown. LDH_5 isoenzyme, predominant in the liver, is not inhibited by the high pyruvate concentrations [3, 18]. Consequently, in addition to an enhanced carbohydrate breakdown, this is one of the factors accounting for the high serum lactic acid level.

The cardiovasopathogenic diet (S. 65) left the blood sugar level unaffected. HEGYVÁRY et al. [6] showed on the isolated heart that the cardiovasopathogenic diet does not interfere with the first step of carbohydrate metabolism, with the entry of glucose, only with the degradation of carbohydrates, that is, the liberation of energy. The present findings have demonstrated *in vivo* that the derangement of tissue metabolism belongs to the essential factors of cardiopathy.

REFERENCES

1. BARKER, S. B., SUMMERSON, W. H.: J. biol. Chem. **138,** 535 (1941).
2. BING, R. J.: Physiol. Rev. **45,** 171 (1965).
3. BUTA, J. L., CONKLIN, J. L., DEWEY, M. M.: J. Histochem. Cytochem. **14,** 658 (1966).
4. EVANS, C. L.: The Metabolism of Cardiac Muscle. In: Recent Advances in Physiology. 5th ed. revised by NEWTON, W. H. Blakiston, Philadelphia 1936. P. 33.
5. FRIEDEMANN, T. E., HAUGEN, G. E.: J. biol. Chem. **147,** 415 (1943).
6. HEGYVÁRY, Cs., NEMESÁNSZKY, E., SÓS, J.: Acta med. Acad. Sci. hung. **23,** 233 (1967).
7. HULTMAN, E.: Nature (Lond.) **183,** 108 (1959).
8. JUVANCZ, I.: Statisztikai eljárások. In: BÁLINT, P.: Klinikai laboratóriumi diagnosztika. Medicina, Budapest 1962. P. 1218.
9. PUCSOK, J., GÁTI, T., SÓS, J., ZELLES, T.: Acta physiol. Acad. Sci. hung. **30,** 338 (1966).
10. PUCSOK, J., SZELÉNYI, I., GÁTI, T., RIGÓ, J., SÓS, J.: To be published.
11. SELMECI, L.: Personal communication.
12. SÓS, J., GÁTI, T., KEMÉNY, T., RIGÓ, J., BUDAVÁRI, I.: Acta med. Acad. Sci. hung. **16,** 189 (1960).
13. SÓS, J., GÁTI, T., RIGÓ, J., KEMÉNY, T., LI BOK NAM, ZELLES, T.: Acta II. Conventus Medicinae Internae Hungaricae, Cardiologia (1963).
14. STRAUB, F. B.: Biokémia. Medicina, Budapest 1961.
15. SZELÉNYI, I.: Unpublished data.
16. UMBREIT, W. W., BURRIS, R. H., STAUFFER, J. F.: Manometric Techniques. Burgess, Minneapolis 1957.
17. VAN DER VIES, J.: Biochem. J. **57,** 410 (1954).
18. WILSON, A. C., COHN, R. O., KAPLAN, N. O.: Nature (Lond.) **197,** 331 (1963).

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EFFECT OF HYDRAZINOPHTHALAZINES ON THE SERUM GLYCOPROTEIN LEVELS

I. EFFECT OF HYDRALAZINE

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A study of the effect of protracted hydrazinophthalazine treatment on the serum glycoprotein and protein levels in rabbits revealed peak hexose and seromucoid levels at 6 weeks, peak hexosamine and sialic acid levels at 12 weeks. With the exception of hexosamine, the changes were highly significant. The serum protein level showed a significant increase at six weeks. At 12 weeks, there was a remarkable increase in the globulin level at the expense of albumin. All these changes occurred before any morphologic abnormality had become manifest. The results were suggestive of changes in carbohydrate metabolism being involved in the pathomechanism of hydralazine lupus syndrome and, by analogy, in that of LED.

Hydrazinophthalazines, i.e. 1-hydrazinophthalazine (Apresoline, Ciba and 1-4-dihydrazinophthalazine (Nepresol, Ciba) have been applied as antihypertensive agents since 1951. Both are known to increase renal blood flow to relieve smooth muscle spasm, to reduce blood pressure and to abolish the pressor effect of norepinephrine. On the evidence of studies with ^{14}C-hydralazine they are eliminated from the organism by the kidneys. They carry the significant hazard of the so-called hydralazine lupus syndrome (HLS) or lupoid syndrome, affecting 10 to 12% of the cases, particularly when used in doses of 500 mg daily over 16 months, but in some instances the syndrome developed after doses of 100 mg daily for three weeks, and even with a delay of six and a half years [3, 8, 12, 13, 18].

In its early stage, HLS is reminiscent of rheumatoid arthritis [5]. As with systemic lupus erythematosus, cutaneous fibrinoid degeneration is characteristic of it. There are typical wire loop changes in the kidney. The complement fixation test may be positive. On discontinuing the drug the syndrome disappears except for the LE phenomenon in the bone marrow which may take one year, occasionally even three to seven years to vanish [8].

The syndrome may be induced in the dog with all its clinical and laboratory features, by hydralazine administration [3, 6], though this experimental method is not infallible [4]. SIGUIER induced the syndrome in guinea-pigs; all animals died in the course of two to four months [17]. LEÖVEY et al. [11] also studied the syndrome in guinea-pigs.

Though HLS lacks certain features of systemic lupus erythematosus, i.e. prevalence in women, gross urinary abnormalities, irreversibility, it shares the majority of its other characters including the abnormal serologic reactions which suggest a definitive relationship between the two syndromes.

The actual pathologic process responsible for the collagen diseases including systemic lupus erythematosus affects the connective tissue. The striking clinical and morphologic similarity between HLS and systemic lupus erythematosus presupposes a similar metabolic disorder in both cases. A study of the effect of hydralazine on the serum glycoprotein and protein level seemed therefore interesting.

Material and method

Rabbits of random sex distribution weighing between 1.3 to 3 kg were used. Twelve animals received a dose of 25 mg hydralazine intragluteally daily over 12 weeks. The serum glycoprotein level was estimated before treatment and after 6 and 12 weeks. Seven animals were alive at the end of 12 weeks, 5 having died in the course of treatment. The survivors were killed by bleeding. The following determinations were made.

Protein-bound galactose and mannose after STARY et al. [19], hexosamine after RIMINGTON by the ELSON-MORGAN reaction [15], sialic acid after AYALA et al. [1], seromucoid after WEIMER and REDLICH-MOSHIN [20]. Serum proteins were estimated by the biuret method at 6 and 12 weeks and electrophoretically at 12 weeks. The interior organs were examined macroscopically and microscopically. For statistical evaluation the one- or two-sample *t*-test was used.

Table I

Effect of hydralazine treatment on serum glycoprotein level

	Hexose (mg/100 ml)	Hexosamine (mg/100 ml)	Sialic acid (mg/100 ml)	Seromucoid (mg/100 ml)	Protein (g/100 ml)
			Before treatment		
x	118.5	79.6	45.6	14.1	—
S	10.5	7.1	6.7	1.9	—
			At 6 weeks		
x	137.2	78.7	57.6	21.0	8.3
S	21.2	11.9	12.2	4.1	1.0
			At 12 weeks		
x	126.8	95.8	74.3	18.7	7.5
S	23.5	18.8	14.8	4.6	0.6

Statistical analysis (one-sample *t*-test)

	Hexose at 6 weeks — initial	Hexosamine at 12 weeks — initial	Sialic acid at 12 weeks — initial	Seromucoid at 6 weeks — initial
t	3.78	1.34	6.24	5.71
p%	<1	>20	<1	<0.1

Results

The serum glycoprotein level increased during hydralazine treatment. Hexose and seromucoid reached peak concentrations at 6 weeks, the changes being significant in both cases; the levels declined during the next six weeks. The hexosamine and sialic acid levels showed a continuous rise until the end

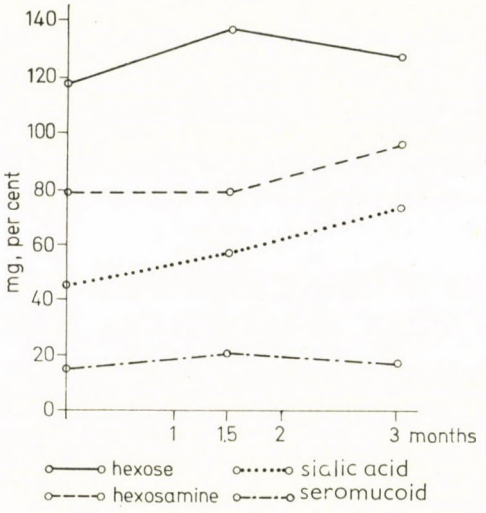

Fig. 1. Effect of hydralazine on serum glycoprotein level

Table II

Effect of hydralazine treatment on serum protein fractions

	Final value (%)				
	Albumin	$Alpha_1$ —	$Alpha_2$ —	Beta —	Gamma-globulin
x	40.3	5.3	17.6	18.6	18.2
S	8.4	2.6	4.2	3.7	4.6

Statistical analysis (two-sample *t*-test)

	Albumin		$Alpha_1$ —		$Alpha_2$ —		Beta —		Gamma-globulin	
	Final	Normal	Final	Normal	Final	Normal	Final	Normal	Final	Normal
x	40.3	67.4	5.3	3.4	17.6	7.7	18.6	13.5	18.2	8.6
t		9.0		2.26		7.8		3.2		5.7
p%		< 0.1		< 5		< 0.1		< 1		< 0.1

of the 12th week; this change was not significant for hexosamine, while definitely so for sialic acid. The serum protein level rose significantly in the first 6 weeks to decline afterwards. Electrophoresis revealed typical changes at the end of three months; the globulin fractions were significantly increased at the expense of albumin. Absolute values displayed similar changes (Tables I and II, Fig. 1).

Apart from a hyperplasia of the lymphatic system, no noteworthy morphologic change was found.

Discussion

The most important, and clinically the most troublesome side-effec of hydralazine treatment is HLS. Its mechanism is still obscure owing to insufficient knowledge concerning the metabolism of hydralazine. It is characteristic of these compounds to form stable chelates with certain trace elements and to act as antienzymes which would account for some of their side-effects, namely tachycardia, headaches, etc. interpreted as possible antihistaminase effects [3].

Observations of HLS induced by therapeutic or sub-therapeutic doses, occasionally even long after completion of treatment, plead against a possible involvement of toxic factors. The syndrome seems to correspond to a hypersensitivity reaction [14], being reproducible by small doses within 24 hrs, and revealing features reminiscent of serum sickness.

The possible hydralazine effect admits of various interpretations including vitamin deficiency, metabolic disorders (particularly in respect to minerals), dietetic factors, and even involvement of mesencephalic functions.

Of the trace elements, manganese seems to be of definite significance, since it has been shown to protect rats from the effect of lethal doses of hydralazine and to inhibit the manifestation of LHS, including its morphological changes [10].

The hydrazinophthalazines seem to affect the metabolism of glycoproteins and of protein-bound polysaccharides, all being structural elements of the ground substance of connective tissue. They may interfere with carbohydrate metabolism at various points. They may affect the biosynthesis of oligosaccharides, i.e. conversion of glucose to monosaccharides, activation of monosaccharides and polymerization of activated monosaccharide units. They may furthermore interfere with the acetylation of glucosamine into UDP-N-acetyl-galactosamine, with sulphatation, and with the formation of protein-bound polysaccharide complexes. These last changes would, however, be more likely to affect the metabolism of glycosaminoglycanes [10, 16]. The possibility of metabolic effects of this kind seems to agree with the observations in respect

to manganese. Being inhibitory to the development of HLS, its deficiency affects carbohydrate metabolism in a characteristic manner by reducing the amount of glycosaminoglycanes.

The present results suggest that the hydrazinophthalazines affect the metabolism of serum glycoproteins. The changes seemed to be significant though the number of animals allowed no definite conclusions. Humoral changes of similar kind are demonstrable in systemic lupus erythematosus as well [2, 7, 9, 21]. The observation that the glycoprotein metabolism is affected before any structural lesion becomes manifest might be regarded as an indication that alterations of the ground substance too small to be detectable microscopically might be present even in the early stage.

The finding that the derangement of carbohydrate metabolism precedes the morphologic changes, connects the mechanism of HLS with changes in the metabolism of glycoproteins and of protein-bound polysaccharides. It is furthermore suggestive of similar changes being involved in the pathomechanism of systemic lupus erythematosus. This, however, awaits confirmation. The observations in respect to manganese raise therapeutic possibilities.

REFERENCES

1. Ayala, W., Moore, L. V., Hess, E. L.: J. clin. Invest. **30,** 781 (1951).
2. Boas, N. F., Soffer, L. J.: J. clin. Endocr. **11,** 39 (1951).
3. Comens, Ph.: Hydralazine Lupus Syndrome. In: Inflammation and Diseases of Connective Tissue. Eds.: Mills, L. C., Moyer, J. H. Saunders, Philadelphia 1961 P. 190.
4. Dubois, E. L., Katz, Y. J., Freeman, V., Garbak, F.: J. Lab. clin. Med. **50,** 119 (1957).
5. Dustan, H. P., Taylor, D., Corcoran, A. C., Page, I. H.: J. Amer. med. Ass. **154,** 23, (1954).
6. Gardner, D. L.: Brit. J. exp. Path. **38,** 227 (1957).
7. Greenspan, E. M.: Arch. intern. Med. **93,** 863 (1954).
8. Hildreth, E. A., Biro, C. E., McCreary, T. A.: J. Amer. med. Ass. **173,** 657 (1960).
9. Jakab, L.: Orv. Hetil. **104,** 17 (1964).
10. Leach, R. M. jr.: Fed. Proc. **26,** 118 (1967).
11. Leövey, A., Szegedi, Gy., Bobory, J.: Magy. belorv. Arch. **19,** 145 (1966).
12. Perey, H. M., Schroeder, H. A.: J. Amer. med. Ass. **154,** 670 (1954).
13. Reinhardt, D. J., Waldron, J. M.: J. Amer. med. Ass. **155,** 1491 (1954).
14. Reynolds, H., Caldwell, J. R.: J. Amer. med. Ass. **165,** 1823 (1957).
15. Rimington, C.: Biochem. J. **34,** 931 (1940).
16. Roseman, S.: Fed. Proc. **21,** 1075 (1962).
17. Siguier, F., Betourme, C., Bonnet de la Tour, J.: Sem. Hôp. Paris **34,** 773 (1958). Cit. Comens.
18. Slonim, N. B.: J. Amer. med. Ass. **154,** 1419 (1954).
19. Stary, Z., Bursa, F., Kaleoglü, Ö., Bilen, M.: Bull. Fac. Med. Istambul **13,** 243 (1950).
20. Weimer, H. E., Redlich-Moshin, J.: Amer. Rev. Tuberc. **68,** 594 (1952).
21. Weiden, S.: J. clin. Path. **11,** 177 (1958).

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EFFECT OF HYDRAZINOPHTHALAZINES ON THE SERUM GLYCOPROTEIN LEVEL

II. EFFECT OF DIHYDRALAZINE

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The effect of dihydralazine on serum glycoproteins and proteins have been studied in rabbits. The compound was found to induce changes similar to those elicited by hydralazine. Serum hexose, hexosamine and seromucoid reached peak levels at the end of the third month, to decline thereafter. Sialic acid reached a peak at the end of the fourth month and declined subsequently. The hexosamine and sialic acid levels remained high till the end of the sixth month. Serum protein levels increased at the end of the third month; their electrophoretic pattern was characterized by a progressive reduction of albumin with a progressive increase of globulins. The mechanism and clinical aspects of the changes have been discussed.

Dihydralazine (1-4-dihydrazinophthalazine) differs from hydralazine with a second hydrazino group at position 4. Though according to certain authors [6] dihydralazine is less toxic than hydralazine, according to others [8] it is of the same toxicity, since BRIGGS and HOLLAND [2] have shown that the hydrazine bond at position 4 of the phthalazine ring does not affect the compound's activity or toxicity.

In an earlier paper [7] we have dealt with the effect of hydralazine on the serum glycoprotein level. Since no data have been found concerning this effect of dihydralazine, a study was performed to clarify whether 1. the changes of the serum glycoprotein level induced by hydralazine are reproducible by dihydralazine? 2. Does protracted dihydralazine treatment induce changes in carbohydrate metabolism? 3. Do the changes bear any relationship to the hydralazine lupus syndrome (HLS) or eventually to lupus erythematosus disseminatus (LED).

Material and method

A total of 20 rabbits of both sexes weighing between 1.6 and 3 kg was used. Dihydralazine was applied intragluteally in doses of 25 mg daily over six months. Serum glycoprotein level was estimated at the start of the study and at 1, 2, 3, 4, and 6 months respectively, the serum proteins at the start and at three months, their electrophoretic pattern at the start and at 1, 2, 3, and 6 months. The respective methods have been described earlier [7].

Of the 20 animals, 12 died in the course of the investigation. The remaining 8 were killed by bleeding at the end of the sixth month. The condition of the animals showed a progressive deterioration with occasional convulsive seizures. The results were statistically evaluated by the one-sample *t*-test.

Results

Results are shown in Fig. 1 and in Tables I and II. From the carbohydrate fractions of the serum glycoproteins, hexose, hexosamine and seromucoid reached peak levels by the end of the third month; the changes were highly significant statistically in all three cases. From the end of the third month onward the levels of these fractions declined and at the end of the sixth month, those of hexose or seromucoid were hardly over the initial values whereas that of hexosamine was still significantly increased. The sialic acid level reached its peak at the end of the fourth month, the change in relation to the initial value being

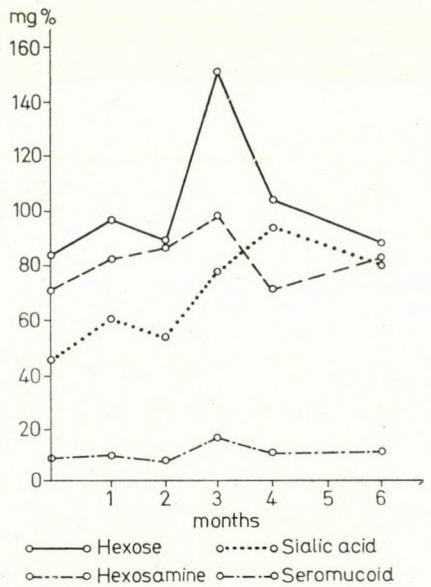

Fig. 1. Effect of dihydrazaline on the serum glycoprotein level

very strongly significant. Though from this time onward it declined, at the end of the sixth month it still was highly significantly increased as compared to the initial value. An increase in the serum protein level was noted at the end of the third month, the change was at the borderline of significance. Its electrophoretic pattern also showed typical changes. The albumin level declined until the end of the sixth month, the reduction was very markedly significant. The α_1-globulin level reached its peak at the end of the third month and despite of declining from this time onward, at the end of the sixth month it was still considerably higher than at the onset. Peak of α_2-globulin and γ-globulin levels occurred at the end of the sixth month, the changes were significant in both

Table I

Effect of dihydralazine treatment on serum glycoprotein levels

		Initial value	At 1 month	At 2 months	At 3 months	At 4 months	At 6 months
Hexose mg per 100 ml	n	20	16	12	12	10	8
	$\bar{x}$	85.40	97.31	89.00	152.42	104.70	88.625
	s	10.57	13.44	15.62	22.35	17.58	22.03
Hexosamine mg per 100 ml	n	20	16	13	12	10	8
	$\bar{x}$	71.85	82.94	87.77	99.58	71.70	83.25
	s	9.069	16.97	12.99	12.29	11.47	11.93
Sialic acid mg per 100 ml	n	20	16	13	12	10	8
	$\bar{x}$	45.25	60.53	54.00	78.92	94.10	80.00
	s	6.672	11.82	7.971	13.87	16.30	14.78
Seromucoid mg per 100 ml	n	20	15	13	11	10	8
	$\bar{x}$	10.10	10.60	8.077	17 18	10.50	10.5
	s	1.021	2.444	1.978	3.345	3.808	2.33
Protein g per 100 ml	n	20			11		
	$\bar{x}$	6.51			7.073		
	s	0.81			0.653		

	3 months-initial				3 months — final			4 months initial	4 months final	Final — Initial			
	He	Ha	Se	Pr	He	Ha	Se	Si	Si	He	Ha	Si	Se
n	12	12	11	11	6	6	6	10	7	8	8	8	8
$\bar{x}$	68.3	28.25	7.2	0.7	52.0	12.0	4.2	49	9.7	2.6	14.2	36.0	0.5
p%	<0.1	<0.1	<0.1	$\gtrsim$5	<1	>20	>10	<0.1	>10	>70	<5	<0.1	>50

n = number of animals
$\bar{x}$ = mean
s = standard deviation

Table II

Effect of dihydralazine treatment on serum protein pattern

		Initial value	At 1 month	At 2 months	At 3 months	Final value
Albumin %	n	15	15	14	12	8
	$\bar{x}$	67.37	64.653	53.23	45.54	39.41
	s	6.310	5.148	9.298	5.688	8.546
α_1-globulin	n	15	15	14	12	8
	$\bar{x}$	3.43	3.79	2.65	6.98	4.91
	s	1.535	1.527	1.058	5.251	2.411
α_2-globulin	n	15	15	14	12	8
	$\bar{x}$	7.69	8.17	4.61	13.00	18.15
	s	1.917	2.087	2.022	4.228	4.113
β-globulin	n	15	15	14	12	8
	$\bar{x}$	13.51	14.14	23.80	18.72	18.88
	s	3.634	3.547	10.7706	7.181	3.885
γ-globulin	n	15	15	14	12	8
	$\bar{x}$	8.65	9.27	15.81	14.37	18.55
	s	3.413	3.880	3.030	3.859	4.820

	Albumin Initial-final	α_1-glob.		α_2-glob. final-initial	β-glob.		γ-glob. final-initial
		3 months initial	final-initial		2 months-initial	final-initial	
n	8	12	8	8	14	8	8
$\bar{x}$	28.51	3.42	0.65	9.72	10.0	5.51	11.32
s	10.94	4.63	1.67	4.99	10.41	5.10	3.70
p%	<0.1	<5	>30	<0.1	<1	<5	<0.1

cases. β-globulin reached its peak at the end of the second month to decline by the end of the sixth month to a level significantly higher than the initial one. The changes were similar if claculated on the basis of absolute protein values.

Apart from lymphatic tissue hyperplasia the organs displayed no noteworthy morphologic change.

Discussion

On the evidence of the present study the question whether the changes induced by hydralazine are strictly typical of the compound may be answered in the negative, since similar changes were produced by dihydralazine as well. The present findings support the view of BRIGGS and HOLLAND [2] in that the activity or toxicity of dihydralazine is identical with that of hydralazine,

or even more intensive. Both drugs induce peak levels of hexose and of seromucoids expressed in hexose, the values subsequently decline to practically normal levels. This change seems to be transitory and if the animal survives the critical phase, the affected fractions are eventually normalized. On the other hand, the hexosamine and sialic acid levels, though declining during the last months of the study, were still increased at the end of six months. This finding may be of particular significance since under physiologic conditions the hexosamine of glycoprotein is made up for the greatest part of glucosamine, moreover, sialic acid is a condensation product of mannosamine and pyruvic acid. Mannosamine on its part is formed by the epimerization of glucosamine.

Prolonged treatment with hydrazinophthalazines thus seems to give rise to a non-progressive process liable to chronicity. This is at least what the changes of glycoprotein metabolism seemed to suggest. The general condition of the animals declined successively and part of them died in the course of treatment.

As to the possible relationship of the changes with HLS and/or LED, no morphological changes pointing to HLS were encountered in the present study. The LE-phenomenon was not examined. The changes in the metabolism of glycoproteins and of serum proteins were, however, remarkable. The former bear an obvious relationship to HLS as well as to LED. As to the abnormalities observed in protein metabolism, they must be regarded as consistent with those encountered in human collagen disease [1, 9]. The results of animal experiments with hydralazine were inconsistent [3, 4]. The basic values obtained in the present study are on the whole in agreement with those to be found in the literature [4, 10]. It was furthermore found that the abnormalities of protein metabolism have a progressive tendency, at least if the drug is administered over a period of six months.

This leads to the further conclusion that the changes of proteins and carbohydrates show no close parallelity which suggests that dihydralazine must have several points of attack and that the liver is closely involved in the mechanism of its effect. This would recommend a prolongation of the period of study in further experiments in the interest of a still greater objectivity in the interpretation of the results.

*

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REFERENCES

1. Bencze, Gy., Lakatos, L.: Rheum. Balneol. Allerg. (Budap.) **4,** 72 (1963).
2. Briggs, A. H., Holland, W. C.: Antihypertensive Drugs. In: Drill's Pharmacology in Medicine. 3rd ed. McGraw Hill, New York 1965. P. 639.
3. Comens, P.: J. Lab. clin. Med. **47,** 444 (1956).
4. Gardner, D. L.: Brit. J. exp. Path. **38,** 227 (1957).
5. Geinitz, W.: Klin. Wschr. **32,** 1108 (1954).

6. ISSEKUTZ, B.: Gyógyszertan és Gyógyítás. Medicina, Budapest 1957.
7. JAKAB, L., FEHÉR, J., VIRÁG, S., SZINAY, GY.: Acta med. Acad. Sci. hung. **25,** 67 (1968).
8. KNOLL, J.: Gyógyszertan. Medicina, Budapest 1965.
9. PETRÁNYI, GY.: A systémás lupus erythematosus és a "rheumás", "collagenosisos" "auto-allergiás-autoimmun" kórképek. D. Sc. Thesis, Budapest 1964.
10. SCHEIFFARTH, F., BERG, G.: Z. ges. exp. Med. **119,** 555 (1952).

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HAEMODYNAMIC CHANGES IN THE SUBCLAVIAN STEAL SYNDROME

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Cerebral and extremital blood flow in the subclavian steal syndromes has been studied in patients and animal experiments. On the evidence of the latter, in the subclavian steal syndrome blood supply to the vascular territory distal to the obstruction is provided by reversed blood flow mainly through the ipsilateral vertebral artery and in the second line through the ipsilateral lower thyroid artery.

It is suggested that where the syndrome combines features of cerebral ischaemia with the absence of ischaemic symptoms of the upper extremity there is a substantial retrograde flow in the vertebral artery providing for a sufficient blood supply to the vascular territory distal to the obstruction. On the other hand, in the rarer type marked by ischaemic symptoms of the upper extremity but none of the brain, retrograde flow in the vertebral artery is presumably insignificant.

In the type marked by cerebral ischaemia, cerebral blood flow was abnormally low and the cerebral fraction of cardiac output was reduced. It is presumed that the vascular resistance in the upper extremity has an important part in the regulation of cerebral blood flow in the subclavin steal syndrome.

The anatomy of the subclavian steal syndrome may be described as an occlusion or stenosis of the subclavian artery proximal to the origin of thever tebral artery. The presenting symptoms comprize of spells of cerebral ischaemia and of discomfort in the upper extremity of the affected side similar in type to intermittent claudication. The syndrome has been attracting interest for the following reasons: (i) In the light of recent investigations, narrowing of the subclavian artery has been emerging as one of the major causes of transient cerebral ischaemia of hitherto obscure origin. (ii) Study of the subclavian steal syndrome offers insight into the regulatory mechanisms of cerebral blood flow. The pathomechanism of the symptoms and haemodynamic changes associated with the subclavian steal syndrome are not fully understood. This has prompted us to join clinical observations with animal experiments for a closer study of the syndrome and of the accompanying haemodynamic changes.

Material and method

Eleven cases of subclavian steal syndrome were detected in our patient material in the period 1964—1965. (Since the completion of this paper six further cases have been identified.) There were 4 males and 7 females, between 32 and 76 years of age. The following investigations were carried out: 1. Measurement of blood pressure in both upper extremities. 2. Bilateral sphygmograms of brachial and radial arteries. 3. Oscillometry in both upper extremities. 4. Phonocardiogram recorded from both supraclavicular fossae. 5. Bilateral ophthalmodyna-

mometry for the measurement of pressure in the central retinal artery. 6. Bilateral digital and temporal rheograms. In 7 cases diagnostic cerebral angiography was carried out, accuracy, in 2 cases the diagnosis was confirmed at autopsy.

Nine of the 11 patients had proximal stenosis of the left, 2 of the right subclavian artery. Three patients had additional stenosis of the carotid of the affected side demonstrated by angiography or at necropsy.

Cerebral blood flow and metabolism were studied in 4 cases in order to establish how the syndrome affected cerebral circulation. Cerebral blood flow was estimated by means of our venous isotope dilution technique [1]. Cardiac output was measured simultaneously by means of Evans blue so as to determine the cerebral fraction of cardiac output. Cerebral O_2 uptake was calculated as the product of cerebral blood flow and of cerebral arteriovenous O_2 difference. O_2 content of blood was determined by a Kipp oxymeter.

Regulation of cerebral and extremital blood flow was studied in dogs weighing between 10 to 15 kg. In the first series of this study (7 dogs) the haemodynamic effects of acute occlusion of the proximal segment of the subclavian artery were examined. The proximal segment of the left subclavian artery was exposed under hexobarbital-ether anaesthesia and ligated above the origin of the vertebral artery so as to reproduce the subclavian steal syndrome. A rotameter was inserted into the initial segment of the left vertebral artery for the registration of blood flow. In 3 animals a rotameter was inserted into the lower thyroid artery, i.e. the second major branch of the subclavian artery. For the estimation of blood flow in the upper limb a T-cannula was inserted into the proximal segment of the subclavian vein on both sides, and venous outflow was measured directly.

The other part of the study was concerned with the haemodynamic effects of chronic obstruction of the subclavian artery. In 7 dogs the left subclavian artery was ligated proximal to the origin of the vertebral artery. A thick thread was placed below the origin of the vertebral artery and its two ends were carried to the outside of the neck by means of a cardiac catheter and fixed there. After local application of penicillin the wound was closed. One week later blood flow in the upper limbs was measured as in the first part. By pulling on the ends of the thread, closure of the vertebral artery could be tightened as desired. Cerebral angiography after acute and chronic closure of the subclavian artery was carried out in 9 dogs.

Table I presents the symptoms and findings in the 11 patients. Characteristic were the symptoms of intermittent cerebral ischaemia, particularly if elicited by movements of the upper extremity. Typical data were furnished by bilateral readings of blood pressure, bilateral radial sphygmography, and phonoarteriograms recorded over the supraclavicular fossa. Diagnosis was decided by cerebral angiography. Data concerning cerebral blood flow are summarized in Table II.

Cerebral blood flow in all the 4 cases was conspicuously less than the normal value of 850 ± 80 ml/min obtained previously on an extensive material. The fall of cerebral blood flow in response to a sudden reduction of vascular resistance in the upper extremity after the intraarterial administration of 0.08 g of papaverine as observed in Case No. 1 was typical. On the sudden elevation of vascular resistance in the upper extremity by application of a tourniquet, a significant increase in cerebral blood flow was demonstrable in Cases No. 2, 3, and 4 and a significant increase in cerebral O_2 uptake in Cases No. 3 and 4. The cerebral fraction of cardiac output was abnormally low in all four cases (normal value, $18.0 \pm 2\%$).

Experimental findings were as follows: Acute occlusion of the subclavian artery resulted in a typical reversal of blood flow in the ipsilateral vertebral artery; this retrograde blood flow exceeded the normal flow prior to occlusion. Blood flow in the upper extremity of the affected side remained nearly equal

Table I

Essential symptoms and diagnostic signs of the subclavian steal syndrome in 11 patients

A) Evidence of individual investigation B) Presenting symptoms	No. of cases studied	No. (%) of positive cases	Note
		A) Investigations	
20 mm Hg difference in blood pressure between the two arms	11	11 (100)	In 4 cases the difference was 50 mm Hg
Systolic sound, thrill over supraclavicular fossa	11	10 (91)	Diamond-shaped ejection murmur over supraclavicular fossa on affected side
Reduced pulse amplitude of brachial and radial arteries on the affected side	9	8 (89)	
Reduction of the rheogram of the fingers and of the arm on affected side	8	5 (62)	
Reduced oscillometric index on affected side	10	4 (40)	No indication of cerebral ischaemia in any of the 4 positive cases
Reduced pressure in central retinal artery on affected side	7	2 (29)	
		B) Symptoms	
Transitory vertigo	11	7 (64)	In 4 cases typical vertigo on physical work
Transitory faint	11	6 (54)	In 3 cases loss of consciousness on physical work
Transient blurring of vision	11	3 (27)	
Ischaemia of upper extremity on affected side	11	3 (27)	In the 3 cases of this type symptoms indicative of cerebral ischaemia were absent
Headache	11	3 (27)	In all 3 cases headache on physical work

to that of the other side. Occlusion of the vertebral artery with reversed blood flow caused a substantial reduction in flow in the ipsilateral upper extremity. A retrograde flow of minor degree was demonstrable in the lower thyroid artery also. Occlusion of the ipsilateral thyroid artery resulted in an insignificant

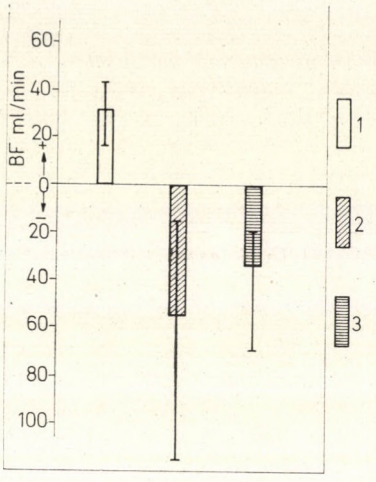

Fig. 1. Reversal of blood flow in vertebral artery following acute occlusion of the proximal segment of the subclavian artery
1. The anterograde flow of the — left — vertebral artery before the ligation of the — left — subclavian artery — 2. The retrograde (reversed) flow of the — left — vertebral artery after the ligation of the — left — subclavian artery — 3. The retrograde flow of the — left — vertebral artery after ligation of the subclavian artery and after occlusion of the right common carotid artery

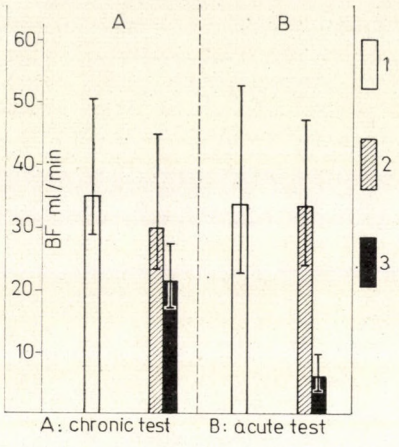

Fig. 2. Change in blood flow of the upper limb following acute or chronic occlusion of the proximal segment of the subclavian artery
B = Acute experiments (immediately after the ligation of the left subclavian artery) — A = Chronic experiments (after a week of the ligation of the left subclavian artery — 1. Blood flow (ml/min) of the right — intact — upper limb — 2. Blood flow of the left upper limb — 3. Blood flow of the left upper limb after the occlusion of the left vertebral artery

Table II

Regulation of cerebral blood flow in subclavian steal (brachiobasilar) syndrome

No.	Name, age (years), sex	P	RR mm Hg	CO l/min.	TPR dyn. sec. cm^{-5}	CBF ml/min.	CVR dyn. sec. cm^{-5}	CO_2C ml/min.	CCOF %
1*	E. J. 59 ♂	A	150/90	5.0	1750	750	11.600	48.0	15.0
		B	140/80	4.8	1600	600	13,200	45.0	12.5
2°	N. Gy. 68 ♀	A	170/95	5.0	1910	710	13,400	49.0	14.2
		B*	180/100	5.3	1910	840	12,000	—	15.5
3°	D. L. 62 ♀	A	220/95	4.5	2400	650	16,600	35.0	14.5
		B*	220/90	4.5	2350	750	14,100	40.0	16.8
4°	N. J. 65 ♀	A	140/90	4.0	2150	650	13,100	45.5	16.2
		B*	140/90	—	—	700	12,200	49.0	—

Abbreviations: P = Period; A = Before experiment; B = 2 minutes after intraarterial administration of 0.08 g papaverine into the left brachial artery; B* = Left upper limb exposed to 220 mm Hg pressure by tourniquet; RR = Blood pressure values refer to the intact (right) upper ex tremity; CO = Cardiac output; CBF = Cerebral (total) blood flow; CVR = Cerebral (total) vascular resistance; CO_2C = Cerebral (total) oxygen consumption; CCOF = Cardiac output fraction of cerebral flow

reduction of upper extremital flow if compared with the effect of closure of the vertebral artery.

Prolonged occlusion of the subclavian artery caused no greater difference in upper extremital flow than did its acute occlusion. In this case, however, there was only a slight reduction in blood flow in the upper limb of the affected side on occlusion of the ipsilateral vertebral artery.

Results of the animal experiments are shown in Figs 1 and 2.

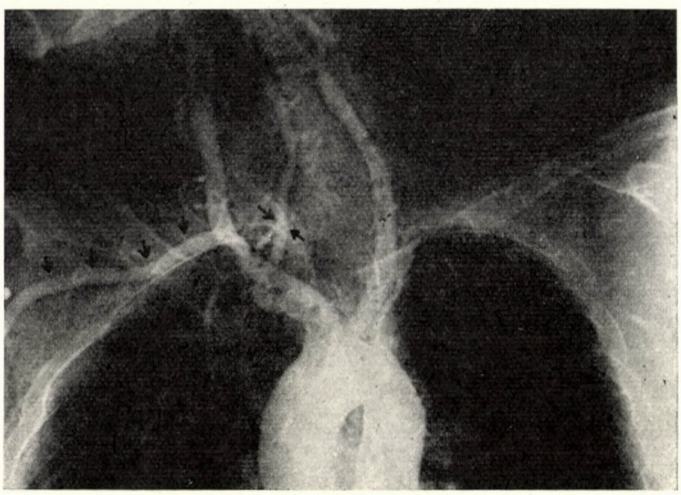

Fig. 3. Cerebral angiogram after introduction of contrast medium into the arch of the aorta. The right subclavian (↓) and vertebral (↕) arteries fill clearly

Cerebral angiography was performed in 7 patients and in 9 dogs. In 5 subjects the distal segment of the subclavian artery filled *via* the vertebral artery, in other words, in this artery there was a characteristic reversal of flow. In 2 cases the distal segment of the subclavian artery received blood through the thyrocervical trunk, presumably by reversal of blood flow through the lower thyroid artery. A typical case with reversed flow in the vertebral artery is presented in Figs 3 and 4.

The angiograms performed in dogs showed that after acute occlusion of the proximal segment of the subclavian artery, its distal segment filled essentially through the ipsilateral vertebral artery (Fig. 5).

In case of chronic occlusion of the proximal segment the distal territory received its blood supply, apart from the vertebral artery, first of all through the thyrocervical trunk.

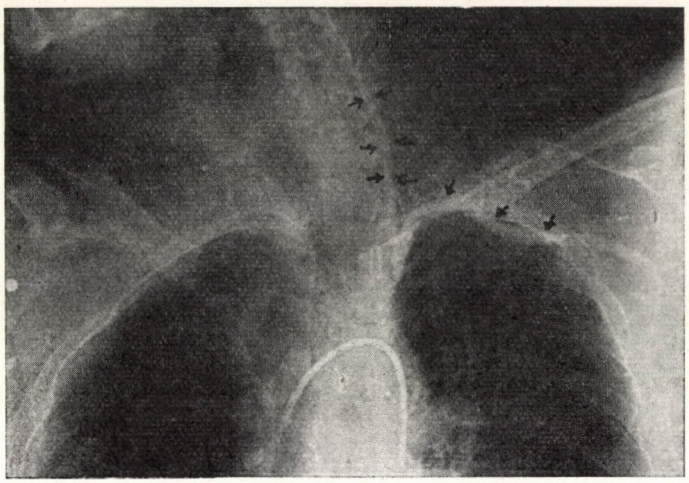

Fig. 4. Angiogram taken 2″ after introduction of contrast medium clearly shows the contrast medium reaching the left subclavian artery (↓) and through retrograde filling of the vertebral artery (↕). (Left subclavian steal syndrome.)

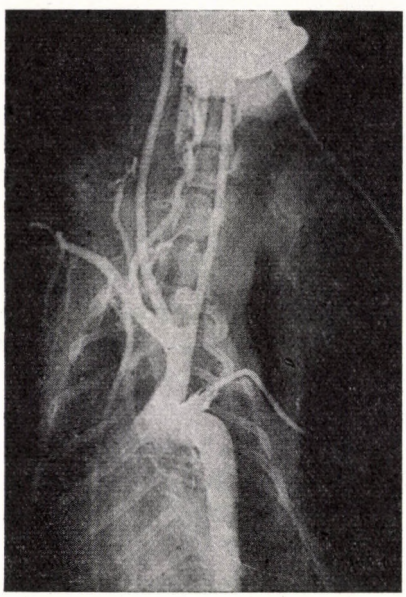

Fig. 5. Cerebral angiogram in dog after occlusion of the proximal segment of the left subclavian artery (artificial left subclavian steal syndrome). After the introduction of contrast medium, filling of the right subclavian and right vertebral arteries are clearly seen

Discussion

The subclavian steal syndrome is due to an occlusion of the proximal segment of the subclavian artery and the consecutive changes in cerebral blood flow [2, 3, 4, 5]. As judged from the present cases, none of the individual symptoms alone seems to be of decisive diagnostic significance. Recurrent spells of cerebral ischaemia comprizing dizziness, clouding of consciousness, blurring of vision were found, in accordance with data in the literature, to provide the most reliable diagnostic clues. The present findings have shown that blood supply to the upper extremity is provided essentially by a reversal of blood flow through the ipsilateral vertebral artery. After occlusion of the proximal segment of the subclavian artery, flow in the upper extremity was scarcely less than on the intact side, in agreement with the findings of CONRAD et al. [6].

The subclavian steal syndrome seems to fall into the following two main types. (i) Symptoms of cerebral ischaemia with fully preserved blood supply to the upper limb. This type, termed also "brachiobasilar insufficiency syndrome" presupposes a massive retrograde blood flow through the vertebral artery. (ii) Ischaemic symptoms of the upper limb similar in type to intermittent claudication, usually with no cerebral phenomena. In these cases cerebral blood supply is little affected because of scanty backward flow through the vertebral artery. On the ground of the symptoms and findings 7 of the present cases belonged to the first, and 4 to the second type. It is the morphology of the cranial vessels, namely of the carotid and vertebral systems, including the circle of Willis which determines the type. Subclavian stenosis combined with narrowing of the carotid or of the vertebral artery results in a great variety of clinical symptoms [7—11].

The subclavian steal syndrome greatly alters circulation in the brain and in the upper limb. After experimental acute or chronic occlusion of the subclavian artery, blood flow in the ipsilateral upper limb was scarcely less than that on the unaffected side. Blood supply to the subclavian segment distal to the occlusion is primarily provided by the ipsilateral vertebral artery. In fact, the backward flow through the vertebral artery of the affected side exceeded the anterograde vertebral flow of the unaffected side, as illustrated in Fig. 1. The present findings are in agreement with those of REIVICH et al. [12], SAMMARTINO and TOOLE [13] as well as those of HANDA et al. [14]. As expected, retrograde flow in the vertebral artery was greatly influenced by the common carotid flow (Fig. 1). In addition to the vertebral artery the thyrocervical trunk also takes part in the blood supply to the upper extremity. After prolonged occlusion of the subclavian artery, its distal portion received blood from the lower cervical artery too, moreover, clamping of the ipsilateral vertebral artery left the blood supply to the upper extremity unaffected (Fig. 2).

Our clinical observations suggest that in the type marked by cerebral

ischaemia even the basic conditions of cerebral circulation are inadequate. Cerebral blood flow was abnormally low and the cerebral fraction of cardiac output reduced. The response of cerebral flow to sudden changes in vascular resistance in the upper extremity was characteristic of the syndrome. In the first patient, an abrupt lowering of the vascular resistance in the upper limb resulted in a definite reduction of cerebral flow. In the second and third case, the sudden elevation of the vascular resistance in the upper extremity was followed by a significant increase in cerebral flow (Table II). It is assumed that vasoconstriction in the distal segment of the subclavian artery leads to a reduction in the pressure differences between the vertebral and the subclavian arteries, which results in an impairment of retrograde blood flow in the vertebral artery to the profit of cerebral circulation. The reverse occurs on vasodilatation in the distal portion of the subclavian artery.

REFERENCES

1. Solti, F., Krasznai, I., Iskum, M., Rév, J., Nagy, J.: Cor et Vasa **8,** 178 (1966).
2. Contorni, L.: Minerva chir. **15,** 268 (1960).
3. Baker, H. J. jr.: in 3rd Princeton Conference on Cerebral Disease 1961. Grune & Stratton, New York 1961.
4. Toole, J. F.: Discussion ibidem, Pp. 31—33.
5. Fields, W. S.: Discussion ibidem, P. 38.
6. Conrad, M. C., Toole, J. F., Janeway, R.: Circulation **32,** 346 (1965).
7. North, R. R., De Bakey, M. E., Fields, W. S., Crawford, E. S.: Neurology **12,** 810 (1962).
8. Heyman, A., Young, W. G. jr., Dillon, M., Gorree, J. A., Klein, L. J., Tindall, G.: Arch. Neurol. (Chic.) **10,** 581 (1964).
9. Nemir, P., Bahabozorgui, S., Wagner, D. E.: J. thorac. Surg. **50,** 534 (1965).
10. Killen, D. A., Gobbel, W. G.: J. thorac. Surg. **50,** 421 (1965).
11. Agee, O. F.: Am. J. Roentgenol. **97,** 447 (1966).
12. Reivich, M., Holling, E. H., Roberts, B., Toole, J. F.: New Engl. J. Med. **265,** 878 (1961).
13. Sammartino, W. F., Toole, J. F.: Arch. Neurol. (Chic.) **10,** 590 (1964).
14. Handa, J., Ishikawa, S., Huber, P., Stirling-Meyer, J.: Surgery **58,** 703 (1965).

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NOUVELLE INTERPRÉTATION PHYSIOPATHOLOGIQUE DES CARACTÉRISTIQUES DE L'HYPERMÉTROPIE «RELATIVE» PRODUISANT L'ÉSOTROPIE, I.

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Résumé

La nouvelle interprétation pathologique de l'hypermétropie «relative» est fondée sur sa corrélation étroite avec la force de fusion. A mesure que la fusion s'affaiblit, le domaine de l'hypermétropie «relative» causant le strabisme s'élargit, car la force de fusion est incapable de conserver la vision binoculaire contre l'impulsion de l'accommodation-convergence à distance.

La conception pathologique de l'hypermétropie «relative» explique aussi les cas exceptionnels et offre des possibilités nouvelles du traitement causal de l'ésotropie. La nouvelle méthode de traitement conservateur du strabisme nous permet d'envisager une réduction considérable du nombre des opérations en cas du strabisme.

Nous traitons la théorie physiopathologique de l'hypermétropie relative en cherchant l'explication étiologique du strabisme convergent et sa thérapeutique causale en nous fondant sur notre expérience.

La notion physiologiquement peu déterminable de l'hypermétropie relative est redevable à DONDERS, qui a démontré avec quelle fréquence elle détermine le strabisme convergent. Ainsi l'hypermétropie relative peut être mise en rapport causal avec un grand chapitre de la pathophysiologie de la vision, notamment avec l'amblyopie, la fixation excentrique, la correspondance anomale, les graves conséquences sensorielles du strabisme.

D'après les principes de DONDERS, du point de vue physiologique et fonctionnel l'hypermétropie peut être divisée en deux parties: l'hypermétropie peu accentuée est facultative car, par l'accommodation binoculaire à distance, le malade est capable de voir convenablement des deux yeux, alors que l'hypermétropie d'au-delà d'environ + 6.0 D est absolue, puisque le malade ne peut sans abandonner la vision binoculaire bien voir ni de l'un, ni de l'autre œil.

A l'aide de l'accommodation monoculaire d'une capacité presque redoublée l'enfant atteint d'hypermétropie absolue serait aussi capable de bien voir de l'un de ses yeux, mais s'en trouve empêché par la force de fusion d'intensité normale, car la convergence se produisant sur l'autre œil simultanément avec l'effort d'accommodation produirait une vision double insuppor-

table. L'emploi prolongé de l'accommodation monoculaire pour la vue à distance n'est possible qu'en cas pathologique, lors de la dégradation de la force de fusion réunissant l'image des deux yeux, le plus souvent en cas d'une hypermétropie moyenne (entre +4.0 D et +6.0 D). Selon DONDERS, une hypermétropie de ce degré est relative par rapport à la convergence. Si, pour une raison quelconque la force de fusion s'affaiblit, un moindre effort d'accommodation peut déjà exciter l'enfant à la convergence à distance (strabisme), et si cela se répète fréquemment, l'exclusion portant du système nerveux central supprime la vision simultanée sur l'œil plus amétrope et fait cesser les images doubles. Dans ce cas l'enfant peut garder l'acuité visuelle d'un œil avec une tension permanente de son accommodation, alors que des altérations pathologiques sensorielles se produisent sur l'autre œil.

Selon DONDERS le strabisme convergent est la conséquence de l'accomodation assurant l'acuité visuelle d'un œil contre l'hypermétropie relative.

La conception actuelle des ophtamologues sur le développement du strabisme convergent et sur son traitement causal est peu affectée par l'enseignement de DONDERS sur l'hypermétropie relative. La raison principale en est l'impossibilité d'expliquer des nombreux cas de l'ésotropie par l'hypermétropie «relative» dans les limites physiologiques indiquée par DONDERS et qu'une hypermétropie de ce degré, considérée comme relative, ne détermine pas de strabisme dans tous les cas. La théorie ne se laissa pas réconcilier avec les faits.

Au cours des derniers 4 ans nous avons tâché de coordonner la théorie de DONDERS avec nos observations. Nous avons examiné la possibilité de modifier la théorie et de nous en servir comme principe directeur dans le traitement des enfants atteints de strabisme. Nous nous proposons de compléter les méthodes conservatrices de traitement pour améliorer considérablement les résultats de cette thérapeutique.

Le domaine de l'hypermétropie relative est généralement placé entre + 4.0 D et + 8.0 D. Or, cette définition ne permet pas d'expliquer de nombreux cas d'ésotropie dont l'hypermétropie se trouve en dehors de ces limites, alors que, d'autre part, il y a beaucoup de cas où l'enfant ne louche pas tout en ayant une hypermétropie de ce degré.

Il est, selon KETTESY, un fait remarquable que la littérature ne définit pas les limites de l'hypermétropie relative, ce qui serait dû à la difficulté de la définition. Par estimation, KETTESY indique les limites de l'hypermétropie relative entre + 2.0 D et + 10.0 D. Certes, le domaine ainsi élargi de l'hypermétropie relative permet d'expliquer plusieurs cas de strabisme, mais pas tous. En même temps, le nombre de ceux qui ne louchent pas malgré leur hypermétropie dite «relative», se voit considérablement agrandi. Afin d'expliquer les exceptions il faudrait donc élargir encore davantage les limites de l'hypermétropie relative mais en même temps les réduire pour arriver à l'ex-

plication d'un autre groupe d'exceptions, ce qui paraît être une tâche impossible à réaliser.

Aucune des définitons n'est conforme à l'expérience pratique. Selon notre statistique, le strabisme convergent se voit entre + 0.5 D et + 11.0 D, avec un maximum de fréquence entre + 4.0 D et + 5.0 D. (Fig. 1)

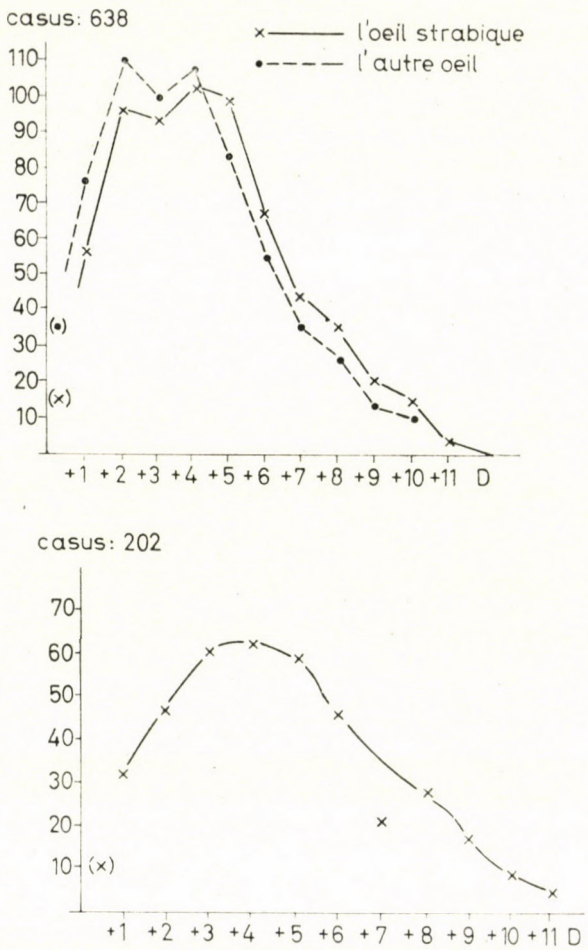

Fig. 1. Courbe de fréquence de l'hypermétropie sphérique de nos cas d'ésotropie. Les valeurs les plus fréquentes sont + 4.0 D et + 5.0 D, mais toutes les valeurs se rencontrent entre +0.5 D et + 11.0 D. Il n'y eut pas d'emmétropie. — L'interprétation physio-pathologique de l'hypermétropie relative explique tous les cas.

Alors, ou bien ce n'est pas vrai que ce n'est que le secteur «relatif» de l'hypermétropie qui cause du strabisme et que l'hypermétropie «relative» le cause toujours, ou bien la conception est fausse selon laquelle le domaine relatif

de l'hypermétropie se laisse limiter dans des cas pathologiques. C'est une solution rassurante de ce dernier problème que nous tâchons de trouver afin que les deux premières hypothèses soient valables dans tous les cas.

L'expérience que nous avons dans le traitement conservateur du strabisme (RÉTHY—GÁL) nous a incité à bien repasser dans l'esprit toutes les corrélations de l'hypermétropie «relative» avec les défauts de la vision binoculaire. Notre hypothèse de travail se fonde sur la conclusion que l'hypermétropie relative étant un concept pathologique, sa définition physiologique n'a pas été effectuée parce que cela n'est pas possible. Il fut prouvé en de nombreux cas qu'aucun degré de l'hypermétropie ne peut causer du strabisme si la force de fusion est durablement d'une intensité normale. Le rôle pathogène de l'hypermétropie ne deviendra manifeste que dans des circonstances que l'on peut appeler pathologiques: la faiblesse ou la dégradation de la force de fusion. L'hypermétropie «relative» ne peut donc être traitée que dans la sphère de la pathologie de la vision.

DONDERS fait également mention du rôle que les facteurs troublant la coopération des deux yeux (macula corneae, anisométropie) jouent dans la genèse du strabisme. La démonstration du rôle décisif joué par la force de fusion dans toute la question ayant rapport au strabisme se rattache au nom de WORTH. L'étroite corrélation des deux théories apparemment contradictoires deviendra manifeste si l'on sait que l'impulsion de l'accommodation est toujours simultanée avec celle de la convergence. Lors de la vision de près la coopération des deux yeux peut se faire comme suit: l'accommodation est suivie instantanément par la convergence. Lors de vision à distance, les conditions se trouvent changées. A cause de l'hypermétropie l'accommodation est nécessaire aussi pour la vision à distance. Dans ce cas l'impulsion nerveuse de l'accommodation et de la convergence est simultanée. Or, l'action de la convergence produirait la diplopie lors de la vision à distance. Dans ce cas, la force de fusion entrave l'action de la convergence qui se sépare de celle de l'accommodation. La force de fusion peut neutraliser une impulsion de convergence d'un degré équivalant à la force par laquelle elle concentre les deux yeux sur le même point. La capacité de la force de fusion est donc en relation directe avec la force de la convergence qu'elle peut entraver. Par «force de fusion» nous comprenons l'activité réflective conditionnelle cérébrale, en résultat de quoi les deux images vues par les deux yeux restent ensemble et fusionnent, malgré certaines différences entre les deux images en matière de grandeur, de forme et d'acuité.

La force de l'impulsion de la convergence peut être mesurée par l'accommodation opérée par une impulsion d'accommodation égale, mais non empêchée dans sa manifestation. L'accommodation imposée par l'hypermétropie est augmentée par l'application de verres concaves au signe négatif, tant que la convergence ne se produit pas. Cette donnée que l'on peut exprimer en dioptries

est la partie positive de l'ampleur relative d'accommodation qui dépend donc de la puissance de fusion.

Il n'y a d'ailleurs rien de nouveau dans cette constatation qui est décrite dans des manuels (CORDS, 1930; KETTESY, 1962). Ce ne sont que les conséquences que nous avons manqué d'en tirer. Car, si la force de fusion est absente dans des cas pathologiques et n'empêche pas l'apparition de la convergence, il s'ensuit que la corrélation étroite de la convergence et de l'accommodation persiste aussi dans la vision à distance (Fig. 2). Dans ce cas il suffit de la moindre impulsion d'accommodation à distance pour produire la convergence à distance, soit le strabisme convergent. Ainsi l'impulsion d'accommodation à

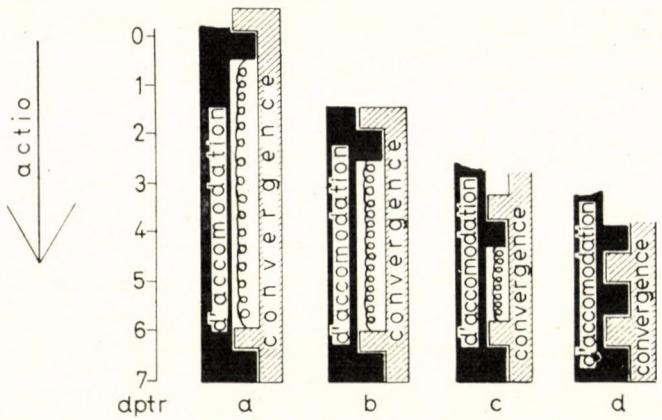

Fig. 2. Le domaine de l'accommodation relative est proportionnel en raison directe avec la capacité de la fusion. Si l'impulsion de la convergence accommodative à distance dépasse la force de fusion, l'ésotropie (convergence à distance) se produit. Si la force de fusion est supprimée, la moindre impulsion d'accommodation peut faire persister l'ésotropie. Fusion: a = normal, b = subnormal, c = HYP, d = sine fusione

distance causée par une hypermétropie non corrigée de + 0.5 D peut déjà maintenir le strabisme.

Pour cette raison nous lions la définition pathologique de l'hypermétropie «relative» à l'apparition de la convergence à distance, sans tenir compte du nombre des dioptries de l'hypermétropie.

L'hypermétropie est «relative» si l'impulsion d'accommodation qu'elle impose déclenche en même temps la convergence à distance. En prêtant à cette thèse une validité générale, nous arrivons à un résultat que l'on peut qualifier de nouveau et surprenant:

Il y a lieu d'élargir et en même temps de réduire les limites de l'hypermétropie «relative» si le domaine de cette dernière est représenté en fonction de la force de fusion (Fig. 3). La figure représente l'image de notre thèse: dans des conditions physiologiques et avec une force de fusion normale aucun degré d'hypermétropie ne peut causer un strabisme convergent. En nous fondant sur l'interprétation physio-pathologique il nous est possible d'expliquer tous les

cas qui restent incompréhensibles pour la conception physiologique de la théorie de DONDERS. Les exceptions rentrent aussi dans les règles. La figure est semi-quantitative puisque les méthodes actuelles d'enregistrement de la force de fusion ne sont pas satisfaisantes ni bien comparables. Il ne nous paraissait pas utile de comparer une certaine qualité de la fusion avec une autre, notamment, en cas de déflexion par prismes, le synchronisme des images des deux yeux jusqu'à une certaine limite avec la résistance contre l'impulsion de la convergence accommodative. N'ayant pas encore une méthode se prêtant à la mesure directe de la force de fusion, il nous fut impossible d'en représenter les unités.

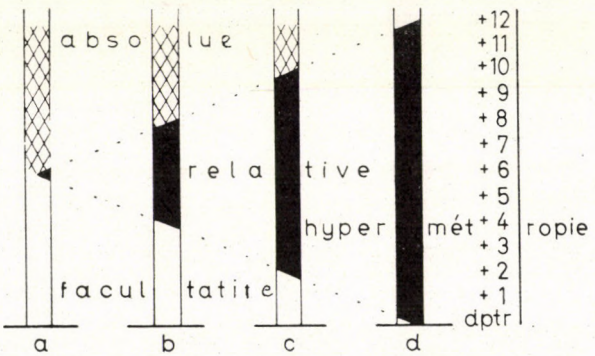

Fig. 3. Le domaine de l'hypermétropie «relative» est inversement proportionnel à la capacité de la fusion. Avec l'affaiblissement de la capacité de fusion apparaît l'hypermétropie relative qui prouve son caractère pathologique. Avec la cessation de la fusion, tout degré d'hypermétropie peut faire persister le strabisme. Dans ce cas les domaines inoffensifs: facultatif et absolu de l'hypermétropie manquent, et seul le domaine dangereux («relatif») est présent.

Sur cette figure, la force de fusion n'est pas exprimée en unités quantitatives. Pour le moment nous ne disposons pas encore d'une unité de mesure qui pourrait exprimer la grandeur, la luminosité et le contraste de l'objet employé pour la fixation, ainsi que sa distance de la personne examinée. Fusion: a = normal, b = subnormal, c = hypo, d = sine fusione

La figure nous fait comprendre que si la capacité de fusion est forte, même une hypermétropie de + 6.0 D ne peut pas déterminer un strabisme, puisque le domaine de l'hypermétropie relative dangereuse fut réduit.

Il ressort également de la figure, la raison pour laquelle une hypermétropie de + 0.5 D peut déjà maintenir un strabisme convergent si la capacité de fusion manque et le domaine dangereux de l'hypermétropie relative est élargi. En nous fondant sur notre théorie modifiée, nous pouvons en tirer des conséquences pratiques de haute importance. Supposons que la fusion est très faible ou manque tout à fait; le domaine de l'hypermétropie relative étend — contrairement à la conception de DONDERS — aussi sur le domaine facultatif inoffensif. Dans ce cas c'est le principe de la correction optique complète qu'il convient d'appliquer d'après notre expérience ophtalmologique dans le traitement conservateur de l'ésotropie.

Il est important de faire une distinction quantitative entre le degré de l'hypermétropie qui cause le strabisme et celui qui le maintient. Le premier dépassera toujours le dernier, car c'est bien l'affaiblissement de la force de fusion qui cause l'apparition du strabisme, mais la fusion est toujours plus forte avant l'apparition du strabisme ou à son début que pendant sa persistance et sa fixation. En effet, le strabisme diminue considérablement la capacité de fusion, voire même la supprime. Ainsi, le maintien du strabisme convergent demande une accommodation produite par une hypermétropie inférieure à celle qui fut nécessaire à sa genèse lorsque la capacité de fusion ne fut pas encore complè-

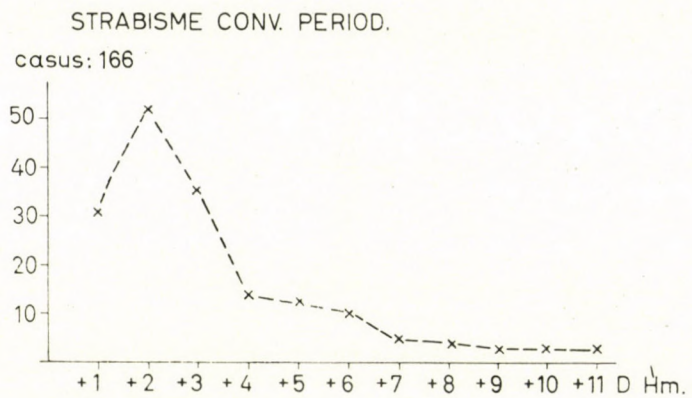

Fig. 4. En cas de strabisme intermittent l'hypermétropie de + 2.0 D est la plus fréquente, à l'opposé des valeurs plus élevées observées en cas de strabisme permanent. En cas d'hypermétropie supérieure à + 2.0 D l'ésotropie ne reste que rarement intermittente. Strabisme conv. period.

tement supprimée. Les lunettes ordonnées pour supprimer le strabisme ne doivent donc pas seulement supprimer le stimulus de l'impulsion accommodation-convergence suffisante pour déclencher le strabisme; mais elles doivent aussi décharger lors de la vision à distance l'impulsion d'accommodation de + 0.5 D, qui fait persister ou fixe éventuellement le strabisme.

Afin que les verres soient plus agréables à porter au début, il n'est pas admissible de faire une concession ne fût-ce que d'une demi-dioptrie au détriment de la correction parfaite à réaliser par les lunettes, comme c'est recommandé encore aujourd'hui à l'étranger. Voir à cet effet les recommandations officielles du cercle de travail «strabisme et amblyopie» publiés en 1965 à Bonn et à Leipzig sur les principes du traitement «moderne» du strabisme. La sous-correction assure la persistance du strabisme ! L'enfant louche ne demande pas mieux que de conserver une situation familière et ne porte donc volontiers au début que des lunettes qui n'assurent qu'une sous-correction. L'ophtalmologiste ne doit guère encourager ces efforts. Une autre publication (RÉTHY—GÁL 1965) décrit une méthode sûre d'habituer l'enfant à porter le verre

correcteur moyennant l'administration prolongée d'atropine. Toutefois, il n'est pas toujours possible de supprimer vite le strabisme de cette manière. Lever les réflexes conditionnés déjà stabilisés qui règlent le tonus de l'accommodation—convergence, et les adapter à la situation optique changée prend généralement un temps assez long.

On peut observer l'élargissement du domaine de l'hypermétropie relative lorsque la fusion est passagèrement détériorée pour une raison quelconque, p. ex. une maladie fébrile (rougeole, grippe). Dans ce cas le strabisme peut déjà se manifester en présence d'une amétropie peu importante. Si la fusion est conservée jusqu'à un certain degré, le strabisme reste intermittent. En appliquant des verres concaves qui augmentent l'impulsion de l'accommodation et par conséquent aussi celle de la convergence, nous avons pu dans ces cas provoquer, augmenter et stabiliser le strabisme pour un temps voulu, comme il ressort de nos prises de vue. Pour cette même raison, le strabisme provoqué par une hypermétropie supérieure à + 2.0 D reste rarement intermittent car, par suite des périodes de strabisme plus intenses et prolongées, la fusion se détériore rapidement et le strabisme peut se stabiliser. Dans les cas de strabisme intermittent observés dans notre pratique, nos statistiques démontrent la plus grande fréquence de l'hypermétropie sphérique de + 2.0 D, à l'opposé des valeurs de + 4.0 et + 5.0 D observées en cas de strabisme permanent.

Selon la publication de DARABOS, la cessation de la force de fusion, par suite de la dégradation de la vue d'un œil à cause d'un accident, a provoqué en quelques mois un strabisme, alors que l'autre œil fut atteint par une hypermétropie peu importante (+ 2.5 D en moyenne). Voici donc encore une expérience qui vérifie notre point de vue selon lequel l'hypermétropie, facultative et inoffensive au début, prend, par suite de la cessation de la fusion, un caractère «relatif» et dangereux causant l'apparition du strabisme. Il est encore intéressant à noter que le développement du strabisme a pris dans tous les cas quelques mois et que la correction complète de l'hypermétropie n'a eu comme résultat instantané qu'une diminution de l'angle strabique. Selon notre expérience, tout comme son développement, la régression complète du strabisme prend également quelques mois ou même plus, si la correction optique est portée constamment. Nous ne pouvons que regretter que l'intéressant travail de DARABOS ne donne pas de renseignements à ce sujet.

En acceptant la définition dynamique et pathologique de l'hypermétropie relative, notre hypothèse selon laquelle l'hypermétropie relative cause dans tous les cas un strabisme convergent se trouve vérifiée. La base du traitement causal de l'ésotropie réside dans la neutralisation des impulsions réflexes de l'accommodation et de garantir à la fusion la possibilité de s'évoluer en premier par une correction optique.

En complétant la théorie de DONDERS par la prise en considération des effets provoqués par les changements de la force de fusion, les nombreux cas

cliniques, inexpliqués et apparemment inexplicables jusqu'à présent, deviennent en vertu de l'interprétation pathologique des cas compréhensibles.

Dans la pratique, aviver la discussion sur le caractère mono- ou polyétiologique du strabisme est dépourvue de sens et mène à des malantendus. En effet, les différents facteurs responsables du strabisme agissent l'un sur l'autre et conjointement. En complétant la théorie de DONDERS il y a lieu de démontrer des corrélations plus profondes. Notre hypothèse se trouve vérifiée: même en cas de myopie, la cause de l'ésotropie est toujours l'impulsion d'accommodation qui provoque la convergence, action que la détérioration de la force de fusion fait prévaloir. Simultanément avec l'accommodation à distance apparaît la convergence à distance (limitant la possibilité de la vision binoculaire).

Toutefois la cause commune provoquant ceci et provenant de plusieurs facteurs ne pourrait être discutée que conjointement avec le caractère de l'accommodation dans tous les cas d'hypermétropie «relative», ce qui dépasserait les cadres de ce travail.

BIBLIOGRAPHIE

CORDS, R.: Die Pathologie der Augenbewegungen. Kurzes Hdbuch der Ophth. Springer, Berlin 1930.

DARABOS, G.: Zur Ätiologie des posttraumatischen Einwärtsschielens. Klin. Mbl. Augenhk. **148,** 351—355 (1966).

DONDERS, F. C.: Die Anomalien der Refraction und Accommodation des Auges. Wien, 1866.

KETTESY, A.: Az összetérő kísérő kancsalság keletkezési módjáról és gyógyításáról. Orv. Hetilap. **100,** 705—712 (1959).

BOROS—KETTESY—KUKÁN: Szemészet. Medicina, Budapest. 1962. p. 351.

RÉTHY, I., GÁL, ZS.: Ergebnisse der konservativen Schielbehandlung durch Überkorrektur der manifesten Hypermetropie. Klin. Mbl. Augenhk. **150,** 170—180 (1967).

WORTH, C.: Squint, London, 1903.

Dr. István RÉTHY Budapest III., Árpád fejedelem u. 49, Hongrie

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AVASCULAR SWELLING OF THE CORNEA

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The presence of vitreous in the anterior chamber with the attendant corneal oedema characteristic of bullous keratopathy has been reproduced in the rabbit eye by the introduction of silicon oil into the anterior chamber. If the corneal oedema thus induced was accompanied by vascularization, this was confined to the limbic area. On the other hand, vascularization was never found in those regions which had been in contact with the oil and where, consequently, swelling was the most intensive. This supports the claim that loosening of the corneal structure is only one of the prerequisites, but not the cause, of vascularization. Avascular oedema of the cornea resulting from substitution of aqueous humour by another medium was found to be accompanied by a significant reduction in the lactic acid content of the cornea. This emphasizes the significance of lactic acid as a vasoproliferative factor and connects the pathomechanism of bullous keratopathy with a poor glucose supply of the cornea.

ASHTON [1] believes that corneal swelling must reach the limbic area to allow ingrowth of new vessels by the agency of the vasoproliferative factor present in the cornea. In earlier studies of the present authors [4, 5] this factor has been identified as lactic acid. The corneal concentration of this substance is high even under normal conditions [6, 7], still more so in case of corneal swelling and vascularization [9]. Nevertheless, swelling of the cornea does not necessarily implicate its vascularization. For instance corneal dystrophy or bullous keratopathy occurring in aphakic eyes, are avascular, though being associated with persistent corneal swelling of considerable intensity.

The aim of the present study was to clarify the causes of avascular swelling of the cornea.

Material and methods

Twelve rabbits weighing between 2.5 and 3.0 kg were used. Under local anaesthesia a small incision into the anterior chamber was made through the upper segment of the limbus of the right eye and two threads were passed. The aqueous humour was left to drain off, then the anterior chamber was filled through the wound with silicon oil of 350 cS viscosity (SISS dimethyl oil, Daw-Corning 200) and the sutures were fastened.

The animals were observed daily and extension of the corneal oedema and appearance of marginal vascularization were registered.

Six animals were killed on the 14th day of the study. Immediately after death circular disks were cut out from the central parts of both corneas with a 8 mm trephine, beginning with the right eye in three animals and with the left in the other three. The wet fragments were placed in a 8% solution of trichloracetic acid of known weight so as to prevent further metabolism, weighed, washed in physiological saline, and placed in a desiccator at room temperature. The residue was weighed and the lactic acid concentration of each specimen was determined by the method of BARKER and SUMMERSON [2].

The arithmetic mean and the standard deviation (SD) of the lactic acid concentration in the test and control fragments were calculated and the results were evaluated by STUDENT's *t*-test.

In two clinical cases of bullous keratopathy associated with aphakia, perforating keratoplasty was carried out using a 8 mm trephine. The cornea was avascular and the anterior chamber was occupied by the vitreous body in both cases. The lactic acid content of the corneal fragments removed in the course of the intervention was estimated by the same method as in the animal tissues.

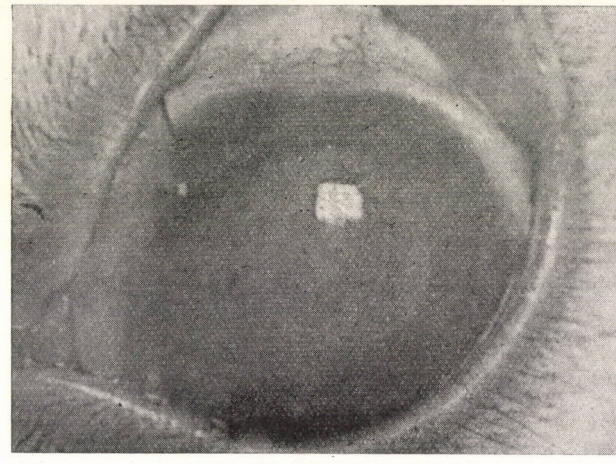

Fig. 1. Rabbit cornea six weeks after introduction of silicon oil into the anterior chamber. The corneal disk being in contact with the oil is cloudy and exhibits marginal scars

Results

At the start of the study the silicon oil seemed to fill the anterior chamber, but by the second day it had collected in the central area as a drop 8 or 9 mm in diameter touching the cornea nearly over its entire surface. By the second or third day the cornea had become cloudy. In half of the cases swelling had spread over the whole cornea involving the limbus too or at least $^2/_3$ of its circumference. From the twelfth day onward the cornea began to clear, but the central area adjoining the accumulated silicon oil remained cloudy and at its edges deep linear scars were left by the end of six weeks (Fig. 1).

In 9 out of 12 corneas a superficial ingrowth of new vessels made its appearance between the third and seventh day, reaching its maximum, i.e. 1.0 ± 0.64 (SD)mm, between the eighth and eleventh day, to diminish subsequently. It never involved the area of maximal swelling contiguous to the silicon oil. On the fourteenth day, a distinct difference was demonstrable in the wet weight of the six sections cut out from the oedematous and those from the control corneas. The mean dry to wet weight ratio of the oedematous corneas was 0.20 ± 0.01 (SD), that of the controls 0.25 ± 0.004 (SD).

The figures relating to corneal lactic acid content are presented in Table I, those derived from human tissue, in Table II.

Table I

Lactic acid content of rabbit corneas
Arithmetic mean ± standard deviation

Lactic acid	Test	Control	
Dry weight μg/mg	1.78±0.15 (6)	2.97±0.90 (6)	$p < 0.02$
Wet weight μg/mg	0.36±0.05 (6)	0.74±0.28 (6)	$p < 0.01$

Table II

Lactic acid content of two human corneas with bullous keratopathy

Wet weight	Dry weight	Dry per wet weight ratio	Lactic acid	
			μg/mg Wet weight	μg/mg Dry weight
—	8.5	—	—	1.8
59.5	8.1	0.13	0.21	1.6

Discussion

Bullous keratopathy, an avascular oedema of the cornea, is prevalent after surgery for cataract if the vitreous intrudes into the anterior chamber in an amount sufficient to form contact with the cornea over a large area. The factor responsible for corneal swelling is supposedly the endothelial damage due to the extensive contact between vitreous and cornea. Corneal swelling can doubtlessly be caused by endothelial damage alone, still more so if there is some additional factor.

The glucose required by the cornea for the maintenance of its metabolism in the dehydrated state is provided by the aqueous humour. According to DAVSON and DUKE-ELDER [3], the vitreous contains less glucose than the aqueous humour, in all likelihood partly as a consequence of its relatively low permeability for glucose. The additional factor of corneal swelling in this case is presumably an inadequate supply of glucose. This is in agreement with the observation that when separation of the cornea from the vitreous was maintained by means of air insufflation or of posterior sclerotomy, corneal oedema eventually ceased [8, 11, 12].

In the present study the presence of vitreous in the anterior chamber was imitated by the introduction of silicon oil into this chamber and this indeed very soon resulted in corneal oedema associated with a significant reduction in lactic acid concentration both in relation to dry and to wet weight ($p < 0.02$; $p < 0.01$) amounting to not more than 59.9 and 48.6% of the respective normal values. In the corneas obtained from the two cases of bullous keratopathy the

lactic acid content was likewise markedly reduced. It emerges from the present study that prolonged deprivation of the cornea of its contact with the aqueous humour will inevitably affect corneal glycolysis, probably in consequence of an inadequate glucose supply. This is what probably happens in bullous keratopathy and also accounts for later endothelial damage even if the endothelium has been unaffected.

It is probably because of a similar nutritional disturbance that the cornea becomes dull in the presence of extensive precipitates. In bulbar atrophy too there is swelling but no vascularization of the otherwise intact cornea. Here the inadequate nutrition of the cornea may be connected with the arrest of secretion of aqueous humour. This is well illustrated by the case of a 78-year-old male patient who was admitted to our department because of complete bilateral occlusion of the ophthalmic arteries, resulting in immediate loss of light sensation. Production of aqueous humour completely ceased. The corneas were swollen to twice their normal thickness, nevertheless neovascularization was absent.

In agreement with the observations of MAURICE, ZAUBERMAN and MICHAELSON [10] the present results suggest that loosening of the corneal tissue, though being one of the prerequisites of its neovascularization, is by no means its cause. If the corneal swelling induced in our animals was associated with neovascularization, this was invariably transient and confined to the marginal areas, never involving the sites of maximal swelling contiguous to the silicon oil. The finding of low lactic acid concentrations in these very areas support the claim that the vasoproliferative factor is identical with lactic acid and explains why swelling of the cornea need not be associated with its vascularization.

Further studies will have to clarify whether in corneal dystrophy associated with avascular swelling the lactic acid content is likewise low or whether in these cases swelling of the cornea does not involve a loosening of its tissue.

REFERENCES

1. ASHTON, N.: Corneal vascularization: In: The Transparency of the Cornea. Symposium. Ed.: DUKE-ELDER, S., PERKINS, E. S., Thomas, Springfield 1960. P. 131.
2. BARKER, S. B., SUMMERSON, W. H.: J. biol. Chem. **138,** 535 (1941).
3. DAVSON, H., DUKE-ELDER, S.: J. Physiol. (Lond.) **107,** 141 (1948).
4. IMRE, GY.: Brit. J. Ophthal. **48,** 75 (1964).
5. IMRE, GY.: Acta morph. Acad. Sci. hung. **14,** 99 (1966).
6. KUHLMANN, R. E., RESNIK, R. A.: Arch. Biochem. **85,** 29 (1959).
7. LANGHAM, M. E.: J. Physiol. (Lond.) **117,** 461 (1952).
8. LEAHEY, B. D.: Arch. Ophthal. (Chic.) **46,** 22 (1951).
9. LEVENE, R., SHAPIRO, A., BAUM, J.: Arch. Ophthal. (Chic.) **70,** 242 (1963).
10. MAURICE, D. M., ZAUBERMAN, H., MICHAELSON, I. C.: Exp. Eye Res. **5,** 168 (1966).
11. OTRADOVEC, J., ZICHA, J.: Čsl. Oftal. **16,** 352 (1960).
12. REESE, A. B.: Trans. Amer. ophthal. Soc. **46,** 73 (1948).

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THE CONJUGATING MECHANISM IN A CASE OF ETIOCHOLANOLONE FEVER

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A female patient had intermittent fever and elevated plasma unconjugated etiocholanolone level. The 17-oxosteroid sulphates in plasma and urine and the glucuronosides in urine have been determined under basal conditions and after the administration of corticotrophin, metopirone and testosterone propionate. The finding revealed that the patient failed to produce 5β steroids preferentially and had no defect of glucuronoside and sulphate conjugation.

It has been shown by KAPPAS et al. [1] that various 5β steroids have an intense pyrogenic activity after intramuscular injection to humans BONDY et al. [2] observed patients with periodic febrile episodes and found an elevated free etiocholanolone level in plasma. When the fever subsided the amount of the steroid decreased to a normal value.

Several abnormalities of steroid metabolism have been suggested to cause fever in patients presenting the above syndrome. Some observations suggested a disorder of the conjugating mechanism, other findings revealed a normal course of the esterification of steroid metabolites [2].

Since few cases of etiocholanolone fever have been reported and almost every case had an unique aetiology, a study has been performed of the conjugating mechanism in a patient with proved etiocholanolone fever. In the course of the study, the plasma level and the excretion of steroid conjugates have been determined under basal conditions as well as after administration of corticotrophin, metopirone and testosterone. Results obtained in this respect will be described below.

Case record

Patient BM, a 18 year-old female, was admitted in July, 1965, with complaints of anorexia, malaise, myalgia, headache, abdominal pain, nausea, vomiting and subfebrility; these symptoms had appeared two years earlier. On numerous occasions she had had fever lasting for weeks. Puberty was uneventful, the patient began to menstruate at 14 years of age. There was no correlation between the menstrual cycle and the complaints.

Stature and body weight below normal (145 cm and 40 kg). Acne vulgaris and slight exophthalmus were observed. Pulse was 108, blood pressure ranged from 140/90 to 200/110. The urine was normal. RBC was 4.2 million, WBC 7200, sedimentation rate 55 during fever. Haemoglobin was 15 g per 100 ml, serum cholesterol 204 mg per 100 ml. Serum protein electrophoresis: albumin, 51.8; alpha-1 globulin, 31.1; alpha-2, 12.0; beta, 14.0; gamma, 19.1 per 100 ml.

Serum electrolytes were within normal limits. Serum protein-bound iodine was 6.7 μg per 100 ml. Glucose tolerance test: 110, 140, 170 and 110 mg per 100 ml. Gynaecological examination revealed underdeveloped external genitalia and uterus. Body hair distribution was normal. Vaginal cytology showed a low oestrogenic and progesterone effect. Immunological and haematological examinations revealed normal values, parasitological examination was negative. All therapies except corticosteroids failed to decrease fever.

Determination of 17-oxosteroids in plasma and urine. Unconjugated 17-oxosteroids in plasma were determined by our own method [3]. In normal individuals no free 17-oxosteroids were isolated by this technique. The level of *conjugated* 17-oxosteroids in plasma was estimated by a similar method following hydrolysis of conjugates, purification of steroids and subsequent thin-layer and paper chromatographic separation [4]. Several authors assume that steroid conjugates determined in this way represent sulphate esters of the human plasma [5].

Excretion of glucuronosides and sulphates of 17-oxosteroids was determined according to SCHENKER et al. [6]. The urinary glucuronosides were subjected to hydrolysis by β-glucuronidase (REANAL, Budapest) and the steroids extracted with ether. The residue of urine was thereafter solvolysed according to BURSTEIN and LIEBERMAN [7]. After cleaving the sulphate esters, the steroids were repeatedly extracted and the extracts evaporated *in vacuo*. Aliquots of the extracts were chromatographed separately on Whatman No 1 paper and the zones of chromatogram evaluated as outlined by FEHÉR [8].

It has been shown [9, 10] that commercial preparations of β-glucuronidase, as also the one employed in the present study, have a considerable sulphatase activity. Consequently, values for "steroid sulphates" appear to be lower than those measured by procedures utilizing first solvolysis and thereafter enzymatic hydrolysis.

For the control of the employed method, we refer to previous papers [3, 4, 8]. Values for plasma were calculated by using authentic standards, those for urine were expressed in androsterone equivalents.

Functional tests. In order to study the patient's conjugating system, various functional tests were performed. The patient and control subjects received *1.* 2 × 80 U ACTH (Exacthin, RICHTER, Budapest) intramuscularly, *2.* 6 × 0.5 g metopirone (CIBA, Basel) orally, for raising the endogenous production of precursors [10]; finally, *3.* testosterone-propionate (Androfort, RICHTER) was injected intramuscularly in doses of 30 mg daily for 5 consecutive days, and the metabolism of exogenous precursors was studied [11]. Blood and urine were examined on the last day of treatment.

Results

17-oxosteroids in plasma

Determination of unconjugated 17-oxosteroids in plasma of the patient during two subsequent episodes of fever (body temperature 37.9° C and 37.6° C) revealed an elevated free etiocholanolone level (11 and 4 μg per 100 ml, respectively). Unconjugated dehydroepiandrosterone and androsterone were present at similar levels. Since the physiological range for all unconjugated 17-oxosteroids is below 1.5 μg per 100 ml, under basal conditions [12, 13] thus undetectable by the applied method, the level of free etiocholanolone in plasma of the patient had a diagnostic value.

Table I contains the values of individual 17-oxosteroid sulphates in plasma of healthy female subjects and patient BM during two episodes of fever. In addition to the increased amount of unconjugated steroids, the levels of dehydroepiandrosterone and androsterone sulphate were at the lower, and etiocholanolone sulphate at the upper, limit of the physiological range, consequently the 5β/5α ratio of conjugated steroids was somewhat increased; the increase did not reach statistical significance.

Table I

17-oxosteroid sulphates in plasma of normal female subjects and of patient BM with etiocholanolone fever

Age (years)	17-oxosteroid sulphates μg/100 ml			
	DEA	E	A	5β/5α
Normal female subjects				
16	33	4	32	0.1
20	42	10	25	0.4
23	53	6	21	0.3
24	29	9	20	0.5
Range:	29—53	4—10	20—35	0.1—0.5
Patient BM				
18	10	8	22	0.4
	23	12	12	1.0

DEA = dehydroepiandrosterone
E = etiocholanolone
A = androsterone

17-oxosteroids in urine

In Table II are shown the urinary values of individual 17-oxosteroid conjugates in control subjects and patient BM during fever. Values of ACTH, metopirone or testosterone treatment indicate increase in mg/24h related to the resting level.

As can be seen from Table II, the excretion of glucuronoside and sulphate conjugates of 17-oxosteroids by patient BM during fever and without any treatment was practically normal. When the conjugating system was loaded by stimulating the production of precursors with ACTH or with exogenous testosterone, the patient's conjugating ability to produce glucuronosides or to form sulphates was similar as in the control subjects. There was a response below normal following metopirone stimulation of patient BM, but this seemed to be pituitary in origin and not due to a disturbance of conjugation, since the response after exogenous ACTH revealed a normal urinary steroid pattern.

Discussion

The elevated plasma level of unconjugated etiocholanolone in patients with "etiocholanolone fever" or "steroid fever" has been ascribed to several aberrations of steroid metabolism [2]. One of the suppositions was that there is a tendency for the preferential production of 5β-steroids; a hypothesis not supported by the present study, since the 5β/5α ratio of 17-oxosteroids was not

Table II

17-oxosteroid glucuronosides and sulphates in urine of normal female subjects and of patient BM with etiocholanolone fever under basal conditions and following administration of ACTH, metopirone and testosterone-propionate

Treatment	Mode of conjugation	DEA	HOE+OE+E	HOA+A	5β/5α
Normal female subjects					
None (5)	G	0.1*	2.6	1.7	1.5
	S	0.4	0.2	0.2	1.0
ACTH (2)	G	+0.2**	+3.6	+1.9	+0.2
	S	+0.4	+0.4	+0.2	+0.5
Metopirone (2)	G	+0.1	+4.8	+1.4	+0.9
	S	+0.1	+0.1	+0.1	0
Testosterone (2)	G	0	+3.5	+2.6	−0.1
	S	0	+0.1	+0.2	−0.2
Patient BM					
None	G	0.2	2.7	2.9	0.9
	S	0	0.1	0.1	1.0
ACTH	G	+0.8*	+3.3	+1.7	+0.4
	S	+2.0	+0.4	+0.4	0
Metopirone	G	+0.2	+1.0	0	+0.4
	S	+0.9	+0.4	+0.4	0
Testosterone	G	0	+2.5	+2.5	0
	S	0	0	+0.1	−0.5

DEA + dehydroepiandrosterone
HOE + OE + E 11-OH-etiocholanolone + 11-keto-etiocholanolone + etiocholanolone
HOA + A 11-OH-androsterone + androsterone
Values in parenthesis represent the number of cases
* mean basal, mg/24h
** increase related to basal value, mg/24h
G glucuronoside
S sulphate

elevated even after exogenous testosterone, a precursor of etiocholanolone, or following ACTH stimulation. The supposition that the patients have an exaggerated tendency to form pyrogenic steroids could not be confirmed, either.

A defect in the conjugating mechanism to produce an elevation of the free etiocholanolone level in blood may be another suggestion. The normal rate of conjugation in the present case as well as in the case of SCHENKER et al. [6] indicates that patients with etiocholanolone fever do not have any detectable defect of glucuronoside and sulphate conjugation of steroid metabolites.

*

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REFERENCES

1. KAPPAS, A., SOYBEL, W., FUKUSHIMA, D. K., GALLAGHER, T. F.: Trans. Ass. Amer. Phycns. **72,** 54 (1959).
2. BONDY, P. K., COHN, G. L., GREGORY, P. B.: Medicine **44,** 249 (1965).
3. FEHÉR, T., HALMY, L.: Europ. J. Steroids, in press
4. FEHÉR, T., KOREF, O., KAZIK, H. M.: Acta endocr. (Kbh.) **51,** 429 (1966).
5. CONRAD, S., MAHESH, V., HERRMANN, W.: J. clin. Invest. **40,** 947 (1961).
6. SCHENKER, S., WILSON, H., SPICKARD, A.: J. clin. Endocr. **23,** 95 (1963).
7. BURSTEIN, S., LIEBERMAN, S.: J. biol. Chem. **233,** 331 (1958).
8. FEHÉR, T.: Clin. chim. Acta **14,** 83 (1966).
9. BAULIEU, E. E., MICHAUD, G., CORPÉCHOT, C.: Ann. Biol. clin. **19,** 291 (1961).
10. FEHÉR, T., KOREF, O.: Proc. VIth Ann. Meet. Biochem. Budapest 1964. P. 25.
11. HOLLÓ, I.: Acta med. Acad. Sci. hung. **20,** 237 (1964).
12. COHN, G. L., BONDY, P. K., CASTIGLIONE, C.: J. clin. Invest. **40,** 400 (1961).
13. TREIBER, L., RINDT, W., OERTEL, G. W.: Z. klin. Chem. Biochem. **5,** 102 (1967).

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IN VITRO CORTICOSTEROID FORMATION BY A BENIGN PHAEOCHROMOCYTOMA

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Formation *in vitro* of 11-deoxycorticosterone, corticosterone, 11-deoxycortisol and cortisol from ^{14}C-progesterone was shown by a benign adrenal phaeochromocytoma. Although in the tumour tissue adrenocortical cells were not detected histologically, the possibility that steroids were synthesized by such cells could not be excluded.

Steroid hydroxylation *in vitro* by phaeochromocytoma tissue was reported by Mulrow et al. (1959), and Beck (1959). Similar results have been described in the papers of Carballeira and Venning (1964), and Acevedo and Beering (1965).

In a recent work Fazekas et al. (1967) reported on corticosteroid biosynthesis and catecholamine formation *in vitro* by an adrenal phaeochromocytoma containing adrenocortical cells.

In the present study we have investigated the corticosteroid formation from 4-^{14}C-progesterone by a benign phaeochromocytoma *in vitro*.

Materials and methods

Progesterone-4-^{14}C (Radiochemical Centre, Amersham) was purified by chromatography, and made up in stock solution (specific activity: 4.29 μC/μM).

The phaeochromocytoma was removed from the left adrenal of a 27-year old male patient with paroxysmal hypertension who at admission complained of headaches, dizziness and cardiac pains. While he was at our department, blood pressure varied from 130/90 to 180/120 mm Hg and, during hypertensive episodes to 250/180 mm Hg, sometimes 300 mm Hg. Blood glucose was usually normal, but rose during the hypertensive episodes to 147—199 mg per 100 ml. Hypertensive episodes could be provoked by injections of histamine. Blood pressure decreased on the effect of sympatholytic drugs. Urinary catecholamines amounted to 1000 μg/24 hrs; urinary VMA to 21—35 mg/24 hrs.

At surgery a 12.6 g encapsulated tumour was removed from the left adrenal. Part of the tumour was used for histological examination; its morphological features were those of a benign, typical phaeochromocytoma (Fig. 1).

The tissue sample used for the biochemical investigation was removed from the inner part of the tumour. Histologically, this part of the tumour was a typical phaeochromocytoma; adrenocortical cells could not definitely be recognized in it. However, in such tumours it is difficult to differentiate between cortical and medullary cells (Walters et al., 1962).

Immediately after removal, 5 g tissue from the tumour was homogenized in 25 ml ice-cold Krebs—Ringer-phosphate solution (pH 7.4) containing 200 mg per 100 ml of glucose.

Incubation was carried out in a 100 ml Erlenmayer flask at 37° C for 3 hours. Progesterone-4-^{14}C (7, 520, 800 dpm, 250 μg) was added to the incubate dissolved in 0.1 ml propylene glycol.

After incubation, 300 μg each of non-radioactive cortisol, corticosterone, 11-deoxycortisol and 11-deoxycorticosterone were added to the incubate.

Extraction and partition were carried out as described elsewhere (FAZEKAS et al., 1966).

The extract was purified on 1.5 g silica-gel (Davison, grade 12) column. Solvent system was ethyl acetate (EA) : benzene in the following sequence:

1. 5% EA in benzene (15 ml)
2. 10% EA in benzene (15 ml)
3. 50% EA in benzene (20 ml)
4. ethylacetate (20 ml)

The first and second fractions were discarded, the third and fourth were combined and evaporated to dryness and examined by thin layer chromatography (TLC).

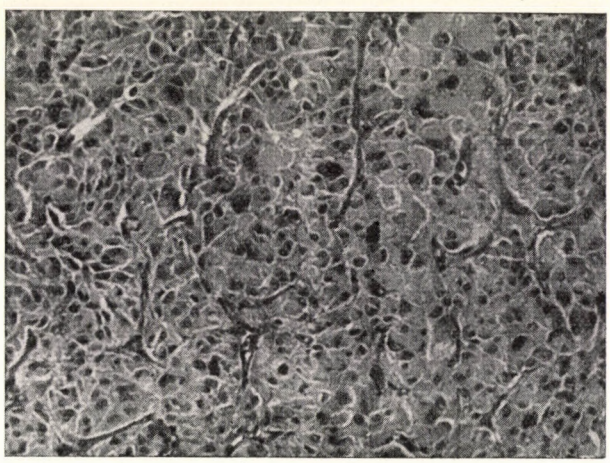

Fig. 1. Histological picture of the phaeochromocytoma. H. E. ×176

0.25 mm thick layers of silica-gel (Silica-gel G, Merck) were used for the chromatography, without activation. The silica-gel was washed with methanol before use (FAZEKAS and KÓKAI, 1967).

Solvent systems were as follows :

TLC-A: chloroform : methanol : water (188 : 12 : 1)
TLC-1: benzene : ethyl acetate (1 : 10)
TLC-5: chloroform : acetone (95 : 5)
TLC-6: cyclohexane : ethyl acetate (50 : 50)
TLC-7: methylene chloride : acetone (70 : 30)
TLC-8: cyclohexane : ethyl acetate (30 : 70)
TLC-9: methylene chloride : acetone (95 : 5)

Steroid spots were detected on the thin layer plates by exposure to I_2 vapour (MATTHEWS et al., 1962). Steroids were eluted with methanol by using a zone extractor (KÓKAI and FAZEKAS, 1967).

The quantity of steroids eluted from the thin layer plates was determined spectrophotometrically by measuring 0. D. at 240 mμ against the eluate of a blank spot on the same chromatoplate.

The quantity of radioactivity was measured with a thin end window G. M. tube at 6% efficiency (FAZEKAS and KÓKAI, 1967).

Identification of the isolated steroids was carried out by purification and derivative formation until the specific activity (desintegrations per minute/μmole; dpm/μM) after at least two consecutive derivatives and chromatographies had differed by less than 10%.

Steroids were acetylated by the method of DECOURCY et al. (1955). Chromic acid oxidation was performed by the method of MATTOX and MASON (1956), and hydrolysis of steroid acetates by that of MEYER (1953).

Table I

Corticosteroids formed from ^{14}C-progesterone by phaeochromocytoma homogenate

Steroid isolated	Carrier added	Derivative formed	TLC-system	Rf values	SA dpm/μmole	Total dpm incorp.	Per cent conversion
11-Deoxycorticosterone	300 μg	Free steroid	TLC—7	0.89	—		
		Free steroid	TLC—9	0.66	163 350		
		Acetate	TLC—5	0.84	54 978	51 480	0.68
		Hydrolysis of acetate	TLC—9	0.66	56 628		
Corticosterone	300 μg	Free steroid	TLC—7	0.64	—		
		Free steroid	TLC—7	0.64	34 647	27 780	0.37
		Acetate	TLC—5	0.31	32 083		
11-Deoxycortisol	300 μg	Free steroid	TLC—7	0.81	—		
		Free steroid	TLC—8	0.55	58 325		
		Acetate	TLC—5	0.55	48 084	30 990	0.41
		Hydrolysis of acetate	TLC—7	0.81	34 650		
		CrO_3 oxidation	TLC—6	0.60	35 793		
Cortisol	300 μg	Free steroid	TLC—7	0.40	—		
		Free steroid	TLC—A	0.20	15 691	7 980	0.10
		Acetate	TLC—1	0.75	9 639		
		CrO_3 oxidation	TLC—6	0.60	9 639		

Results

4-^{14}C-steroids isolated from the homogenate of phaeochromocytoma incubated with 4^{14}-C-progesterone were: 11-deoxycorticosterone, corticosterone, 11-deoxycortisol and cortisol.

Non-radioactive carrier steroids were added to the incubates before extraction. After extraction, partition and column chromatographic purification the steroids were separated on thin layer plates in system TLC-7. Steroids eluted from these chromatoplates were rechromatographed in TLC systems shown in Table 1, which shows the specific activities (dpm/μM) of the identified steroids and their derivatives, and total dpm incorporated into the isolated corticosteroids. In Table I the percentual conversions of the progesterone-4-^{14}C substrate into 11-deoxycorticosterone, corticosterone, 11-deoxycortisol and cortisol are also given.

Discussion

The presence of cortisol in the extract of a phaeochromocytoma was reported by RAMSEY and LANGLANDS (1962). Other investigations revealed that phaeochromatocytomas can form steroid hormones by hydroxylating

suitable precursors at positions 17 α, 21 and 11β, and by converting Δ^5-3-hydroxysteroids to their corresponding Δ^4-3-ketosteroids (MULROW et al., 1959, BECK, 1959, CARBALLEIRA and VENNING, 1964). ACEVEDO and BEERING (1965) showed that an active 2-hydroxylase and 17 β-oestradiol dehydrogenase may also be present. FAZEKAS et al. (1967) reported that a benign adrenal phaeochromocytoma containing adrenocortical cells can produce cortisol, 11-deoxycortisol and corticosterone from cholesterol -4-^{14}C and Δ^5-pregnenolone-16-^{3}H *in vitro*.

The present phaeochromocytoma was found to convert progesterone-4-^{14}C into 11-deoxycorticosterone, corticosterone, 11-deoxycortisol and cortisol.

Comparison of the relative dpm incorporated into cortisol and corticosterone showed that the preferential pathway had shifted in favour of corticosterone biosynthesis in this tumor. The percentage conversions of the substrate progesterone-4-^{14}C into the steroids isolated were very low as compared to the values obtained in human adrenocortical tumour tissue (44%) (FAZEKAS et al., 1967). In this phaeochromocytoma, only 1.56% of the substrate was converted into the four identified metabolites indicating a very limited steroid biosynthetical capacity which obviously cannot have a clinical significance (9.56 μg corticosteroids synthesized by the 12.6 g tumour in 3 hours).

The fact that we failed to demonstrate the presence of adrenocortical cells in the tumour does not necessarily exclude the possibility that the steroids were synthesized by such cells. As indicated previously, in phaeochromocytomas it is difficult to differentiate between cortical and medullary cells.

REFERENCES

ACEVEDO, H. F., BEERING, S. C.: Steroids **6**, 531 (1965).
BECK, J.: quoted by MULROW et al. (1959).
CARBALLEIRA, A., VENNING, E. H.: Steroids **4**, 329 (1964).
DECOURCY, C., BUSH, I., GREY, C. M., LUNNON, J. B.: J. Endocr. **9**, 401 (1955).
FAZEKAS, Á. G., WEBB, J. L., SYMINGTON, T.: Kísérl. Orvostud. **18**, 480 (1966).
FAZEKAS, Á. G., KÓKAI, K.: Steroids **9**, 177 (1967).
FAZEKAS, Á. G., NEVILLE, A. M., SYMINGTON, T.: In preparation.
FAZEKAS, Á. G., KÓKAI, K., WEBB, J. L., SYMINGTON, T.: Acta biochim. biophys. Acad. Sci. hung. **3**, 53 (1968).
KÓKAI, K., FAZEKAS, Á. G.: Kísérl. Orvostud. In press.
MATTHEWS, J. S., PEREDA, A. L., AQUILERA, V. A.: J. Chromatogr. **9**, 331 (1962).
MATTOX, V. R., MASON, H. L.: J. biol. Chem. **223**, 215 (1956).
MEYER, A. S.: J. biol. Chem. **203**, 469 (1953).
MULROW, P. J., COHEN, G. L., YESNER, R.: Yale J. Biol. Med. **31**, 363 (1959).
RAMSEY, I. D., LANGLANDS, J. H. M.: Lancet **2**, 126 (1962).
WALTERS, G., WYATT, G. B., KELLEHER, J.: J. clin. Endocr. 22, 575 (1962).

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INFLUENCE OF VITAMIN E ON LIVER INJURY AND FAT METABOLISM

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In rats, acute or repeated liver injury was induced by a single or repeated administration of carbon tetrachloride. Subcutaneous tocopherol application exerted no protective effect against the liver damage. Increased liver lipid values were observed after a few weeks of tocopherol treatment. After 10 months treatment no liver steatosis could be found, the liver glycogen and collagen content was unchanged, but serum lipids were significantly elevated. The results indicate a necessity for more careful indication of vitamin E treatment.

Liver tissue plays an important role in metabolism, uptake, storage, utilization and excretion of vitamin E. The liver tocopherol content depends to a great extent on the intake with food of the vitamin the largest depot of which in the organism is the liver. As vitamin E is excreted mainly in bile and feaces, liver function regulates its catabolism. Vitamin E exerts an influence on respiration, protein and fat metabolism, and the detoxicating processes of the liver.

The therapeutic use of vitamin E in liver diseases is a controversial subject. For this reason we have performed a series of experiments, in order to contribute to these open questions.

Materials and methods

In the experiments white Wistar rats were used. In some experiments a vitamin E deficient diet was given, to follow up the metabolic changes in vitamin E deficiency. Composition of the vitamin E deficient diet was:

casein	200 g
starch	640
cod liver oil	60
Osborne salt mixture	40
cellulose powder	40
dried brewer's yeast	20

Casein and starch were extracted with acetone to remove naturally occurring tocopherols. The starch was then enriched with a mixture of vitamin B, choline, p-benzoic acid and ascorbic acid [21]. Dried yeast was extracted with a solution of $FeCl_3$ in ether.

To secure sufficient vitamin E saturation in some groups of animals, 30 mg per 100 g body weight of tocopherol acetate (Erevit, Spofa) was injected subcutaneously once weekly.

Blood tocopherol was estimated using a photometric method based on the reaction of ferric chloride with 2, 2′-dipyridile [7].

Serum and tissue esterified fatty acids were estimated according to STERN and SHAPIRO [22], phospholipids using the STEWART and HENDRY procedure [23], and the cholesterol content with Liebermann-Burchard's colour reaction. For lipid analysis the tissue samples were homogenized and extracted with Folch's mixture of chloroform and methanol.

Liver glycogen was estimated after alkaline hydrolysis using anthrone reagent. For liver collagen determination we used the method based on hydroxyproline analysis [14, 5].

Serum glutamic-oxalacetic transaminase (SGOT) activity was estimated using the colour reaction of dinitrophenylhydrazine with pyruvic acid after incubation of serum with substrates [27].

Experimental liver injury was induced by the administration of carbon tetrachloride in an equal amount of sunflower oil through a stomach tube.

In experiment No. 1 we observed the influence of acute carbon-tetrachloride liver injury on the serum vitamin E level.

Experiment No. 2 was designed to study the signs of vitamin E deficiency in adult and weanling rats kept on a vitamin E deficient diet. Thus a model of animal tocopherol deficiency was created, on the basis of which we examined the metabolic effect of liver injury.

Experiment No. 3 had to prove the effect of acute liver injury in adult vitamin E deficient rats.

Experiment No. 4 had to reveal the metabolic effect of repeated liver injury in weanling vitamin E deficient rats.

In experiment No. 5 we tried to exclude the possibility of liver steatofibrosis induced by repeated and prolonged dosage of tocopherol.

Experimental design and results

1. To follow the influence of hepatic injury on the serum vitamin E level we examined in fasting rats of 200 g body weight the blood tocopherol level before and after a single intragastric dose of 0.1 ml CCl_4 per 100 g body weight (Table I). The results indicated a significant fall (in all instances $P < 0.001$) of blood tocopherol following acute liver injury.

2. In this experiment, we could observe in the first six months symptoms of tocopherol deficiency (absorption of pregnancy, sterility, creatinuria). A complete picture of avitaminosis E (paralyses of trunk and lower extremities, microscopical fragmentation of muscle bundles) could be observed only in weanling rats.

Table I

Serum vitamin E level in rats given a single intragastric dose of carbon tetrachloride
Mean values ± S. E. M.

Group of rats	Number of rats	Serum vitamin E (mg per 100 ml)
Control	17	0.60±0.01
18 hours after CCl_4	15	0.45±0.01
24 hours after CCl_4	10	0.46±0.02
48 hours after CCl_4	15	0.43±0.01

3. Next, we studied the effect of different vitamin E saturation on the course of acute liver injury in male rats weighing 200 g. The animals were fed the vitamin E deficient diet for 60 days. In the control group, sufficient vitamin E saturation was secured by weekly doses of tocopherol acetate. After 60 days, liver injury was induced by a single intragastric dose of 0.25 ml per 100 g body weight of CCl_4. The animals were sacrificed 18 hours after CCl_4 administration.

Tocopherol saturation in this experiment had no effect on liver dry matter. Tocopherol saturated rats had a significantly higher liver weight, both before and after liver injury. Tocopherol treatment resulted in an increase of esterified fatty acids and phospholipids in the liver (Table II). In injured livers this increase was not significant. Tocopherol administration had no effect on liver cholesterol in either intact or CCl_4-poisoned rats.

Though tocopherol saturation had no effect on SGOT activity in the rats with an intact liver (Fig. 1), the liver damage induced a remarkably steep rise of SGOT activity in tocopherol deficient rats.

Serum protein fractions determined by paper electrophoresis did not show any significant change due to tocopherol saturation. Microscopic examination of CCl_4 poisoned livers did not reveal any remarkable difference under the effect of tocopherol saturation.

4. Conditions of this experiment were similar to those in experiment No. 3, where male rats weighing 70 g were fed the vitamin E deficient diet for 40 days. The preventive dose of tocopherol in the control animals was the same as in experiment No. 3. Liver injury was induced by four intragastric doses of

Table II

Effect of vitamin E saturation on liver lipids of rats after a single intragastric CCl_4 *dose*

	Group of rats	Liver esterified fatty acids mEq/100 g	Liver lipoid phosphorus mg per 100 g	Liver cholesterol mg per 100 g
1	Saturated with vitamin E	58.9±5.0	438.5± 9.5	1242±71
2	Saturated with vitamin CCl_4 liver injury	108.3±9.7	290.4±13.2	945±38
3	Vitamin E deficient	39.8±2.7	358.1±28.9	1413±84
4	Vitamin E deficient CCl_4 liver injury	89.0±9.8	264.6±16.3	873±33
	Significance	1 : 3 $P < 0.01$ 2 : 4 $P > 0.05$	1 : 3 $P < 0.01$ 2 : 4 $P > 0.05$	1 : 3 $P > 0.05$ 2 : 4 $P > 0.05$

0.05 ml per 100 g body weight of CCl_4 each in four day intervals. Seven days after the last CCl_4 dose the animals were sacrificed in the fasting state.

This experiment revealed similar biochemical and microscopical results as experiment No. 3. Remarkable was the significant increase of esterified fatty acids and phospholipids in the liver of the rats saturated with tocopherol.

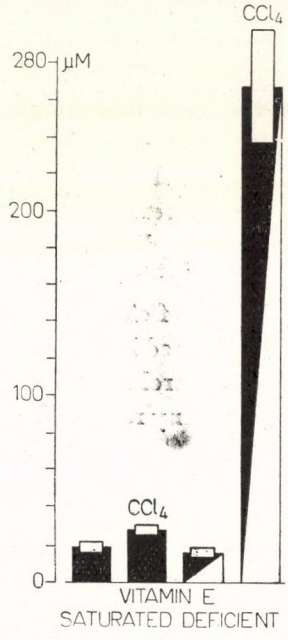

Fig. 1. Significantly increased serum glutamic-oxalacetic transaminase activity after liver injury in vitamin E deficient rats

There was no significant difference in mortality according to the degree of tocopherol saturation. When the liver had been injured repeatedly, SGOT activity did not show any remarkable difference due to different vitamin E saturation.

5. The liver lipid accumulation as a result of tocopherol treatment (experiments No. 3 and No. 4) was important enough to exclude liver steatosis and steatofibrosis due to tocopherol.

Young male rats of 120 g starting weight were fed a natural diet of constant composition in an experiment lasting 305 days. The control group had physiological tocopherol intake in the diet. The high tocopherol animals were given the usual tocopherol doses subcutaneously every week for 43 weeks. Animals of the control group were given equal amounts of sterile vegetable oil subcutaneously at appropriate intervals (tocopherol acetate is dissolved in sterile vegetable oil). Each group consisted of 15 animals. In this experiment

no liver injury was induced. After 305 days the animals were sacrificed after an overnight fast.

The weight curves of the highly saturated rats showed a decline from the 31st week onwards. At the end of the experimental period the body weight of high tocopherol rats averaged 70 g less than that of the control group. Some of the hypersaturated rats died in the course of the experiment. At the site of the tocopherol injections hard subcutaneous nodules developed in a number of rats. Similar observations were made in earlier experiments with repeated subcutaneous tocopherol application. In the control rats, injection of sterile oil caused no such nodules to appear. The nodules consisted microscopically of calcium deposits with lipod-like cysts.

Table III

Prolonged vitamin E hypersaturation results in elevation of serum lipids, but there are no signs of fatty infiltration in the liver and no changes in liver glycogen and hydroxyproline content

	Group of rats	Liver esterified fatty acids mEq/100 g	Liver glycogen g per 100 g	Liver hydroxyproline γ/1 g	Serum esterified fatty acids mEq/100 ml	Serum cholesterol mg per 100 ml
1	Control	7.4±0.5	2.5±0.3	512.1±17.1	0.48±0.04	39.2±1.8
2	Vitamin E saturated	8.2±0.8	2.6±0.2	526.7±34.6	0.83±0.07	58.1±4.5
Significance		$P > 0.05$	$P > 0.05$	$P > 0.05$	$P < 0.001$	$P < 0.001$

Prolonged tocopherol treatment (Table III) caused no significant change in esterified fatty acid, collagen or glycogen content in the liver, nor in hepatic dry matter. In the rats treated with high tocopherol doses, serum cholesterol and esterified fatty acid levels were higher (both $P < 0.001$) than in the control group.

Microscopic examination of liver tissue did not reveal any remarkable differences in structure, in the amount of lipids and connective tissue after repeated tocopherol application.

Discussion

Acute liver injury significantly influences the serum vitamin E level. Though a fall of this level was observed in the present experiment, the possibility of a transient rise in liver injury has not been excluded [11]. A decisive factor may be the mechanism producing liver injury and the extent of its action. In this respect, acute CCl_4 poisoning is not quite comparable with the usual human hepatocellular damage.

We failed to find a decisive protective action of vitamin E when trying to influence the liver damage induced by different applications of CCl_4. The only finding supporting the possibility of a favourable influence of vitamin E could be the behaviour of SGOT, which was increased in CCl_4 treated vitamin E deficient rats. Other biochemical and microscopical observations, however, did not support this possibility. Changes in transaminase activity are perhaps due to the specific influence of tocopherol on protein metabolism.

Tocopherol saturation undoubtedly plays an important role in lipid metabolism. This could be stated on the basis of results of several own experiments, using either a vitamin E deficient diet, a natural diet, or vitamin E hypersaturation. Only some differences in the lipid level could be explained with differences in the type of diet. Nevertheless, liver lipids increase if the rats receive high parenteral doses of vitamin E. It seems that the tendency to fatty infiltration of the liver appears only in the first few weeks of tocopherol application, since the liver lipids were unchanged after repeated vitamin E injections for more than 10 months. After this period we failed to find changes in liver glycogen and connective tissue.

The present results were in accordance with the rise of liver lipids reported in tocopherol saturated mice [13], guinea pigs [20], and other animals [10], and thus contradicted the reports describing a smaller extent of lipid infiltration after liver injury in tocopherol treated animals [16, 9, 3]. The possibility of the antioxidative influence of tocopherol in relation to liver lipids [26] should be taken into account as an explanation of the higher lipid levels in vitamin E saturated rats.

A similar metabolic effect of vitamin E may be observed also when examining the lipid level of other organs. Repeated subcutaneous application of vitamin E led in our experiments to higher serum lipid levels and to the evolution of subcutaneous nodules consisting of degenerated fatty tissue. Increased values of cholesterol after tocopherol application have been observed in blood [2, 20, 24, 8], liver and aorta [4], thus contributing to the evolution of experimental atherosclerosis. Patients suffering from arteriosclerosis have in their aorta an inreased vitamin E content, the amount of which seems to be proportional to the degree of aortic sclerosis [28]. Treatment of these patients with vitamin E resulted in an increase of total lipids, cholesterol and phospholipids in blood.

PELKONEN [17] reported on a significant regression between the serum cholesterol and blood tocopherol levels in coronary patients as well as in healthy subjects. Immediately after myocardial infarction the blood levels of tocopherol and cholesterol show similar changes. DARBY [6] observed a frequent elevation of blood tocopherol in diseases associated with hypercholesterolaemia. Similar findings were made by POPPER [18] in patients with diseases of the liver and biliary tract. A positive correlation between the plasma tocopherol and

cholesterol levels was observed in diabetes [1, 25] and in thyroid disorders [19]. NIKITIN [15] on the other hand observed the decrease of cholesterolaemia in atherosclerotic patients treated with vitamin E.

It may be concluded from the above that the prescription of such a potent metabolic factor as vitamin E should be carefully weighed. The results of animal and clinical experiments have been so contradictory that the indications for tocopherol should be preceded by further research.

After protracted tocopherol administration most of the rats in our experiment lost weight and some of them died. As regards the influence on liver function of vitamin E, its subcutaneous application in high doses for longer periods is of a dubious value in cases of both acute and chronic liver injury.

Conclusions

In experiments on rats we observed no distinct influence of tocopherol saturation on the course of CCl_4 induced liver injury. The steep rise of SGOT in vitamin E deficient rats with injured livers cannot be considered a favourable influence of the vitamin on the course of liver injury, since other biochemical and morphological findings failed to support that possibility.

Vitamin E saturated rats had higher values for esterified fatty acids and phospholipids in the liver than the deficient rats. This tendency to liver steatosis did not occur when vitamin E had been applied for 10 months. The liver of rats hypersaturated with vitamin E showed no differences in glycogen and collagen content nor in microscopic structure. Serum esterified fatty acids and cholesterol values were increased after repeated vitamin E application. The adverse biological effect of this treatment was indicated by the lower body weight and the higher mortality of these animals as compared to the controls.

REFERENCES

1. BENSLEY, E. H., FOWLER, A. F.: Plasma tocopherol level in diabetes mellitus. J. Nutr. **40,** 323 (1950).
2. BURRIER, C.: Pren. med. argent. **47,** 88 (1960).
3. BUTTURINI, U., VOLTA, A.: Cit. [12].
4. CHAKRAVARTI, R. N., BALKRISHNA, S. H.: Effect of alfa tocopherol on experimental atherosclerosis. Indian J. med. Res. **48,** 356 (1960).
5. CHVAPIL, M.: Možnosti kvantitatívního stanovení kolagenních bílkovin a elastinu v biologickém materiálu. Cs. Fysiol. **8,** 81 (1959).
6. DARBY, W. J. FERGUSON, M. E.: Plasma tocopherols in health and disease. Ann. N. Y. Acad. Sci. **52,** 328 (1949).
7. FELDHEIM, W.: Schnellbestimmung zur Ermittlung des Vitamin E Gehaltes im Serum. Int. Z. Vitaminforsch. **27,** 8 (1956).
8. GRAY, D. E., LOH, S. M.: Influence of alfa-tocopheryl acetate on some lipids and nitrogen compounds of plasma in human subjects. Canad. J. Biochem. **36,** 269 (1958).
9. HARTMAN, F., HERTEL, R.: Über die lipotrope Wirkung von Vitamin E und Cystin, Methionin und Inosit an CCl_4 vergifteten Ratten. Naunyn-Schmiedebergs Arch. exp. Path. Pharmak. **214,** 152 (1952).

10. HOVE, E. L., HARDIN, J. O.: Effect of vitamin E and CCl_4 on fat, respiration, choline oxidase of rat livers. Proc. Soc. exp. Biol. (N. Y.) **78,** 858 (1951).
11. KLATSKIN, G., TISDALE, W. A.: The effects of experimental hepatobiliary disease on certain aspects of tocopherol metabolism in the rat. J. clin. Invest. **36,** 1627 (1957).
12. KRONE, H. A.: Über den Einfluss von Vitamin E auf den Ablauf der histologischen Leberveränderungen bei Ratten nach CCl_4 Vergiftung. Int. Z. Vitaminforsch. **24,** 12 (1952).
13. MENSCHIK, Z., SZCESNIAK, T. J.: Vitamin E and liver lipids in mice. Anat. Rec. **103,** 349 (1949).
14. NEUMANN, R. E., LOGAN, M. A.: J. biol. Chem. **186,** 549 (1950). Cit. [5].
15. NIKITIN, J. P.: Vop. Pit. **21/6,** 22 (1962).
16. PACILLI, N.: L'azione dell'alfa-tocoferolo e degli acidi grassi insaturati sul fegato in rigenerazione. Osped. maggiore **41,** 324 (1953).
17. PELKONEN, R.: Plasma vitamin A and E in the study of lipid and lipoprotein metabolism in coronary heart disease. Acta med. scand., Suppl. 399, 1 (1963).
18. POPPER, H., DUBIN, A.: Plasma tocopherol levels in various pathological conditions. J. Lab. clin. Med. **34,** 648 (1949).
19. POSTEL, S.: Total free tocopherols in the serum of patients with thyroid disease. J. clin. Invest. **35,** 1345 (1956).
20. SCHULL, R., ERSHOFF, B. H.: Effects of dietary cholesterol on guinea- pigs fed a vitamin E deficient diet. Exp. Med. Surg. **17,** 198 (1959).
21. ŠIMKO, V.: Pokusná dietetická hypovitaminóza E u krýs. Čs. Fysiol. **12,** 417 (1963).
22. STERN, J., SHAPIRO, B.: A rapid and simple method for the determination of esterified fatty acids. J. clin. Path. **6,** 158 (1953).
23. STEWART, C. P., HENDRY, E. B.: The phospholipids of blood. Biochem. J. **29,** 1683 (1935).
24. TARASOVA, L. S., TROICKIJ, G. V.: Vop. med. Khim. **6,** 62 (1960).
25. VANZETTI, G., MORPURGO, M.: Le taux plasmatique de vitamin E chez le sujet normal, diabetique et l'atherosclérotique. Presse méd. **2,** 1246 (1956).
26. ZALKIN, H., TAPPEL, A. L.: Lipid peroxidation in vitamin E deficient rabbit. Arch. Biochem. **88,** 113 (1960).
27. REITMAN, S., FRANKEL, S.: A colorimetric method for the determination of SGOT and GPT. Amer. J. clin. Path. **28,** 56 (1957).
28. VANOTTI, A., GERVASONI, L. A.: Action des vitamines liposolubles A et E sur le métabolisme des lipides chez les artérioscléreux. Bull. schweiz. Akad. med. Wiss. **13,** 363 (1957).

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RECENSIO

Experimental Diabetes Mellitus in the Rat (Selected Chapters). Rudolf KOREC M. D., Professor of Pathophysiology and Experimental Pathology, Faculty of Medicine, P. J. Safárik University, Kosice Publishing House of the Slovak Academy of Sciences, Bratislava 1967. 150 pages, 70 illustrations, 18 tables.

The range of the book, greatly understated by its title, comprises all the essentials the worker embarking upon studies of carbohydrate metabolism in rats may wish to know. The various issues of the subject are presented in the light of the author's observations supported by reliable statistical figures.

In the introductory section the experimental methods, including nutrition and management of the animals as well as laboratory investigations are described in full detail. The rat's carbohydrate metabolism, the various types of glucose tolerance test (intragastric, intraduodenal, intraperitoneal, subcutaneous, intravenous) are presented in their theoretical and practical aspects. The author on the evidence of his own studies asserts that in the case of intravenous glucose tolerance tests the liver has no significant part in the extraction of glucose, the fall in the blood sugar level being determined by the peripheral tissues.

After discussing the biological properties of insulin, the insulin tolerance tests are described with attention to the effect under various conditions of dosage and of routes of administration, also after ligation of the portal vein and of the hepatic artery. Special sections are devoted to the questions of insulin hypoglycaemia, neuroglycopenia, and insulin coma. On the basis of own experiments, the special features of hypoglycaemia in adrenodemedullated rats are dealt with. The glucagon and adrenaline tolerance tests are combined with insulin and dihydroergotamine. In these studies, adrenaline clearly appears as a constant factor in maintaining normoglycaemia.

The volume contains a historical survey of experimental diabetes research. This is followed by a detailed account on the hypoglycaemic conditions induced by 2-deoxy-D-glucose through a metabolic inhibitory mechanism ("metabolic inhibitor diabetes"). The significance of aurothioglucose in the production of experimental diabetes is pointed out. The experimental issues of subtotal and total pancreatectomy including their techniques are presented in detail. The author describes the rat's response patterns to glucose and tolbutamine tolerance tests on the basis of own studies, which also point to the difficulty in eliciting immunodiabetes in rats.

The section devoted to alloxan diabetes bears the authenticity of the author's personal experience. In the discussion of experimental diabetes the study of antidiabetic drugs finds its proper place. The significance of the hyperglycaemic effect of benzothiadizines is pointed out. In the final chapter dealing with the question of calciphylaxis the author has shown that in dihydrotachysterol-pretreated rats even subdiabetogenic doses of alloxan are liable to produce glycosuria.

It is a particular merit of the author to have kept the length of the individual sections in harmony with the importance of the issues to be discussed. In this manner he offers a source of competent critical information on practically every issue of diabetology. His personal observations made in more than one thousand rat experiments add to the value of the book.

The volume should appeal to the physician concerned with the clinical questions of diabetes as well as to the research worker engaged in this field of study. The Publishing House of the Slovak Academy of Sciences merits every praise for its production.

L. BARTA

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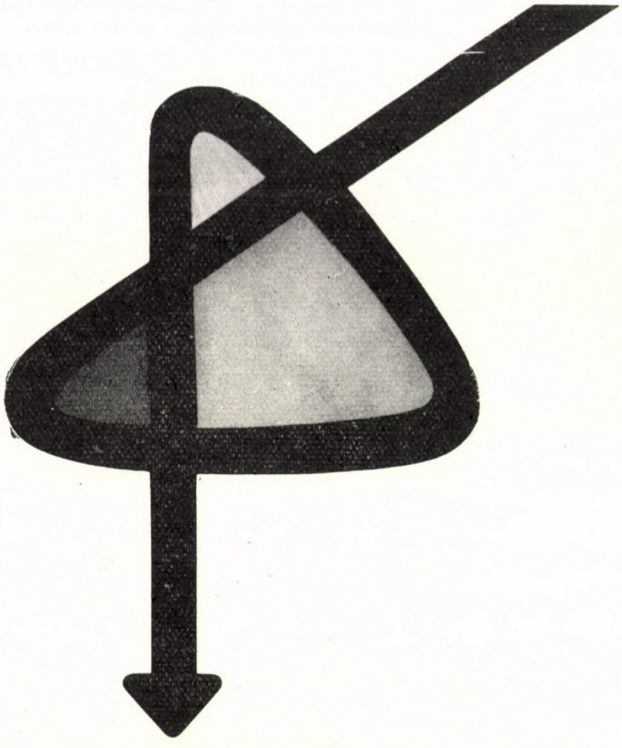

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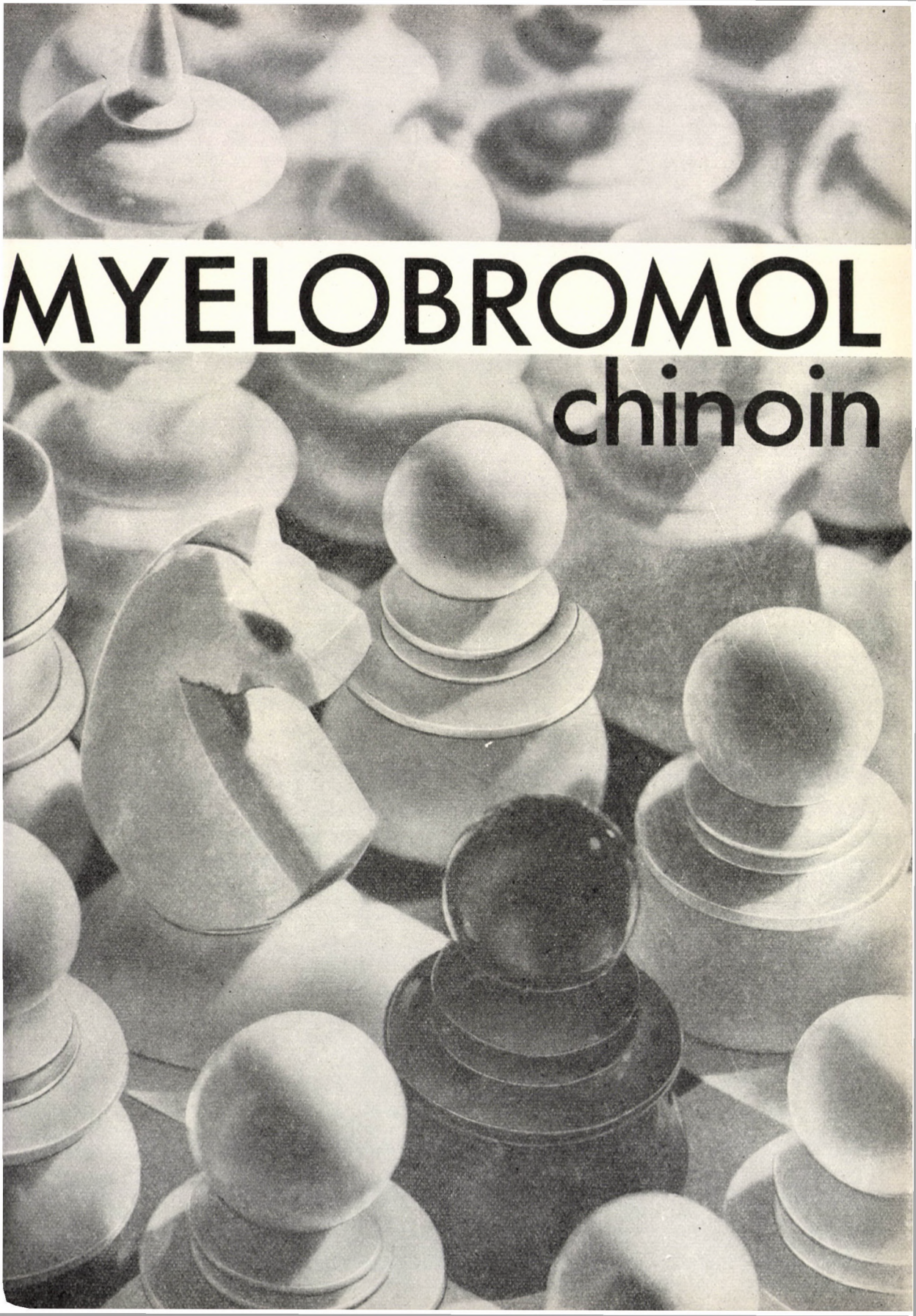
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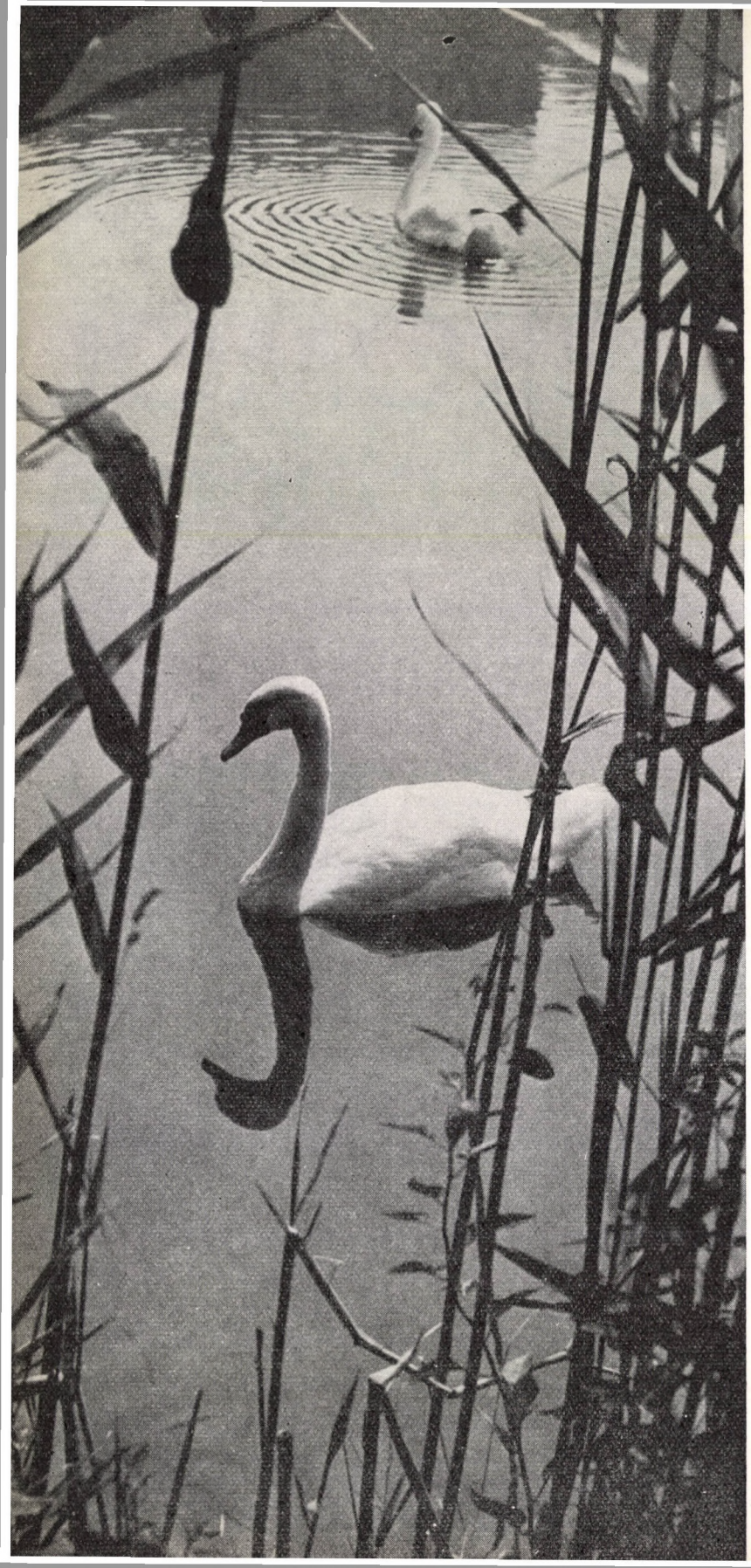

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РЕЗЮМЕ

МЕХАНИЗМ ГЕНЕРАЛИЗОВАННОЙ СПАСТИЧЕСКОЙ СПАЙК-ВОЛНЫ И РЕТИКУЛЯРНАЯ СИСТЕМА ПРОБУЖДЕНИЯ

П. ХАЛАС и Й. ХИДАШИ

В связи с изложением атипичного случая эпилептического состояния, слагающегося отчасти из генерализованных спайк-волн и отчасти из генерализованных приступов десинхронизации, обсуждается связь между механизмом генерализованной спайк-волны и ретикулярной системой пробуждения. На основе экспериментальных данных и клинического опыта, а также результатов обследования больного автора, предполагается, что торможение ретикулярной системы пробуждения играет существенную роль в механизме спайк-волны.

СОДЕРЖАНИЕ CO_2 ТКАНИ ГОЛОВНОГО МОЗГА И ИЗМЕНЕНИЯ КИСЛОТНО-ЩЕЛОЧНОГО РАВНОВЕСИЯ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ШОКЕ ОТ ОБЕСКРОВЛИВАНИЯ ПОСЛЕ ПРЕДВАРИТЕЛЬНОЙ ДАЧИ ДИБЕНЗИЛИНА

Е. МАКЛАРИ и А. Г. Б. КОВАЧ

Авторы исследовали изменения pH, содержания бикарбоната и pCO_2 в крови сонной артерии и сагиттальной вены в различных стадиях экспериментального шока от обескровливания, у контрольных собак и у животных, предварительно получивших дибензилин. В идентичных фазах они определяли также содержание CO_2 ткани лобной и затылочной частей коры, таламуса, гипоталамуса, моста и продолговатого мозга.

Было установлено, что у контрольных животных в ходе обескровливания развивается значительный метаболический ацидоз; на участие центральной нервной системы указывает, что по сравнению с артериальной кровью в крови сагиттальной вены наблюдалась более значительная кислотность и очень существенное повышение pCO_2. Ретрансфузия не вызвала существенного изменения. К концу обескровливания и в состоянии шока в ткани головного мозга авторы определяли значительную тканевую гиперкапнию. Повышение pCO_2 в сагиттальной вене по всей вероятности является результатом гиперкапнии. В исследованных участках мозга тканевая гиперкапния была неодинаковой степени: наиболее выраженной она являлась в гипоталамусе и коре, в то время как в таламусе она была менее значительной. Этот факт указывает на то, что в патологическом процессе шока от обескровливания отдельные части центральной нервной системы участвуют в различной степени.

У собак, предварительно получивших дибензилин, в ходе экспериментов не наблюдалось изменения pH, был выявлен лишь незначительный, компенсированный метаболический ацидоз. Отсутствовало также повышение pCO_2 в сагиттальной вене, и гиперкапния в ткани головного мозга. По-видимому, предварительная дача дибензилина в большой мере уменьшает изменения тканевого обмена веществ, вызванные понижением кровообращения и гипоксией, и оказывает благоприятное действие и на центральную нервную систему.

ИНДУЦИРОВАННОЕ ВОССТАНОВЛЕНИЕ ПЕЧЕНИ КРЫС ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ЦИРРОЗЕ

Я. МЕНЬХАРТ и Л. ШИМОН

В физиологических условиях частичное удаление печени вызывает в остаточной печеночной ткани интенсивное восстановление.

Авторы старались использовать индуциремую частичной гепатектомией регенерацию для воздействия на цирротические процессы различной степени тяжести, вызванные у крыс введением CCl_4. Степень цирроза и эффективность индуцированного восстановления они проверяли измерением веса регенерированной печеночной ткани, определением соотношения веса печени к весу тела, а также с помощью анализа митотической реакции.

Было выявлено, что частичная гепатектомия оказывает благоприятное действие на течение циррозов меньшей тяжести. При далеко зашедшем циррозе частичная гепатектомия оказывается безэффективной.

Анализ данных показал, что эффективность частичного удаления печени зависит от интактности обмена веществ печеночной ткани. Во всех случаях, в которых цирротическая ткань еще сохранила свою реактивную способность в отношении стимулов митоза, частичная гепатектомия оказала благоприятное действие. Когда же вследствие прогрессирования процесса митотическая реактивность утрачивается, частичная гепатектомия оказывается уже неэффективной. Первому состоянию соответствует гипертрофическая фаза, а второму — атрофическая фаза цирроза.

ДАННЫЕ К ПАТОФИЗИОЛОГИЧЕСКИМ И ДИАГНОСТИЧЕСКИМ ВОПРОСАМ ГЕПАТОГЕННОЙ АНЕМИИ

Исследование содержания энзимов в эритроцитах

Ш. ДАН, Б. ШАРИ, С. КИРИЛЛИНА и А. КИШ

У больных хроническим гепатитом авторы определили содержание кислой фосфатазы, ALD, трансаминазы глютаминовой-щавелеуксусной кислот, LDH, G—6—PDH и PE в эритроцитах. На основе полученных результатов и литературных данных они проводили опыты на модели и исследовали влияние экспериментального поражения печени на реактивный эритропоэз, на ретикулоцитокризис и на изменения содержания энзимов в популяции эритроцитов, наблюдаемые в процессе созревания эритроцитов. На основе совместного анализа клинических и экспериментальных данных устанавливается, что с точки зрения практики приходится дифференцировать *потенциальную гепатогенную анемию* (нормальное число эритроцитов) от *явной гепатогенной анемии* (пониженное число эритроцитов). В первой стадии определение содержания энзимов в эритроцитах имеет большое значение для постановки раннего диагноза, а во второй стадии — для оценки реактивной эритропоэтической способности.

ВОПРОС О РОЖЕ

III. Первые результаты вакцинации стрептококковой вакциной, больных рецидивирующей рожей

Ш. КОРОШИ, Э. РАЙКА, Ю. БЁСЁРМЕНЬИ и М. ГОЗОНЬ

Авторы у 68 повторного рецидировавших (2 — 72 раза) больных рожей проводили вакцинацию поливалентной стрептококковой вакциной группы А, изготовленной из вариантов вирулентных на белых мышах штаммов микробов типа 14, содержащей М-протеиновые антигены, чаще всего встречающихся в Венгрии. В большинстве случаев авторы проводили 6 иммунизирующих и 6 добавочных прививок. С учетом ритма рецидивов, в течение 8 — 27 месячного периода наблюдения у 28 больных (41%) в среднем 4,5 рецидивов не проявлялось. У 19 больных (29%) рецидивы появлялись вопреки вакцинации. У 21 больного (30,9) срок рецидива еще не наступил, но в период наблюдения

обострения болезни не наблюдалось. Реакция позднего типа, наблюдаемая после внутрикожной прививки стрептококковой вакциной, в 7,3% случаев была повышенной. В то же время она после внутрикожного введения термостабильного фактора в 20,4% случаев была менее значительной или стала отрицательной. Среди циркулирующих гуморальных противотел количество антистрептолизина-О в 20,3%, а титр антистрептокиназы в 11,6% случаев были повышенными.

НОВЫЕ ДАННЫЕ ОБ ИЗМЕНЕНИЯХ УГЛЕВОДНОГО ОБМЕНА ЖИВОТНЫХ, СОДЕРЖАННЫХ НА КАРДИОВАЗОПАТОГЕННОЙ ДИЕТЕ

И. СЕЛЕНИ, Ю. ШОШ, Э. НЕМЕШАНСКИ, ЛИ БОК НАМ
и Ю. РИГО

Авторы исследовали изменения, возникающие в углеводном обмене крыс под влиянием кардиовазопатогенной диеты (S. 65). Было установлено, что соотвенно результатам прежних исследований in vitro, нарушается фаза расщепления в углеводном обмене, предоставляющая энергию, и это приводит к значительному повышению внеклеточного содержания пировиноградной кислоты. Наблюдалось и умеренное повышение содержания молочной кислоты. Под влиянием диеты сильно нарушается углеводный обмен не только сердца, но и печени.

ДЕЙСТВИЕ ГИДРАЗИНОФТАЛАЗИНОВ НА СОДЕРЖАНИЕ ГЛИКОПРОТЕИНА В СЫВОРОТКЕ

I. и II. Исследование действия Апрезолина

Л. ЯКАБ, Я. ФЕХЕР, Ш. ВИРАГ и Д. СИНАИ

Авторы исследовали на кроликах действие дачи 1-гидразинофталазина на содержание гликопротеинов и белков в сыворотке. Было установлено, что под влиянием препарата в сыворотке максимальная концентрация гексозы и серомукоида достигается по истечении полтора месяца, а максимальное содержание сиалиновой кислоты — по истечении 3 месяцев. В отношении гексозамина эти изменения незначительны, а изменения концентрации остальных веществ очень достоверные. Через полтора месяца после введения 1-гидразинофталазина концентрация сывороточных белков значительно повысилась, через 3 месяца наблюдалось существенное понижение содержания альбумина, при значительном повышении концентрации глобулинов. Эти изменения выявляемы еще до появления морфологических изменений. Результаты экспериментов указывают на то, что изменения углеводного обмена играют роль в развитии HLS и per analogiam в развитии LED.

ИЗМЕНЕНИЯ ГЕМОДИНАМИКИ ПРИ «SUBCLAVIAN STEAL SYNDROME»

Ф. ШОЛЬТИ, М. ИШКУМ, Е. ЗАДОРИ, А. ХАРТАИ,
Й. НАДЬ и Ю. РЕВ

Авторы исследовали у людей и в опытах на животных (искусственное создание «subclavian steal syndrome» у собак) особенности изменений мозгового кровообращения и кровообращения в конечностях, наблюдаемых при этом синдроме.

Согласно результатам опытов на животных при данном синдроме кровоснабжение сосудистой области верхней конечности, находящейся дистально от места закупорки, обеспечивается в первую очередь ретроградным кровотоком в позвоночной артерии той же стороны, а во вторую очередь, ретроградным кровотоком в нижней щитовидной артерии, той же стороны.

На основе своих собственных наблюдений и исследований авторы полагают, что в случаях с выраженными симптомами ишемии головного мозга в позвоночной артерии ретроградный кровоток значителен и в данной верхней конечности кровоснабжение от-

носительно очень хорошее. Случаи с ишемией в верхних конечностях наблюдаются реже, и в таких случаях симптомы церебральной ишемии отсутствуют. Предполагается, что у этих больных ретроградный кровоток в позвоночной артерии незначительный.

Согласно результатам проводившихся исследований церебрального кровообращения при «subclavian steal syndrome», сопровождаемом симптомами гипоксии головного мозга, церебральный кровоток патологически низкий и сильно уменьшена также фракция церебральная минутного объема. В регулировании церебрального кровообращения большое значение имеет изменение сосудистого сопротивления в верхней конечности, на стороне стеноза подключичной артерии.

НОВОЕ ПАТОФИЗИОЛОГИЧЕСКОЕ ТОЛКОВАНИЕ ОСОБЕННОСТЕЙ ОТНОСИТЕЛЬНОЙ ГИПЕРМЕТРОПИИ, ОБУСЛАВЛИВАЮЩЕЙ СХОДЯЩЕЕСЯ КОСОГЛАЗИЕ, I.

И. РЕТИ

Новая патологическая интерпретация относительной гиперметропии основывается на тесной корреляции со способностью к сливанию. Чем слабее эта способность, тем более значительной будет степень относительной гиперметропии, вызывающей страбизм, так как сила сливания неспособна обеспечить бинокулярное зрение, против импульса отдаленного схождения, симультанного с аккомодацией.

Патологическая концепция относительной гиперметропии объясняет также случаи исключения и предоставляет новые возможности для причинного лечения сходящегося косоглазия. Новый метод консервативного лечения страбизма позволяет надеяться на значительное уменьшение операций, производимых по поводу сходящегося косоглазия.

НАБУХАНИЕ РОГОВИЦЫ БЕЗ ВАСКУЛЯРИЗАЦИИ

Д. ИМРЕ и М. ПАЛ

Введением силиконового масла в камеру глаза кроликов авторы создали состояние, соответствующее камере типа стекловидного тела, в результате чего образовался отек роговой оболочки глаза, подобный пузырчатой кератопатии. В набухших роговицах возникла лишь минимальная краевая васкуляризация. Соприкасающаяся с силиконом и больше всего набухшая часть роговицы нигде не показала васкуляризации. Этим фактом доказывается, что уменьшение плотности роговицы не является причиной, а лишь одной из предпосылок васкуляризации. В набухших роговицах без васкуляризации, соприкасающихся с камерной влагой, концентрация молочной кислоты была достоверно пониженной. Этот факт говорит за то, что высокая концентрация молочной кислоты имеет значение в процессе васкуляризации, а также за то, что в развитии пузырчатой кератопатии по всей вероятности играет роль понижение снабжения роговицы глюкозой.

ИЗМЕНЕНИЕ МЕХАНИЗМА КОНЪЮГАЦИИ У БОЛЬНОЙ С ЭТИОХОЛАНОЛОНОВОЙ ЛИХОРАДКОЙ

Т. ФЕХЕР и Л. ХАЛМИ

Авторы наблюдали у больной женщины периодическую лихорадку и повышение содержания неконъюгированного этиохоланолона в плазме. В настоящей работе они определили содержание сульфата 17-оксостероида в плазме и моче, содержание глюкуронозида 17-оксостероида в моче, в основном состоянии или после дачи кортикотропина, метопирона и тестостерон-пропионата. Они нашли, что у больной с этиохоланолоновой лихорадкой производство 5-стероидов, способность конъюгировать сульфат и глюкуронозид были нормальными.

ОБРАЗОВАНИЕ КОРТИКОСТЕРОИДОВ В ДОБРОКАЧЕСТВЕННОЙ ФЕОХРОМОЦИТОМЕ IN VITRO

К. КОКАИ, А. Г. ФАЗЕКАШ и К. КОВАЧ

Авторы выявили, что доброкачественная феохромоцитотома синтезировала из 4—C^{14}-прогестерона 11-дезоксикортикостерон, кортикостерон, 11-дезоксикортизол и кортизон in vitro. Вопреки тому, что при гистологическом исследовании в опухолевой ткани не удалось доказать присутствия клеток коры надпочечников, нельзя исключить возможность, что указанные стероиды все же были синтезированы клетками коры надпочечников.

ДЕЙСТВИЕ ВИТАМИНА Е НА ПОРАЖЕНИЕ ПЕЧЕНИ И НА ЖИРОВОЙ ОБМЕН

В. ШИМКО

В опытах на крысах введением четыреххлористого углерода было вызвано острое или повторное отравление печени.

Подкожное введение токоферола не оказало защитного действия против поражения печени. После введения токоферола на протяжении нескольких недель были определены более высокие величины липоидов в печени. В случае введения витамина Е в течение 10 месяцев жирового перерождения печени не наблюдалось, содержание гликогена и коллагена в печени было неизменным. Однако величины сывороточных липоидов были достоверно повышенными.

Эти результаты указывают на необходимость повышенной осторожности при назначении лечения витамином Е.

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Acta Medica Academiae Scientiarum Hungaricae Tomus 25 (2), pp. 121—126 (1968)

ÜBER DIE WIRKUNG VON DESOXYKORTIKOSTERON-GLYKOSID AUF DIE SCHILDDRÜSENFUNKTION

Von

KLARA GÓLIÁN-BARTHA

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DER MEDIZINISCHEN UNIVERSITÄT, BUDAPEST

(Eingegangen am 16. Januar 1967)

Die Nebenwirkungen von wasserlöslichem Desoxycorton auf die Jodaufnahme und Hormonsynthese der Schilddrüsen wurden untersucht. Zwecks Registrierung der Wirkung des Mittels auf den Jodstoffwechsel wurde mit der radioaktiven Indikator-Methode die Jodspeicherung der Schilddrüse sowie die prozentuale Zusammensetzung der Jodhormon- und Plasmahormonfraktionen analysiert. Anhand der qualitativen und quantitativen Analyse der Jodspeicherungskurven war eine hochgradige Hemmung der Jodaufnahme festzustellen: Bei den mit einer Tagesdosis von 10 mg/kg behandelten Tieren sanken die Werte von 33% auf 20%. In der Schilddrüse erhöhte sich die anorganische Jodphase — zu Lasten der Dijodtyrosinphase — fast auf das Doppelte. Die Zusammensetzung der Plasmahormone wies auf eine Mobilisationsstörung hin: Unter der Wirkung von 10 mg/kg/Tag Desoxycorton erhöhten sich die Di- und Monojodtyrosinphasen auf das Fünffache, während sich die Thyroxinkonzentration auf weniger als die Hälfte verminderte.

In Anbetracht der weitverbreiteten therapeutischen Anwendung der Kortikosteroide ist es notwendig, daß alle möglichen Gefahren bzw. Nebenwirkungen dieser Medikamente geklärt werden. Die anhaltende Überdosierung der Kortikosteroide hemmt die ACTH-Produktion in derartig bedeutendem Maße, daß die gesamte Hormonproduktion des Hypophyse-Nebennierenrindensystems eine Störung erleidet. Selbstverständlich betrifft diese Wirkung durch Vermittlung der Hypophyse sämtliche unter der Kontrolle irgendeines in der Adenohypophyse produzierten Trophormons stehende endokrine Drüsen und so auch die Schilddrüse.

Mit dem zwischen den Nebennierenrindenhormonen und der Schilddrüsenfunktion bestehenden Zusammenhang haben sich im vergangenen Jahrzehnt zahlreiche Forscher befaßt ohne daß sich auf diesem Gebiet eine einheitliche Anschauung entwickelt hätte. In manchen Fragen sind sogar widerspruchsvolle Meinungen laut geworden. Während einige Verfasser über die auf Wirkung der Kortikosteroide eintreffende Steigerung der Jodaufnahme insbesondere aber der Jodmobilisation berichteten [3, 7, 15, 18], beobachtete eine andere Gruppe der Forscher nach Verabfolgung von Cortisonderivaten eine Verminderung des Jodstoffwechsels [10, 11, 12, 17, 20]. FÖLDES und Mitarb. [6], sowie PITT-RIVERS und Mitarb. [19] stellten fest, daß die erwähnten Wirkstoffe den Jodstoffwechsel des Organismus nicht signifikant verändern. In der Mitteilung von FELT [5] wird über die durch Thyroxinbehandlung ausgelöste Störung der Nebennierenrindenhormonproduktion berichtet.

Die angeführten Daten veranlaßten uns zur Untersuchung der sekundären Nebenwirkungen des wasserlöslichen Desoxycortons auf die Schilddrüsenfunktion.

Methodik

Die auf den Jodstoffwechsel ausgeübte Wirkung des Mittels haben wir in allen 3 Phasen des Jodumsatzes mit der radioaktiven Indikatormethode registriert [21, 22].

1. Die Jodspeicherungskurven wurden qualitativ und quantitativ bewertet;
2. die in der Schilddrüse synthetisierten und gespeicherten Jodhormonkomponenten wurden prozentuell analysiert;
3. die prozentuale Zusammensetzung der mobiliserten — in das Blutplasma transportierten — Jodhormonfraktionen wurde bestimmt.

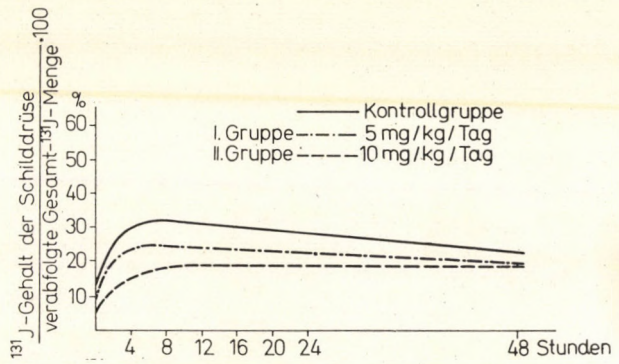

Abb. 1. ^{131}J-Speicherungskurven der mit wasserlöslichem Desoxycorton behandelten Tiere

Die Experimente erfolgten an durchschnittlich 200 g schweren, an jodarmer Diät (nach Remington) gehaltenen Albinorattenmännchen. Die Tiere wurden in 3 Gruppen eingeteilt; die zu der ersten Gruppe gehörenden 30 Ratten erhielten 2 Tage hindurch täglich 5 mg/kg Körpergewicht wasserlösliches Desoxycorton i.p., während den zur zweiten Gruppe gehörenden 30 Ratten ebenfalls 2 Tage hindurch 10 mg/kg Körpergewicht wasserlösliches Desoxycorton i. p. injiziert wurde; noch kleinere Dosen verursachten keine signifikante Wirkung. Die Tiere der dritten Gruppe dienten als Kontrolle. Gleichzeitig mit dem Beginn der Behandlung wurde allen Tieren 100 μCi ^{131}J i.p. injiziert.

Die Jodspeicherungskurven wurden anhand von 2, 6, 24 und 48 Stunden nach der ^{131}J-Verabfolgung durchgeführten in vivo Messungen aufgenommen [16] (Abb. 1).

Ergebnisse

Die Jodaufnahme der Schilddrüse erleidete bei den behandelten Tieren bereits nach Verabfolgung einer Dose von 5 mg/kg/Tag eine etwa 8%ige Hemmung ($P < 0{,}05$). Diese Wirkung steigt mit der Erhöhung der Dosis an, die Verabreichung von 10 mg/kg/Tag hatte bereits eine fast 50%ige Verminderung der Jodaufnahme zur Folge ($P < 0{,}02$). Die quantitative Verminderung war 6 Stunden nach Injizierung des Mittels am ausgeprägtesten, die P-Werte beziehen sich ebenfalls auf diesen Zeitpunkt.

Die Hemmung manifestierte sich jedoch nicht nur in der quantitativen Verminderung der Jodspeicherung, sondern auch darin, daß sich der Verlauf der Speicherungskurven in charakteristischer Weise veränderte. Die den

Aufnahmeabschnitt der Kurve charakterisierende Angabe, der organische Bindungskoeffizient (α) wurde bei den Versuchs- bzw. Kontrolltieren auf Grund folgender Formel berechnet [13, 14]:

$$\frac{dT}{dt} = \alpha S - \sigma T$$

wobei T den Jodgehalt der Thyreoidea, S den Jodgehalt des Serums und σ den sog. Mobilisationskoeffizient bedeutet. Der Wert des α-Koeffizienten, dessen Schwankungen eine Funktion der Aufnahmeverhältnisse sind, beträgt bei normaler Schilddrüsenfunktion durchschnittlich 0,1 [4].

Bei der Berechnung der α-Werte, die anhand der auf den Aufnahmekurven ersichtlichen Meßpunkte erfolgte, erhielten wir folgende Ergebnisse:

$$\alpha_{\text{Kontrolle}} = 0{,}14\ \text{h}^{-1}$$

$$\alpha_{5\ \text{mg/kg/Tag}} = 0{,}085\ \text{h}^{-1}$$

$$\alpha_{10\ \text{mg/kg/Tag}} = 0{,}026\ \text{h}^{-1}$$

Die bei den unbehandelten Kontrolltieren ermittelten Resultate entsprachen annähernd dem in der Literatur angegebenen Wert von 0,1, während die auf Grund der Aufnahmekurven der behandelten Tiere berechneten Koeffizienten auf Hypofunktion hinwiesen.

Angesichts des Umstands, daß für die auf den Kontrollkurven nach 24 Stunden wahrnehmbare Aktivitätsverminderung die Mobilisation der Hormone verantwortlich ist, dürfte angenommen werden, daß das Ausbleiben dieser Verminderung, welche Erscheinung besonders bei den mit großen Dosen behandelten Tieren zu beobachten war — mit der gehemmten Mobilisation der aktiven Hormone zusammenhängt.

Für die Entstehung einer auf Hypofunktion weisenden Speicherungskurve können folgende Faktoren verantwortlich sein: 1. der gehemmte Einbau des aktiven Jods in die Hormone, 2. die gehemmte Mobilisation der Hormone, und 3. die gemeinsame Wirkung beider Ursachen.

Zwecks Klärung des Problems wurden radiopapierchromatographische und autoradiographische Plasmaanalysen vorgenommen [1, 8, 9]. Die Resultate veranschaulicht Abb. 2.

Wie ersichtlich, änderte sich die prozentuale Zusammensetzung der verschiedenen Hormonfraktionen bei den behandelten Ratten in bedeutendem Maße. Besonders auffallend war die Veränderung bei den mit einer Tagesdosis von 10 mg/kg Körpergewicht behandelten Tieren, bei denen sich die Menge des Dijodtyrosins von 33% auf 20% verminderte, während sich der Wert der anorganischen Jodphase auf mehr als das Doppelte erhöhte. Die für die

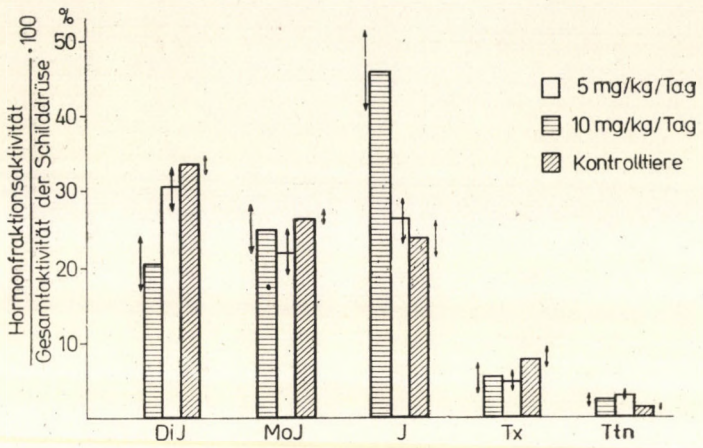

Abb. 2. Prozentualer Jodhormongehalt der Schilddrüse der mit wasserlöslichem Desoxycorton behandelten Tiere 48 Stunden nach ^{131}J-Verabfolgung. Die Pfeile zeigen die Standarddeviation

Änderung der einzelnen Komponenten charakteristischen *s*-, *t*- und *P*-Werte sind in Tabelle I dargestellt.

Tabelle I

Signifikanz der Veränderungen der Abbildung 2

	Dijodtyrosin		Monojod-tyrosin		Jodid		Thyroxin		Trijod-thyronin	
	Gruppe I	Gruppe II	Gruppe I	Gruppe II	Gruppe I	Gruppe II	Gruppe I	Gruppe II	Gruppe I	Gruppe II
s	2,6	3,58	2,85	2,92	2,74	5,14	1,18	1,68	0,25	0,66
t	3,25	8,45	2,70	0,99	0,98	8,55	4,25	3,45	6,80	4,46
P	$<0{,}01$	$\leqslant 0{,}01$	$<0{,}02$	$>0{,}05$	$>0{,}05$	$\leqslant 0{,}01$	$<0{,}01$	$<0{,}01$	$<0{,}01$	$<0{,}01$

Die bei den mit einer Dosis von 10 mg/kg/Tag vorbehandelten Tieren erhaltenen Ergebnisse der Plasmaanalyse sind in Abb. 3 zusammengefaßt.

Wie darauf die Angaben der Abb. 3 hinweisen, war die Di- bzw. Monojodtyrosinkonzentration im Plasma der behandelten Tiere etwa 4—5mal höher als bei den Kontrolltieren, während sich der Thyroxingehalt des Plasmas auf etwa die Hälfte verminderte. Die Gesamtmenge des sich in organischer Verbindung befindlichen Jods war somit sowohl bei den behandelten als auch bei den unbehandelten Tieren annähernd gleich groß. Diese Erscheinung wurde auch durch die Ergebnisse der im Plasma organisch gebundenen Jod-Bestimmungen unterstützt.

Diese Resultate wiesen darauf hin, daß die organisch gebundene Jodmenge im Laufe der Mobilisation in das Plasma der mit Desoxycorton behandelten Tiere in einer ganz anderen Zusammensetzung gelangt. Die von den Kontrollwerten abweichenden Resultate enthält Tabelle II.

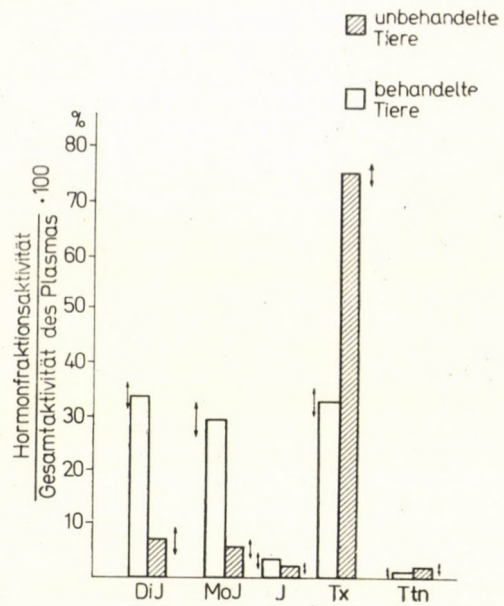

Abb. 3. Prozentualer Plasma-Jodhormongehalt der mit 10 mg/kg/Tag wasserlöslichem Desoxycorton behandelten Tiere 48 Stunden nach ^{131}J-Verabfolgung. Die Pfeile zeigen die Standarddeviation

Tabelle II

Signifikanz der Veränderungen der Abbildung 3

	Dijodtyrosin	Monojodtyrosin	Jodid	Thyroxin	Trijodthyronin
s	1,84	3,38	0,30	3,78	0,26
t	13,23	15,63	8,92	24,53	0,17
P	$\leqslant$0,01	$\leqslant$0,01	$\leqslant$0,01	$\leqslant$0,01	$\geqslant$0,05

Sämtliche angeführten Ergebnisse stellen die in einem gewissen Zeitpunkt — namentlich 48 Stunden nach Verabfolgung des ^{131}Jods — ermittelten Werte dar. Die prozentuale Zusammensetzung der inaktiven Serum-Jodhormone ist in den auf die Verabreichung folgenden verschiedenen Zeitpunkten unterschiedlich [2]. Da jedoch sämtliche unserer Untersuchungen nach 48 Stunden stattfanden, können die Resultate ohne Korrektion verglichen werden.

Besprechung

Die Verminderung der Jodaufnahme und die signifikante Erhöhung des anorganischen Jodids zu Lasten der Hormonfraktionen in der Schilddrüse, fer-

ner die hochgradige Verminderung des Plasmathyroxingehalts, erlauben die Folgerung, daß unter Wirkung von wasserlöslichem Desoxycorton die Jodhormonproduktion gehemmt ist. Die bedeutendste Störung verursachte die Verabfolgung des Mittels in der prozentualen Zusammensetzung der Plasmajodhormonfraktionen. Derartige Störungen kommen im Organismus in dem Fall vor, wenn sich der TSH-Spiegel vermindert. In Anbetracht dessen, daß das Desoxykortikosteron-Glykosid primär die ACTH-Produktion der Adenohypophyse hemmt, kann angenommen werden, daß sich auf Wirkung des Mittels auch die Ausscheidung des TSH vermindert. Infolge der verringerten TSH-Blutkonzentration ändern sich alle Phasen der Jodhormonsynthese in Richtung der Hypofunktion.

Das Ziel unserer Untersuchungen war nicht die genaue Bestimmung des sekundären Nebenwirkungsmechanismus bzw. Angriffspunkts des Desoxycortons, sondern lediglich die Erweiterung der mit dem Medikament zusammenhängenden Kenntnisse.

LITERATUR

1. Block, R. J., Werner, S. C., Mandl, R. H.: Arch. Biochem. **73,** 9 (1958).
2. Block, R. J., Werner, S. C., Mandl, R. H., Row, V. V., Radichevich, J.: Arch. Biochem. **88,** 98 (1960).
3. Dunlop, D., Davidson, S., Alstead, S.: Textbook of Medical Treatment. Livingstone Edinburgh 1961, p. 395.
4. Eichhorn, O.: Schweiz. med. Wschr. **85,** 879 (1955).
5. Felt, V.: Čs. Gastroent. Výž. **19,** 3 (1965).
6. Földes, J., Krasznai, I., Papp, M., Meggyesi, K., Gyertyánffy, G.: Acta med. Acad. Sci. hung. **20,** 23 (1964).
7. Galbraith, H. J. B., Richardson, J. S.: Brit. med. J. **2,** 671 (1958).
8. Gólián-Bartha, K.: Kiserl. Orvostud. **15,** 622 (1963).
9. Gólián-Bartha, K.: Acta pharm. hung. **35,** 129 (1965).
10. Hill, S. R., Reiss, R. S., Forsham, P. H., Thorn, G. W.: J. Clin. Endocr. **10,** 1375 (1950).
11. Ingbar, S. H., Freinkel, N.: Metabolism **5,** 652 (1956).
12. Krawutschke, R., Radenbach, K. L.: Med. Klin. **57,** 512 (1962).
13. Kutka, M., Németh, S.: Endokr. pol. **16,** 493 (1965).
14. Kutka, M., Licko, V.: Naturally Occurring Goitrogens and Thyroid Function. Publishing House of the Slovak Academy of Sciences. Bratislava 1964, p. 141.
15. Mosonyi, L.: 10. Weimarer Therapietagung 1964.
16. Nagy, J.: Magy. Radiol. **9,** 39 (1957).
17. O'Neal, L. W., Heinbecker, P.: Endocrinology **53,** 358 (1953).
18. Perlmutter, M., Cohm, H.: Amer. J. Med. **36,** 883 (1964).
19. Pitt-Rivers, R., Tata, J. R.: The Thyroid Hormones. Pergamon Press, London 1959, p. 34.
20. Radenbach, K. L., Heinrich, E.: Hippokrates (Stuttg.) **32,** 267 (1961).
21. Remenár-Balogh, I., Szuchovszky, I., Kertai, P.: Kisérl. Orvostud. **12,** 655 (1960).
22. Remenár-Balogh, I., Kertai, P.: Orv. Hetil. **102,** 2141 (1961).

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BACTERIAL FLORA OF BRONCHIAL SECRETION

By

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(Received July 12, 1967)

A study was performed of 3867 bacterial cultures and 1407 sensitivity tests of 3682 bronchial secretion specimens obtained at 3602 bronchoscopies in 2432 children aged from 0 to 18 years. The patients were grouped as follows: 1. healthy controls, 2. active tuberculosis, 3. inactive tuberculosis, 4. post-tuberculous bronchiectasis, 5. rupture of lymph gland into a bronchus, 6. bronchial asthma, 7. chronic pneumonia, 8. chronic bronchitis, 9. bronchiectasis unrelated to tuberculosis.

Analysis of the results allowed the following conclusions.

1. There exists no obligate sterility of bronchial secretion even in healthy individuals.

2. The presence of *Neisseria* and streptococci in bronchial secretion should be regarded as definitely abnormal instead of being ascribed to bronchoscopic contamination.

3. There seem to be definite relationships between the bacterial flora of bronchial secretion and the severity of the bronchial process whether tuberculous or non-tuberculous in origin.

4. The access of pathogens to the bronchial lumen may be ascribed to morphological changes of the wall as well as to biochemical changes of the mucus.

5. Similar bacterial strains isolated from tuberculous and non-tuberculous subjects show different patterns of drug sensitivity.

6. Chloramphenicol and erythromycin may be regarded as the antibiotics of choice for haphazard treatment in acute recurrences of chronic respiratory disease.

7. Erythromycin seems to be specially suited for long-term preventive use in chronic respiratory disease, particularly in children.

The aetiologic role of bacteria in chronic respiratory disease presents many a problem. Nevertheless it seems certain that bacteria play a definite part in its maintenance and progression. No successful therapy is therefore conceivable without a reliable identification of the bacterial agents and the determination of their sensitivity to antibiotics [60, 61, 62]. The aim of the present study was to identify the bacteria occurring in the bronchial secretion of children, to establish their relationships with the severity of the condition, and to test their sensitivity to antimicrobial agents. This large-scale study was performed for the following reasons.

1. The diagnostic significance of bacteria demonstrable in bronchial secretion is still controversial. The bacterial population of the air passages has first been identified from the sputum by BEZANÇON and DE JONG in 1913 [8].

Owing to its simplicity, examination of the sputum is still one of the most currently employed procedures though there is ample proof of its limited value. LORIAN [45] reported inconsistent bacteriological findings of sputum and of bronchial secretion in no less than 73% of 900 comparative tests. PECORA et al. [53, 54] also obtained greatly varying results on comparing sputum, bronchial secretion and material obtained by intraoperative aspiration from the lower air passages. MISKOVITS et al. [47] found basically different bacterial populations in sputum, bronchoscopic samples and transtracheal punctates from subjects with chronic bronchitis. There is also evidence to the contrary. BARTMANN et al. [5] noted a uniformity of bacterial flora of sputum and of bacterial secretion in 90%, and LAPINSKY et al. [37] found a high percentage of correspondence between the bacterial populations of specially prepared sputum and of transtracheal aspirates.

2. The tests referred to in most of the pertaining papers are too small in number to be suited for conclusive statistical evaluation (Table II).

Material and method

The present study involved 3867 bacterial cultures and 1407 sensitivity tests of 3682 specimens derived from 3602 examinations of 2432 patients out of 4471 subjected to bronchoscopy in the period 1961 to 1966. The age of the patients ranged from 0 to 18 years.

After bronchoscopic intubation the secretion seen in the tracheal lumen was removed by a sterile suction tube as being potentially contaminated from the pharynx. This tube was discarded, a fresh sterile suction tube was introduced, connected with a glass container and the secretion was removed by means of a mechanic aspirator from the area of the bronchial tree where discharge was seen or where radiologic changes were present. In the absence of any manifest abnormality, the discharge was removed bilaterally from several sites of the main bronchi. In 80 cases the samples of each side were cultured separately. From certain samples several cultures were isolated. This is why the examinations and the cultures differ in number. The bacterial combinations are shown in Table III.

The samples were examined in the bacteriological laboratory of this Institute (Dr. G. MÁTYÁS). Each sample was inoculated on blood agar and on chocolate agar, moreover on broth. If after 24 hrs the solid media proved sterile whereas the broth culture showed bacterial growth, this was subcultured on blood agar. If the broth was also sterile at 24 hrs, it was incubated for another 24 hrs and if by the end of this period no bacterial growth was seen, this particular sample was considered sterile.

Drug resistance was tested on blood agar plates using Biotest discs. The strain to be tested was inoculated on the medium and this was kept at room temperature for 30 min. Then the paper discs impregnated with the drugs were laid on the surface of the media. This was followed by incubation at 37° C for 24 hrs. Zones of inhibition more than 20 mm in diameter were graded as "sensitive", those between 20 and 10 mm as "moderately sensitive", between 7 and 10 mm as "relatively resistant", and those of less than 7 mm as "resistant". In the case of polymyxin, "sensitivity" was scored from 11 mm onward. Sensitivity was tested against the following antimicrobial agents: chloramphenicol, erythromycin, oxytetracycline, tetracycline, chlortetracycline, neomycin, penicillin, streptomycin, polymyxin, sulphathiourea, sulphadimidine.

Distribution of the material was as follows:

Children with no evidence of pulmonary disease	372,	cultures 388
Children with pulmonary tuberculosis	1060,	cultures 1511
Children with non-specific respiratory disease	901,	cultures 1806

Table I

Results of 3867 cultures from 3682 bronchoscopical specimens in relation to disease groups

Disease group	No. of cases	No. of tests	No. of culturing	Sterile	Sterile %	No. of bacteria	Neisseria			Streptococcus non haemolyticus			Streptococcus haemolyticus			Staphylococcus aureus			Staphylococcus albus			Diplococcus pneumoniae			Escherichia coli			Klebsiella pneumoniae			Proteus vulgaris			Pseudomonas pyocyanea			Haemophilus influenzae			Non-identified, mostly gram-negative bacteria		
							No.	Rel. %	Abs. %	No.	Rel. %	Abs. %	No.	Rel. %	Abs. %	No.	Rel. %	Abs. %	No.	Rel. %	Abs. %	No.	Rel. %	Abs. %	No.	Rel. %	Abs. %	No.	Rel. %	Abs. %	No.	Rel. %	Abs. %	No.	Rel. %	Abs. %	No.	Rel. %	Abs. %	No.	Rel. %	Abs. %
No evidence of respiratory disease	372	384	388	292	75.26	96	51	13.14	53.13	33	8.51	34.38	0	0	0	2	0.52	2.08	2	0.52	2.08	3	0.75	3.13	2	0.52	2.08	0	0	0	1	0.26	1.04	0	0	0	1	0.26	1.04	1	0.26	1.04
Bronchial perforation	100	100	162	38	23.46	124	38	23.46	30.65	17	10.49	13.71	4	2.47	3.22	26	16.05	20.97	15	9.26	12.10	17	10.49	13.71	2	1.23	1.61	0	0	0	3	1.86	2.42	0	0	0	2	1.23	1.61	0	0	0
Active tuberculosis	803	1167	1222	649	53.11	573	199	16.29	34.73	216	17.67	37.70	4	0.33	0.70	49	4.01	8.54	36	2.94	6.29	29	2.37	5.06	10	0.82	1.75	2	0.16	0.35	3	0.24	0.51	6	0.50	1.05	10	0.82	1.75	9	0.74	1.57
Inactive tuberculosis	175	186	189	122	64.55	67	23	12.17	34.33	25	13.23	37.32	1	0.53	1.49	5	2.64	7.46	4	2.12	5.97	5	2.64	7.46	1	0.53	1.49	0	0	0	0	0	0	0	0	0	2	1.06	2.99	1	0.53	1.49
Post-tuberculous bronchiectasis	82	97	100	50	50.00	50	25	25.0	50.00	19	19.00	38.00	0	0	0	1	1.00	2.00	2	2.00	4.00	1	1.00	2.00	1	1.00	2.00	0	0	0	0	0	0	0	0	0	1	1.00	2.00	0	0	0
Bronchial asthma	135	166	175	88	50.29	87	34	19.43	39.07	30	17.14	34.48	0	0	0	7	4.00	8.05	3	1.71	3.45	6	3.43	6.09	4	2.29	4.06	0	0	0	1	0.57	1.15	0	0	0	2	1.14	2.30	0	0	0
Chronic bronchitis	159	262	283	77	27.21	206	67	23.68	32.52	64	22.61	31.07	0	0	0	23	8.13	11.17	3	1.06	1.46	18	6.36	8.74	6	2.12	2.91	0	0	0	1	0.35	0.48	0	0	0	23	8.13	11.17	1	0.35	0.48
Chronic pneumonia	383	522	557	241	43.27	316	97	17.41	30.70	89	15.98	28.16	1	0.18	0.32	40	7.18	12.66	21	3.77	6.65	28	5.03	8.86	13	2.33	4.11	1	0.18	0.32	2	0.36	0.63	7	1.26	2.21	13	2.33	4.11	4	0.72	1.27
Bronchiectasis unrelated to tuberculosis	224	718	791	123	15.55	668	147	18.81	22.00	165	20.86	24.70	6	0.76	0.90	57	7.20	8.53	21	2.66	3.14	73	9.23	10.93	13	1.64	1.95	2	0.25	0.30	1	0.13	0.15	11	1.39	1.65	156	19.72	23.35	16	2.03	2.40

Legends

Rel.% (relative percentage): global figure for all the cultures.
Abs.% (absolute percentage) refers to positive cultures.

Table II

Survey of the literature

Author	Clinical diagnosis	No. of cases	Source of specimen	No. of cultures	Sterile	Neisseria	Streptococcus non haemolyticus	Streptococcus haemolyticus	Diplococcus pneumoniae	Klebsiella pneumoniae	Haemophilus influenzae	Escherichia coli	Staphylococcus aureus	Staphylococcus albus	Proteus vulgaris	Pseudomonas pyocyanea	Non-identified	Non-pathologic	Miscellaneous	Aerobacter.	Other bacteria
Allibone et al.	bronchiectasis (child)	32	bronchoscopy	32							84%										
Allison et al.	bronchiectasis	100	bronchoscopy	100					11%	sometimes	63%		9%					7%			
Bacsa et al.	?	47	bronchial discharge	47	12								4		4	19			8		
Benstead	pulmonary tuberculosis	12	bronchoscopy		5	2	9				1			2							
	bronchitis	11	bronchoscopy		7	2	4				1			1							
	residual pneumonia	8	bronchoscopy		2	2	5														
Corell et al.	?	100	bronchoscopy	100	—	4	36		9				25	61	1	1				3	—
			surgical discharge	100	80	—	4		5				8	6	—	—				1	2
Csernohorszky et al.	?	151	bronchial discharge			115	203	16	21	9	2	8	22		1	3				1	30
Dowling et al.	chronic bronchitis + bronchiectasis	23	sputum	301					17%	13%	43%		11%					39%			
	bronchiectasis	14	sputum	203					14%	12%	53%		10%					32%			
Halász	acute bronchitis	126	?				70%		5%		5%	2%	10%		1%						
Kovács	pulmonary tuberculosis		bronchoscopy	630	435	46	11	17	30	7	5	10	9	24	16	17					18
	non-specific pulmonary disease		bronchoscopy	370	140	10	—	17	43	—	5	5	61	33	—	25					35
Lackner	chronic bronchitis	102	sputum			55.9%	61.9%				2%	13.7%	21.6%			3.9%				8.8%	16.6%
Lányi	chronic bronchitis	288	sputum	288	6 2%	5 2%	40 14%	18 6%	16 6%	15 5%	2 1%	3 1%	13 4%	22 8%	9 3%	4 1%			125 43%		
	chronic bronchitis	159	bronchoscopy	159	13 8%	2 1%	16 10%	6 4%	19 12%	14 9%	3 2%	5 3%	7 4%	12 7%	3 2%	5 3%			50 32%		
	chronic bronchitis	71	transtracheal puncture	71	24 34%	0	7 10%	4 7%	5 7%	5 7%	5 7%	1 1%	6 8%	11 15%	3 4%	0			0		
Miskovits et al.	chronic bronchitis	71	transtracheal puncture	71	24		7	4	5	5	4	1	6	11	3				3		
Rytel et al.	bronchiectasis	108	bronchoscopy		11		153		1	12	6		5			3				1	
	pneumonia	42	bronchoscopy		9		55		1	1	—		1			—				—	
	bronchitis	7	bronchoscopy		1		9		—	—	—		—			—				—	
	other pulmonary disease	11	bronchoscopy		2		18			1	—		2			—				—	
	no pulmonary disease	5	bronchoscopy		1		5			1	—		1			—				—	
Stuart-Harris	chronic bronchitis	113	sputum	172				8	87 50.5%	2	26 15%	5	20 11.6%								
Szita et al.	bronchiectasis	195	bronchoscopy	281		71 15.8%	215 47.7%	9 2%	24 5.3%	8 1.8%	4 0.89%	30 6.7%	13 2.9%		12 2.7%	15 3.3%	8 1.8%				41 9.1%

In addition we evaluated 162 cultures of bronchial specimens from 100 cases with lymphoglandular bronchial perforation. Owing to the relative rarity of this complication, the material from 1961/66 had to be completed with cases from the previous years, to make up one hundred cases. Otherwise there was no selection or grouping whatsoever.

The patients were divided into the following diagnostic sub-groups (numerical data being presented in Table I).

I. Normal

Healthy controls, judged by the standards of negative radiographic and bronchoscopic findings.

II. Pulmonary tuberculosis

1. *Active*

This group comprised patients with primary or post-primary tuberculosis— with the exception of bronchial perforation—regarded as active in the moment of bronchoscopy on grounds of the clinical course confirmed by radiographic evidence and by cultures positive for tubercle bacilli.

2. *Inactive*

This group comprised cases where the process, according to the above criteria, was no longer considered active.

3. *Post-tuberculous bronchiectasis*

The patients had been under care for bronchoscopically verified bronchial perforation five to ten years earlier. All were symptom-free. Residual bronchiectasis was diagnosed on the evidence of bronchography.

4. *Lymphoglandular bronchial perforation*

In the patients of this group bronchoscopically confirmed bronchial perforation had taken place at the time of the study.

III. Non-specific respiratory disease

1. *Bronchial asthma*

These were patients with bronchial asthma confirmed beyond any doubt during their stay but with no evidence of other bronchopulmonary disease.

2. *Chronic bronchitis*

The criteria of this group were: a history suggestive of bronchitis of at least three months in duration, consistent with the clinical and radiological signs, but exclusive of other respiratory disease and with bronchoscopic evidence of chronic inflammation of the bronchial mucosa.

3. *Chronic pneumonia*

In these patients changes of the pulmonary parenchyma typical of pneumonia and exclusive of tuberculosis or of bronchiectasis had been present for more than four weeks. The majority had been referred to us from other institutes after long-continued antibiotic treatment. Patients with pulmonary abscess, gangrene or empyema were excluded from this group.

4. *Bronchiectasis unrelated to tuberculosis.*

The criteria included bronchographic evidence of bronchiectasis and proof of its non-tuberculous origin.

Results

The cultures yielded 12 bacterial strains. Table 1 presents the numerical data in terms of relative and absolute percentage.

The incidence of sterile bronchial secretion in the individual groups has been found to diminish in the following order: normal controls, inactive tuberculosis, active tuberculosis, post-tuberculous bronchiectasis, bronchial asthma, chronic pneumonia, chronic bronchitis, bronchial perforation, bronchiectasis unrelated to tuberculosis. It was, however, the severity of the bronchial process rather than its aetiology which seemed to determine the order of succession. In the group with no evidence of pulmonary disease 75% of the

Table III

Bacterial associations in bronchial secretion

Bacterial associations Upper: specific Lower: non-specific	Neisseria	Streptococcus non haemolyticus	Streptococcus haemolyticus	Staphylococcus aureus
Neisseria	■	33	2	5
Streptococcus non haemolyticus	37	■	1	1
Streptococcus haemolyticus	1	0	■	0
Staphylococcus aureus	12	5	1	■
Staphylococcus albus	5	3	0	0
Diplococcus pneumoniae	21	1	0	8
Escherichia coli	1	0	0	3
Klebsiella pneumoniae	0	0	0	0
Proteus vulgaris	0	0	0	0
Pseudomonas pyocyanea	1	0	0	0
Haemophilus influenzae	13	1	1	1
Unidentified, gram-negative	0	0	0	0

The right side of the dividing line is occupied by the tuberculous, its left side by the non-tuberculous material.

specimens were found sterile as against 15% in the group with non-tuberculous bronchiectasis. The groups of bronchial asthma and of post-tuberculous bronchiectasis, each yielding 50% sterility, i.e. 50% positive cultures, occupy a borderline position. It is remarkable that the incidence of *Neisseria* and of Streptococcus was significantly higher in the groups with pulmonary changes than in the normal controls, and particularly high in post-tuberculous bronchiectasis and in chronic bronchitis.

Fig. 2 gives a comparative survey of the occurrence of bacteria generally considered pathogenic as well as of *Neisseria* and Streptococcus. These figures represent the absolute percentage of these organisms in the positive cultures. The incidence of the pathogens in the individual groups increased in the following order: healthy controls, post-tuberculous bronchiectasis, bronchial asthma, active tuberculosis, inactive tuberculosis, chronic bronchitis, chronic pneumonia, bronchiectasis unrelated to tuberculosis, bronchial perforation.

The absolute and relative figures of sensitivity have been tabulated globally instead of giving the values separately for each group (Table IV). The 12 strains grown from the specimens were tested against 11 antimicrobial agents.

of 208 patients

Staphylococcus albus	Diplococcus pneumoniae	Escherichia coli	Klebsiella pneumoniae	Proteus vulgaris	Pseudomonas pyocyanea	Haemophilus influenzae	Unidentified, Gram-negative
7	5	1	0	0	1	0	0
0	4	0	0	0	0	0	0
2	0	0	0	0	0	0	0
0	9	0	0	0	0	0	0
■	2	0	0	0	0	0	1
1	■	0	0	1	0	0	0
1	0	■	0	0	0	0	0
0	1	0	■	0	0	0	0
0	0	1	0	■	0	0	0
0	0	0	1	0	■	0	0
3	6	1	0	0	1	■	0
0	1	0	0	0	0	1	■

Fig. 3 presents the comparative values for the groups of tuberculosis (exclusive of bronchial perforation), bronchial perforation, and non-specific respiratory disease. These values represent the overall figures of the "sensitive" and "moderately sensitive" groups.

Discussion

The present analysis was concerned with the following questions:

1. In the healthy individual, is the bronchial tree sterile?

2. Is the presence in bronchial secretion of bacteria of the normal pharyngeal population of any pathogenic significance or is it merely a result of contamination?

3. Is there any relationship between the severity of the bronchial process and the bacterial flora?

4. Is the growth of a bacterial population in the bronchial lumen sufficiently explained by morphologic changes of the wall of bronchi and of the mucous membrane or are there any additional factors involved?

5. Do identical bacterial strains grown from tuberculous and non-tuberculous subjects exhibit the same pattern of drug resistance?

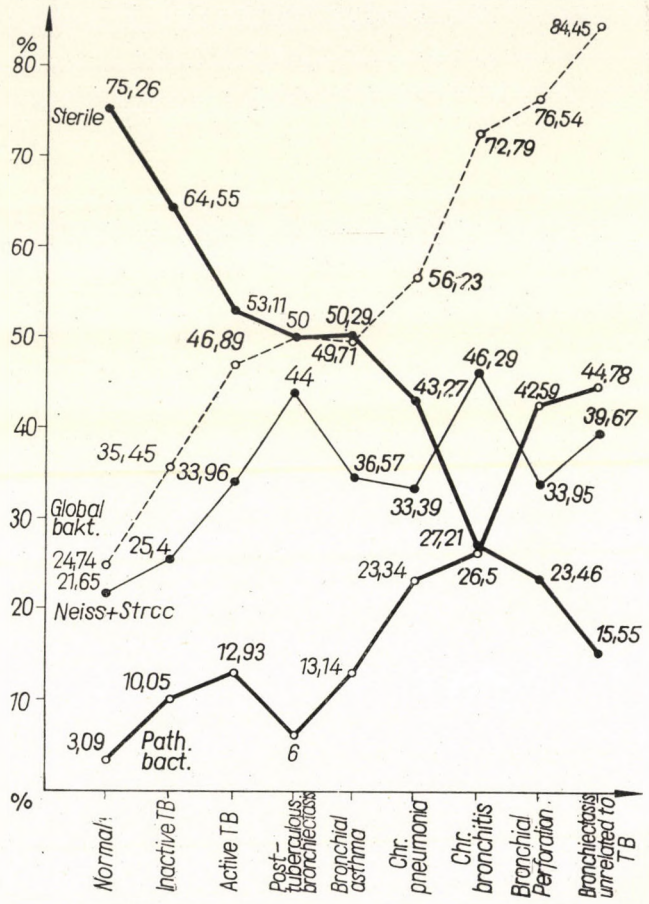

Fig. 1. Distribution of sterile and positive cultures in relation to disease groups. The overall percentage of sterile cultures is represented by the descending heavy line, that of the totality of bacterial cultures by the broken line, that of *Neisseria* and Streptococcus by the thin line, that of pathogens (*Streptococcus haemolyticus*, *Staphylococcus aureus*, *Staphylococcus albus*, *Diplococcus pneumoniae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas pyocyanea*, *Haemophilus influenza* and unidentifiable bacteria) by the ascending heavy line

6. Which are the most potent antibiotics for the control of exacerbation of chronic respiratory disease in children and which are best suited for long-term preventive use?

Ad 1. Bronchial secretions of healthy children yielded bacteria in 25%, i.e. *Neisseriae* and streptococci which belong to the normal bacterial population of the pharynx, in 13% and 8.5%, respectively, and other strains in 3% (Table I and Fig. 1). These findings contradict the view represented by LEES et al. [42], LAURENZI et al. [38], KOVÁTS and NYIREDY [31] and SZITA et al. [65] that the bronchial secretion of healthy individuals is sterile, while supporting

the observations of ROHMER [56] and CSERNOHORSZKY et al. [14] that the bacteria of the pharynx may occur also in the bronchial lumen and become pathogenic under special conditions.

Since the bronchial tree communicates with the outside world it is of necessity subject to contamination with microbial agents. These fall, however, very soon victim to the physiologic functions of the ciliated epithelium, the bactericidal activity of normal bronchial mucosa and other factors. As demonstrated by LAURENZI et al. [40] in animal experiments, 95% of the bacteria introduced into the bronchi disappear from there in six hours. In the healthy subject bacterial clearance proceeds at a normal rate. This, naturally, implies that the bronchial lumen may at any time contain bacteria which have not yet been cleared away [23, 29, 39, 40]. It is therefore obvious that in case of large-scale culturing there is a probability of demonstrating bacteria in the bronchial secretion of healthy individuals, particularly of the normal pharyngeal population carried by the respiratory flow into the bronchial lumen.

Ad 2. There is a certain controversy concerning this point, too. CORELL et al. [13] as well as GERNEZ-RIEUX et al. [20] ascribed the occurrence of streptococci in bronchial secretion to contamination. LÁNYI [36] disagrees with these authors. BARTMANN et al. [6] stressed the pathogenicity of *Neisseria.* Our finding that the incidence of these bacteria was significantly higher in the respiratory disease groups than in the controls also contradicts the role of contamination (Fig. 1), unless we had any acceptable reason to consider bronchoscopy as a more probable source of contamination in chronic respiratory disease — particularly in post-tuberculous bronchiectasis and in chronic bronchitis — than in the absence of respiratory disease. We therefore regard *Neisseria* or streptococci as part of the bronchial population without having to incriminate a possible contamination by the bronchoscope for this finding.

Ad 3 and 4. Analysis of the present evidence decides the question about the relationship between the severity of the bronchial process and the bronchial flora in the affirmative. Fig. 1 clearly shows that the bacterial content of bronchial secretion increases in proportion to the severity of the process. In the four groups of greatest severity the incidence of bacteria greatly exceeded the incidence of the sterile cultures. Damage to the bronchial mucosa affects its clearing activity thus giving access to the bacterial population of the pharynx. This is at least what the high proportion of *Neisseria* and of streptococci found in the groups of post-tuberculous bronchiectasis and of chronic bronchitis seems to suggest.

The above relationship provides us with clues to the fourth question, namely whether the morphologic changes of the mucous membrane and of the wall of bronchi are the sole factors responsible for bacterial invasion of the lower airways. Confrontation of the data for the group of post-tuberculous bronchiectasis with those of the normal group clearly indicates that even re-

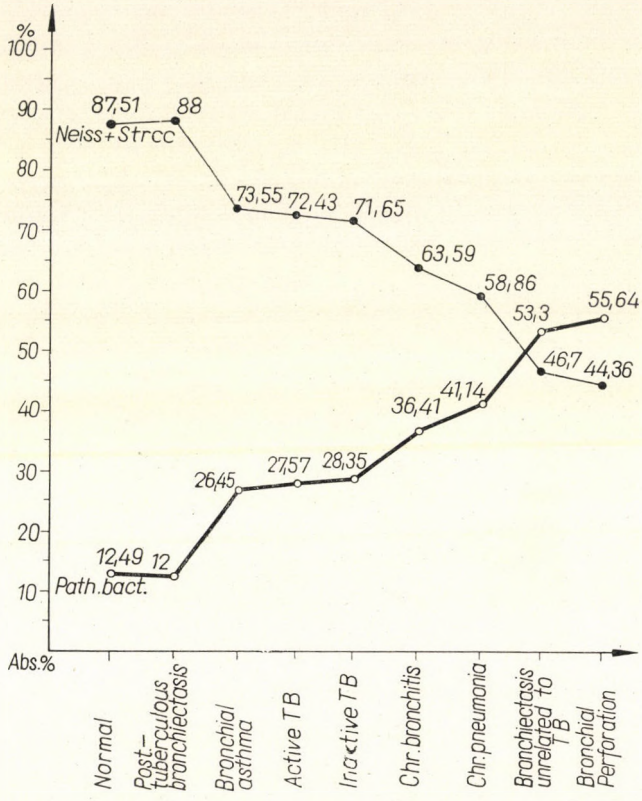

Fig. 2. Global figures of *Neisseria* and Streptococcus (upper line) and of pathogens (see legend for Fig. 1) in relation to disease groups, expressed in absolute percentage

sidual changes of healed bronchopulmonary processes may well account for bacterial invasion, though prevalently by non-pathogenic strains (Fig. 2). In the group of chronic bronchitis the incidence of positive cultures was still higher. Though here too the proportion of pathogens showed a substantial increase, it is nearly doubled by that of *Neisseria* and of streptococci. All this emphasizes the significance of the histological changes of the mucous membrane [25, 29, 35, 67]. The more extensive the changes affecting bronchial tissue and secretion, the more favourable the soil for bacterial growth. In the groups of bronchiectasis and of bronchial perforation not only the high incidence of bacteria, but also the prevalence of pathogens over *Neisseria* and streptococci was conspicuous.

The frequent occurrence of *H. influenzae* in chronic bronchitis and in bronchiectasis has been stressed by numerous authors [2, 3, 11, 16, 20, 25, 49]. This is in agreement with the present findings, though our figures are lower than those quoted in the literature, especially as far as chronic bronchitis is

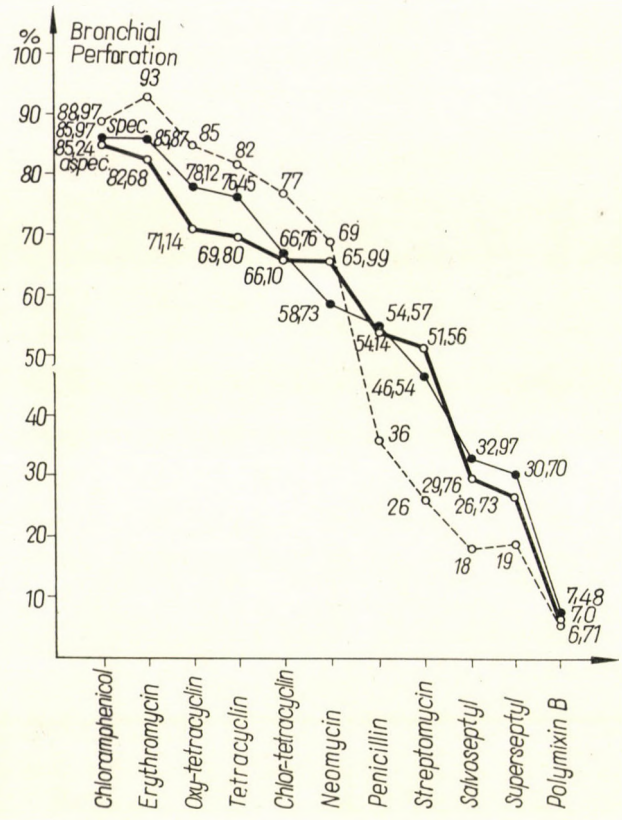

Fig. 3. Comparison of drug sensitivity of bacteria in three disease groups. Thin line: tuberculosis without bronchial perforation. Broken line: bronchial perforation. Heavy line: non-tuberculous respiratory disease. The figures refer to the global percentage of "sensitivity" and "moderate sensitivity"

concerned, in all probability because the authors referred to made no sharp distinction between chronic bronchitis and bronchiectasis.

The high incidence of staphylococci in the group with bronchial perforation points to the significance of staphylococcal superinfection in this complication involving gross destructive tissular changes.

Ad 5. We could find no comparative evidence in the literature concerning the drug resistance of identical strains isolated from tuberculous and non-tuberculous cases. Our finding that bacteria isolated from tuberculous cases are more sensitive to large-spectrum antibiotics than are similar strains originating from non-tuberculous material, furthermore the inverse response to streptomycin and to neomycin provided further proof that the long-continued use of antibiotics will invariably lead to the development of bacterial resistance. This is bound to frustrate the benefits of classical long-term antibiotic treat-

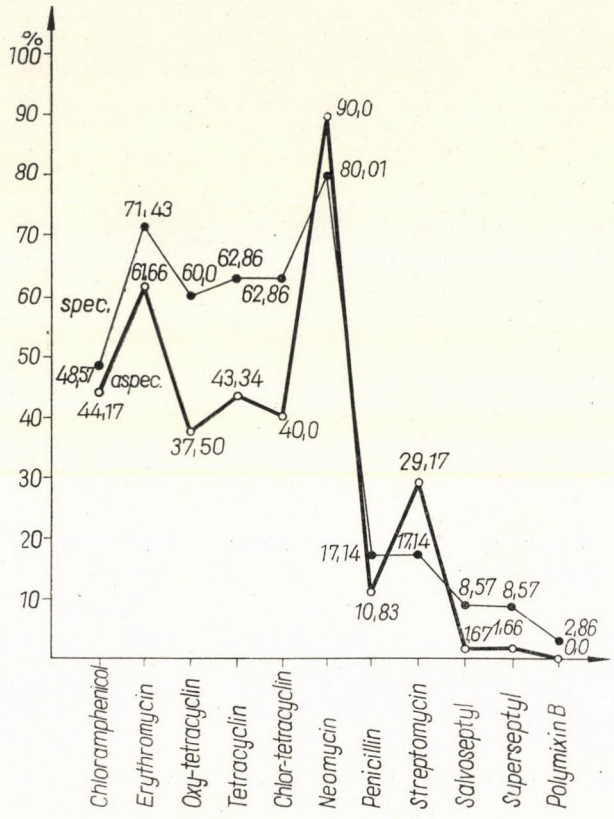

Fig. 4. Comparison of drug sensitivity of *Staphylococcus aureus* cultured from tuberculous and non-tuberculous material. Thin line: tuberculous, heavy line: non-tuberculous cases. The figures represent the global percentage of "sensitivity" and "moderate sensitivity"

ment of chronic respiratory disease and explains the demands for newer and newer antibiotics [41, 50, 66, 69] (Fig. 3). That the different patterns of drug sensitivity are entirely unrelated to the different bacterial populations of the two groups of disease is clearly illustrated by the example of *Staphylococcus aureus* (Fig. 4). The reduced resistance to streptomycin and to neomycin in the tuberculous groups finds its explanation in long-term streptomycin therapy of the primary disease and in the cross-resistance between the two antibiotics.

Ad 6. It is seen in Table IV and Fig. 3 that the overall sensitivity of the bacterial strains tested was highest to chloramphenicol and erythromycin while significantly lower to oxytetracycline and tetracycline and still less to the other drugs tested. It emerges from Fig. 5 that the bacterial strains generally considered pathogenic exhibited practically the same degree of sensitivity to chloramphenicol, erythromycin, oxytetracycline and neomycin.

Decision on the type of drug and on the duration of its use in chronic respiratory disease involves no little responsibility. Their prescription accord-

Table IV

Global results of 1407 sensitivity tests

Drug	No. of tests	Chlortetracycline				Oxytetracycline				Tetracycline				Chloramphenicol				Erythromycin				Neomycin				Penicillin				Polymyxin				Streptomycin				Sulphatiourea				Sulphadimidine			
Bacteria		S	MS	RR	R	S	MS	RR	R	S	MS	RR	R	S	MS	RR	R	S	MS	RR	R	S	MS	RR	R	S	MS	RR	R	S	MS	RR	R	S	MS	RR	R	S	MS	RR	R	S	MS	RR	R
Neisseria	204	57 27.94	71 34.80	22 10.79	54 26.47	100 49.02	67 32.84	13 6.37	24 11.77	90 44.12	71 34.8	18 8.82	25 12.26	188 92.16	8 3.92	2 0.98	6 2.94	106 51.96	64 31.37	4 1.96	30 14.71	78 38.24	63 30.38	18 8.82	45 22.06	44 21.57	44 21.57	18 8.82	98 48.04	4 1.96	11 5.39	20 9.81	169 82.84	54 26.47	40 19.61	14 6.86	96 47.06	27 13.23	28 13.73	16 7.84	133 65.20	26 12.75	23 11.27	18 8.82	137 67.16
Streptococcus non haemolyticus	498	216 43.37	144 28.92	21 4.21	117 23.50	280 56.23	107 21.48	25 5.02	86 17.27	273 54.82	103 20.68	24 4.82	98 19.68	422 84.74	54 10.84	6 1.21	16 3.21	444 89.16	32 6.43	7 1.40	15 3.01	75 15.06	153 30.72	60 12.05	210 42.17	279 56.02	81 16.27	29 5.82	109 21.89	7 1.40	6 1.21	11 2.21	474 95.18	81 16.27	168 33.73	67 13.45	182 36.55	100 20.08	95 19.07	36 7.24	267 53.61	79 15.86	90 18.07	40 8.03	289 58.04
Streptococcus haemolyticus	9	2	5	0	2	5	3	0	1	4	4	0	1	7	1	0	1	9	0	0	0	3	3	0	3	7	0	0	2	0	0	3	6	1	3	0	5	0	1	0	8	0	1	0	8
Staphylococcus aureus	179	70 39.11	13 7.26	3 1.68	93 51.95	67 37.43	13 7.26	4 2.23	95 53.08	74 41.34	14 7.82	3 1.68	88 49.16	68 37.99	18 10.06	5 2.79	88 49.16	104 58.10	15 8.38	16 8.94	44 24.58	106 59.22	53 29.61	4 2.23	16 8.94	15 8.38	5 2.79	5 2.79	154 86.04	1 0.56	0 0	1 0.56	177 98.88	25 13.97	21 11.73	14 7.82	119 66.48	3 1.68	2 1.11	3 1.68	171 95.53	2 1.11	4 2.23	4 2.23	169 94.43
Staphylococcus albus	76	52 68.43	11 14.47	2 2.63	11 14.47	52 68.43	12 15.79	1 1.31	11 14.47	54 71.06	11 14.47	1 1.31	10 13.16	57 75.0	5 6.58	0 0	14 18.42	68 89.47	0 0	3 3.95	5 6.58	36 47.37	34 44.74	1 1.31	5 6.58	16 21.05	6 7.90	1 1.31	53 69.74	7 9.21	0 0	7 9.21	62 81.58	31 40.79	15 19.74	9 11.84	21 27.63	3 3.95	5 6.58	2 2.63	66 86.84	4 5.26	7 9.21	2 2.63	63 82.90
Diplococcus pneumoniae	162	103 63.58	24 14.81	5 3.09	30 18.52	125 77.16	20 12.35	4 2.47	13 8.02	119 73.46	14 8.64	6 3.70	23 14.20	148 91.36	10 6.18	2 1.23	2 1.23	152 93.83	4 2.47	0 0	6 3.70	19 11.73	34 20.99	17 10.49	92 56.79	117 72.22	19 11.73	4 2.47	22 13.58	2 1.23	2 1.23	2 1.23	156 96.31	17 10.49	42 25.93	16 9.88	87 53.70	45 27.78	33 20.37	8 4.94	76 46.91	44 27.16	35 21.60	9 5.56	74 45.68
Escherichia coli	45	2	8	0	35	9	11	3	22	6	12	3	24	22	6	1	16	12	8	1	24	16	19	3	7	1	1	2	41	2	1	5	37	14	6	6	19	3	1	2	39	3	1	2	39
Klebsiella pneumoniae	4	1	0	0	3	1	0	0	3	1	0	0	3	1	0	0	3	0	0	0	4	4	0	0	0	0	0	0	4	1	0	0	3	3	1	0	0	0	0	0	4	0	0	0	4
Proteus vulgaris	11	0	1	1	9	1	2	1	7	1	2	1	7	8	0	0	3	1	1	0	9	6	3	0	2	0	0	0	11	0	0	0	11	5	3	0	3	0	0	0	11	0	0	0	11
Pseudomonas pyocyanea	24	2	0	2	20	2	0	4	18	2	0	2	20	2	2	5	15	0	0	2	22	13	1	9	1	0	0	3	21	5	0	7	12	5	2	3	14	2	3	1	18	0	1	3	20
Haemophilus influenzae	167	71 42.51	76 45.51	1 0.60	19 11.38	106 63.47	46 27.54	4 2.40	11 6.59	101 60.48	51 30.54	8 4.79	7 4.19	151 90.42	11 6.59	0 0	5 2.99	112 67.07	38 22.75	5 2.99	12 7.19	86 51.50	64 38.32	4 2.40	13 7.78	51 30.54	56 33.53	10 5.99	50 29.94	7 4.19	37 22.16	27 16.17	96 57.48	86 51.50	42 25.15	9 5.39	30 17.96	23 13.77	39 23.36	11 8.38	91 54.49	23 13.77	37 22.16	14 8.38	93 55.69
Unidentifiable, prevalently gram-negative	28	9	8	1	10	11	9	5	3	12	7	5	4	18	3	1	6	16	2	4	6	17	8	1	2	10	0	3	15	2	0	5	21	8	5	6	9	8	0	0	20	5	1	0	22
Total	1407	585 41.58	361 25.66	58 4.12	403 28.64	759 53.94	290 20.61	64 4.55	294 20.90	737 52.38	289 20.54	71 5.05	310 22.03	1092 77.61	118 8.39	22 1.56	175 12.44	1024 72.78	164 11.66	32 2.27	187 13.29	459 32.62	435 30.92	117 8.31	396 28.15	540 38.38	212 15.07	75 5.33	580 41.22	38 2.70	57 4.05	88 6.25	1224 87.0	330 23.46	348 24.73	144 10.23	585 41.58	214 15.21	207 14.71	82 5.83	904 64.25	186 13.22	200 14.21	92 6.54	929 66.03

Signs: S = sensitive MS = moderately sensitive
R = resistant RR = relatively resistant

Legend: The upper figures represent the absolute values, the lower ones the percentage.
The per cent values of absolute figures below 50 are not represented.

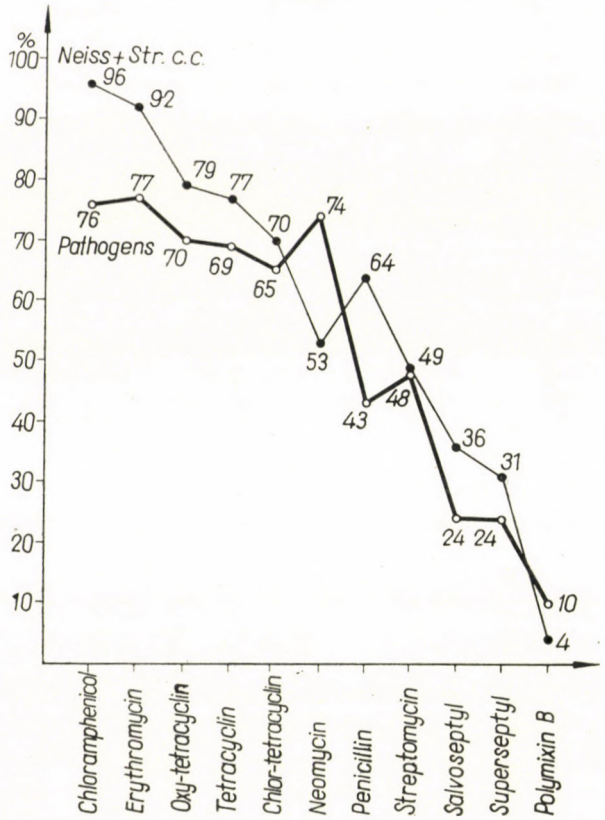

Fig. 5. Comparison of drug sensitivity of *Neisseria* and streptococci (thin line) with that of pathogens (heavy line). The figures represent the global percentage of "sensitivity" and "moderate sensitivity"

ing to the results of previous sensitivity tests [28, 31] is of course correct, but has the following practical limitations:

1. The bacterial flora of the sputum does not represent that of the bronchial tree.

2. The procedure of transtracheal puncture to obtain adequate specimens is unsuited for routine use.

3. Bronchoscopy allows to obtain reliable specimens but has not been universally adopted for this purpose.

4. In grave cases, especially in small children, bronchoscopy cannot be performed.

5. The patients, particularly infants or small children may be in a condition which warrants no delay involved by the sensitivity tests.

In this manner the physician has in many cases the only choice of a haphazard treatment for which chloramphenicol, erythromycin, oxytetra-

cycline and neomycin seem to be best suited [9, 13, 44, 57]. The toxicity of chloramphenicol and of neomycin limits, however, the use of these drugs and warrants particular caution [1]. In contrast to numerous authors who reported on a high sensitivity to sulphonamides, these drugs were rarely of benefit in the present cases [26, 52]. The low sensitivity to polymyxin found in the present study was likewise at variance with other results [57]. This also applies to *H. influenzae*, in contrast to the findings of FÜZI et al. [19] who found a 100% polymyxin sensitivity of *H. influenzae* strains isolated from tonsils. In our view, with the possible exception of a few particular cases, polymyxin has no place in the treatment of chronic respiratory disease.

As regards the proper selection of antibiotica for long-term prevention [46] we agree with KERTES [28] in prescribing the most specific drug if we know the result of sensitivity tests while resorting to the broadest possible spectrum in case of haphazard therapy. The widest range of efficiency is provided by chloramphenicol and erythromycin [9, 15, 24, 33, 48, 55, 57, 64, 69]. The toxicity of chloramphenicol makes it unsuited for long-term use [32]. Numerous authors [16, 17, 18, 48, 55, 68, 70] emphasize the benefits as well as the safety of chronic treatment with oxytetracycline or tetracycline. There is on the other hand evidence pointing to an increase of resistance to these antibiotics [16, 57]. STAUFFER [59] described severe dental changes produced by tetracycline during intrauterine life or later and cautions against the use of this antibiotic during pregnancy and in children before the sixth year. Apparently, erythromycin is the most efficient and at the same time the safest of the currently used drugs, therefore it is particularly suited for long-term treatment. However, to be of optimal benefit, this kind of treatment would have to be employed within the framework of a well-organized care of non-tuberculous chronic respiratory disease. This would have to include a bronchoscopic follow-up with repeated bacterial sensitivity tests providing for a correct selection of specific drugs as well as for the necessary adjustments during their use.

REFERENCES

1. Round table conference on the ototoxic effect of streptomycin (dihydrostreptomycin), Budapest 1967.
2. ALLIBONE, E. C., ALLISON, P. R., ZINNEMANN, K.: Brit. med. J. **1,** 1457 (1956).
3. ALLISON, P. R., GORDON, J., ZINNEMANN, K.: J. Path. Bact. **55,** 465 (1943).
4. BACSA, S., BÖLCS, M.: Tuberkulózis **16,** 341 (1963).
5. BARTMANN, K., BRANDT, H. J.: Tuberk.-Arzt **17,** 506 (1963).
6. BARTMANN, K., BRANDT, H. J., BLISSE, A.: Med. Thorac. **20,** 341 (1963).
7. BENSTEAD, J. G.: Lancet **1,** 206 (1950).
8. BEZANÇON, F., DE JONG, S. I.: cit. GERNEZ-RIEUX.
9. BOPP, K. PH., MANNS, KL., J. DÖRNER, G.: Med. Klin. **60,** 190 (1965).
10. BURMAN, I. O.: J. thorac. cardiovasc. Surg. **40,** 635 (1960).
11. BURNS, M. W., MAY, J. R.: Lancet **18,** 354 (1967).
12. CARSON, S., GOLDHAMER, R., CARPENTER, R.: Amer. Rev. resp. Dis. **93,** 86 (1966).

13. Corell, N. O., Johnston, K. C., Langston, H. T., Hubble, R. H.: J. thorac. cardiovasc. Surg. **37,** 367 (1959).
14. Csernohorszky, V., M. Ádám, M.: Orv. Hetil. **102,** 1376 (1961).
15. Davis, A. L., Drabow, E. J., Kaminski, Th., Tompsett, R., Mc Clement, J. H.: Amer. Rev. resp. Dis. **92,** 900 (1965).
16. Dowling, H. F., Mellody, M., Lepper, H., Jackson, G. G.: Amer Rev. resp. Dis. **81,** 329 (1960).
17. Francis, R. S., May, J. R., Spicer, C. C.: Brit. med. J. **1,** 979 (1961).
18. Francis, R. S., May, J. R., Spicer, C. C.: Brit. med. J. **1,** 728 (1964).
19. Füzi, M., Dénes, B., Szentgyörgyi, Zs.: Orv. Hetil. **105,** 391 (1964).
20. Gernez-Rieux, C. H., Voisin, C., Wattel, F., Mahieu, P.: Poumon **20,** 675 (1964).
21. Gernez-Rieux, C. H., Voisin, C., Havez, R., Watel, F., Aerts, C.: Sel. Papers (Hague) **10,** 21 (1967).
22. Gondkiewicz, M., Széll, E.: Orv. Hetil. **104,** 312 (1963).
23. Gosselin, R. E.: Amer. Rev. resp. Dis. **93,** 41 (1966).
24. Halász, Gy.: Tuberkulózis **16,** 211 (1963).
25. Holdaway, M. D., Turk, D. C.: Lancet **1,** 358 (1967).
26. Höhne, C., Patsch, R.: Zbl. Bakt. I. Abt. Orig. **202,** 227 (1967).
27. Kerrebijn, K. F., Van Geuns, H. A.: Sel. Papers (Hague) **9,** 70 (1965).
28. Kertes, I.: Továbbképző jegyzet. Budapest 1965.
29. Kovács, K.: Z. Tbk **124,** 16 (1965).
30. Kováts, F. sen., Nyiredy, G.: Bronches **9,** 403 (1959).
31. Kováts, F., Nyiredy, G.: A hörgők betegségei. Medicina, Budapest 1966.
32. Lackner, M.: Wien. med. Wschr. **117,** 123 (1967).
33. Lampe, W. T.: Dis. Chest **46,** 599 (1964).
34. Lányi, M.: Mschr. Tuberk.-Bekämpf. **7,** 411 (1964).
35. Lányi, M.: Orv. Közl. **2,** 22 (1966).
36. Lányi, M.: Orv. Hetil. **108,** 303 (1967).
37. Lapinsky, E. M., Flakas, E. D., Taylor, B. C.: Amer. Rev. resp. Dis. **89,** 760 (1964).
38. Laurenzi, G. A., Potter, R. T., Kass, E. H.: New Engl. J. Med. **265,** 1273 (1961).
39. Laurenzi, G. A., Guarneri, J. J., Endriga, R. B.: Med.Thorac. **22,** 48 (1965).
40. Laurenzi, G. A., Guarneri, J. J.: Amer. Rev. resp. Dis. **93,** 134 (1966).
41. Leduc, A.: Chemotherapia (Basel) **11,** 186 (1966).
42. Lees, A. W., McNaught, W.: Lancet **2,** 1112 (1959).
43. Lees, A. W., McNaught, W.: Lancet **2,** 1115 (1959).
44. Le Melletier, J., Browaeys, J.: Poumon **23,** 1 (1967).
45. Lorian, V.: Acta tuberc. scand. **43,** 159 (1963).
46. Meister, W.: Z. ärztl. Fortbild. **61,** 247 (1967).
47. Miskovits, G., Lányi, M., Dubay, M.: Orv. Hetil. **108,** 9 (1967).
48. Morgan, W. K. C.: Postgrad. Med **41,** 190 (1967).
49. Mulder, J.; cit. Lányi.
50. Orrego-Puelma, H., Botteselle-Pahul, O., Gajardo-Henriquez, J., Olivarez-Corona, J.: Dis. Chest **46,** 276 (1964).
51. Palva, T., Grönroos, J. A., Elo, R.: Acta tuberc. scand. **35,** 204 (1958).
52. Patsch, R., Höhne, C.: Zbl. Bakt. I. Abt. Orig. **202,** 220 (1967).
53. Pecora, D. V., Yegian, D.: New Engl. J. Med. **258,** 71 (1958).
54. Pecora, D. V., Kohl, M.: Amer. Rev. resp. Dis. **86,** 755 (1962).
55. Protivisky, R.: Med. Klin **59,** 285 (1964).
56. Rohmer: cit. Csernohorszky.
57. Rozgonyi, F., Valenta, B., Bratovics, I., Csira, B.: Orv. Hetil. **108,** 337 (1967).
58. Rytel, M. W., Conner, G. H., Welch, Ch. C., Kraybill, W. H., Edwards, E. A., Rosenbaum, M. G., Frank, P. F., Miller, L. F.: Dis. Chest **46,** 23 (1964).
59. Stauffer, U. G.: Helv. paediat. Acta **21,** 397 (1966).
60. Steiner, B., Putnoky, Gy., Kovács, K., Szabon, J., Földes, Gy.: Orv. Hetil. **102,** 244 (1961).
61. Steiner, B., Putnoky, Gy., Kovács, K., Földes, Gy.: Orv. Hetil. **102,** 1501 (1961).
62. Steiner, B., Putnoky, Gy., Kovács, K., Földes, Gy.: Orv. Hetil. **102,** 2407 (1961).
63. Steinert, Gy.: Orv. Hetil. **103,** 10 (1962).
64. Stuart-Harris, Ch.: Med. Thorac. **22,** 39 (1965).

65. Szita, J., Nyiredy, Gy., Ferenczy, S.: Acta microbiol. Acad. Sci. hung. **7,** 137 (1960).
66. Szövérffy, L., Miczbán, I.: Gyógyszereink **17,** 249 (1967).
67. Thiemann, H. H., Wohlrab, W.: XVIIth Congress of Bronchology, Prague 1967.
68. Wade, O. L., Elmes, P. C., Barteley, E.: Thorax **22,** 265 (1967).
69. Wendler, H.: Paediat. Prax. **6,** 215 (1967).
70. Zöllner, N., Parrisius, G., Linzenmeier, G.: Dtsch. med. Wschr. **88,** 1457 (1963).

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RELATIONSHIP BETWEEN THE MUCOPOLYSACCHARIDE CONTENT OF GASTRIC JUICE AND THE COMPOSITION OF SERUM LIPIDS

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A total of 75 patients with diverse abnormalities of lipid metabolism and 150 controls have been studied.

1. The correlations of the concentrations of gastric hexosamine, sialic acid and WINZLER's mucoid with the serum cholesterol level were practically the same in the controls and in hypercholesterolaemic subjects.

2. In clinical conditions associated with hypercholesterolaemia, the mean concentrations of hexosamine, protein-bound hexose, WINZLER-mucoid, and sialic acid in gastric juice were significantly lower than in the controls.

3. The lowest concentrations of the gastric MPS components were found in the group with atherosclerosis.

4. As concerns the relationship between the individual MPS components in gastric juice and serum of the same subject, hexosamine concentration showed a linear correlation in the controls as well as in the group with hypercholesterolaemia, whereas in the control group the curve was of a paraboloid character.

5. No relationship was demonstrable between the acidity of gastric juice and its MPS composition.

6. The MPS pattern of the gastric wall was similar in the group with atherosclerosis and the control group.

Atherosclerosis is known to change the composition of serum lipids, particularly to increase the low-density beta-lipoprotein (beta LP) fraction. There are, furthermore, indications in the literature that alimentary lipaemia is higher in subjects with ischaemic heart disease than in healthy individuals [1, 2, 3]. Certain authors ascribe this difference to a reduced clearing activity [4, 5, 6], an assumption lacking uniform experimental support [7, 8, 9, 10]. This inconsistency together with the fact that after intravenous administration of lipids no difference in alimentary lipaemia was demonstrable between subjects with coronary disease and healthy individuals [11, 12] has directed attention to the possible significance of fat absorption and of gastric secretion in atherosclerosis.

The studies of BRONTE-STEWART et al. [13, 14] are suggestive of the existence of inverse relationships between the degree of gastric acidity and that of alimentary lipaemia, thus linking up fat tolerance with gastric secretion. The finding that gastric anacidity is more frequent in subjects with a history of myocardial infarction than in healthy controls has been connected with a possible role of reduced gastric secretion in the production of ischaemic heart disease. It has been found furthermore that in individuals of blood group 0

the frequency of cardiac infarction was lower while that of gastric hyperacidity higher than in those of blood groups A and B [15, 16]. In this manner, ischaemic heart disease makes no exception to the other hitherto detected blood-group-associated diseases which are invariably related to the upper gastrointestinal tract [17].

Apart from hydrochloric acid, enzymes, etc., gastric juice also contains mucoids with diverse, partly well-known, biologic functions [18]. CANTONE et al. [19, 20, 21, 22] have shown the antilipaemic, clearing, and lipotropic activity of a complex of aminopolysaccharides isolated from gastric mucosa and gastric juice. This is in agreement with other observations [23, 24, 25, 26]. There is, in addition, evidence of a stimulating effect of this mixture on fat absorption [27]. The duodenal heparinoids also possess antilipaemic properties [28, 29, 30] and the drug Ateroid (Crinos, Milan), one of the numerous products serving for the reduction of the blood cholesterol level, contains heparinoids of duodenal origin.

It is an established fact that the blood group substance is also a mucopolysaccharide (MPS), demonstrable in the saliva and in the gastric juice of secretors [31, 32]. GLASS et al. were the first to identify an MPS-containing substance extracted from the gastric wall as being responsible for intrinsic factor activity [33, 34]. It has long been known that in pernicious anaemia which is inseparable from atrophy of the gastric mucosa, the blood cholesterol level is low and atherosclerosis is relatively rare though there are observations to the contrary [35]. Despite the high incidence of malabsorption including steatorrhoea after gastric resection, the serum lipid level may be high [36, 37, 38, 39]. Finally, in clinical or experimental atherosclerosis there is an increase in the blood MPS level [40, 41, 42, 43].

The above observations have prompted us to examine the possible relationship between the changes in the composition of serum lipids and in those of the MPS pattern of gastric secretion. Some of the results have already been published [44, 45]. In the present study we examined the MPS composition of gastric secretion in relation to the composition of MPS and of lipids in the blood serum partly of subjects with normal blood lipids, partly in clinical conditions affecting lipid metabolism. Finally the human gastric wall was studied for its MPS composition.

Material and methods

The MPS patterns of gastric secretion and blood serum as well as the total lipids, cholesterol, and beta-lipoproteins of blood serum were examined in a miscellaneous material of 150 subjects (87 females and 63 males from 19 to 76 years of age). The same parameters were determined in 75 patients, i.e. 47 females, 28 males, aged between 18 and 78, with the following clinical conditions: atherosclerosis (with myocardial infarction in the history), 24 cases; diabetes, 10 cases; diabetes with myocardial infarction in the history, 10 cases; hypercholesterolaemia of unidentified origin (probably connected with coronary sclerosis), 31 cases. Subjects with demonstrable gastrointestinal abnormality were excluded from the study.

Gastric juice was collected by means of a duodenal tube after an overnight fast. No secretory stimulants were applied, these having been found to stimulate MPS secretion too [46, 47]. Gastric juice contaminated with blood, bile, or residues of ingested food was discarded. The material immediately after collection was filtered and homogenized in a turmix-type homogenizer. The MPS components were estimated by the following methods: protein-bound hexose: STARY et al. [48]; WINZLER's mucoid: WEIMER and REDLICH-MOSHIN [49]; sialic acid: AYALA et al. [50]; fucose: JACUBEIT et al. [51]; hexuronic acid: DISCHE [52]; hexosamine: RIMINGTON [53]. The same MPS components of blood serum were estimated in parallel tests. In addition, serum-total lipids (KUNKEL et al. [54]), total cholesterol (RAPPAPORT and EICHHORN [55]) and beta-LP (BOYLE and MOORE [56]) were determined. The same methods were used for the assay of the MPS mixtures prepared from the gastric wall in our laboratory by the modified [57] method of DYRBYE-KIRK [58], using structurally intact gastric walls obtained partly from atherosclerotic subjects, partly from individuals with no evidence of vascular abnormality. The acid output of gastric juice was studied, blood group estimations (A, AB, B, 0) were performed as well.

Results

1. Relationship between MPS composition of gastric juice and serum lipid values

Fig. 1 shows that in healthy controls with serum cholesterol levels between 180 and 210 mg per 100 ml the hexosamine concentration of gastric juice was around 90 mg/g protein. Reduction in the hexosamine content of

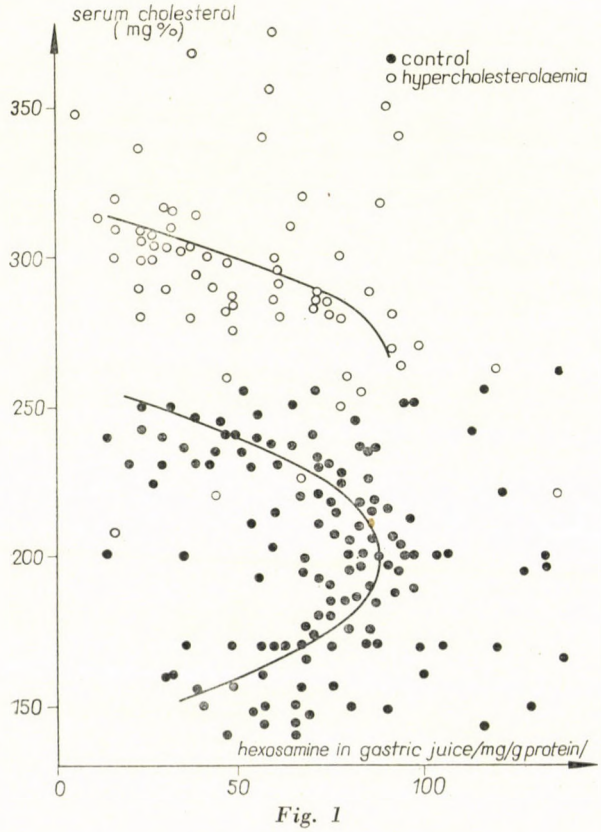

Fig. 1

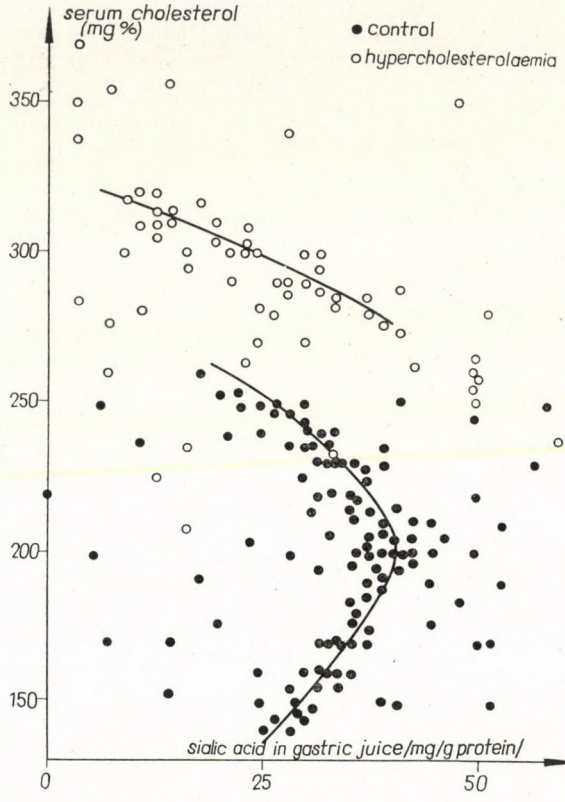

Fig. 2

gastric juice was associated with a rise in the serum cholesterol level. Similar correlations were found in the subjects with hypercholesterolaemia, with the only difference that here the graph was shifted to higher cholesterol values, although only one half of the parabola-shaped graph expressing these relationships was manifest, therefore the correlations may be considered linear. In hypercholesterolaemia the graph started with cholesterol values ranging between 260 and 290 mg per 100 ml, but the shape of the curve was essentially the same as in the former case.

Similar correlations were found between the sialic acid concentration in gastric juice and the blood cholesterol level (Fig. 2).

In correlation to the WINZLER-mucoid content of gastric secretion, the serum cholesterol level showed a similar distribution (Fig. 3). (The graphs were plotted on the basis of regression calculations.)

No correlation was found between serum cholesterol and the other gastric MPS components, further between the other serum lipids and gastric MPS.

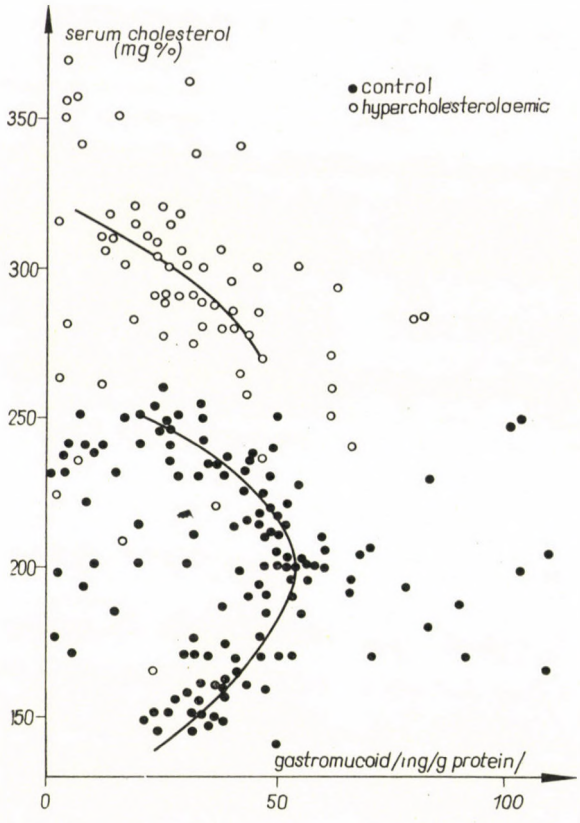

Fig. 3

2. MPS composition of gastric juice in lipid metabolism disturbances

The results presented in Table I show that in diseases associated with hypercholesterolaemia the MPS pattern in gastric juice was widely divergent from that found in persons with normal cholesterol levels. Evaluated by the two-sample *t*-test, mean hexose, hexosamine, sialic acid, and WINZLER-mucoid concentrations were significantly lower than in the controls, whereas the other MPS components revealed no significant change.

Table II shows values for various conditions, not including the 10 diabetic subjects with a history of myocardial infarction. On the grounds of mathematical analysis comprising analysis of variance (i.e. evaluation of overall scatter of the respective groups) and paired two-sample *t*-tests, the changes in the individual MPS components were as follows.

Hexose. In the diabetic group the mean values were slightly higher, whereas in the two other groups they were slightly lower (overall scatter 69) than in the control group.

Table I

(a) *Mean values of MPS components in gastric juice*
[mg/g protein]

	Hexose	Hexosamine	Sialic acid	Fucose	Mucoid	Hexuronic acid
Controls (150 cases)	143.2	76.4	37.7	2.23	42.0	3.35
Hypercholesterolaemia (75 cases)	123.6	62.0	28.8	2.56	30.1	2.82

(b) *Statistical significance of differences of mean values of the hypercholesterolaemic group from the control values*

	Hexose	Hexosamine	Sialic acid	Fucose	Mucoid	Hexuronic acid
t	1.99	3.3	3.6	not signif.	3.99	not signif.
p%	<5*	<1**	<0.1***		<0.1***	

* = significant
** = markedly significant
*** = highly significant

Hexosamine. In the atherosclerosis group mean values were significantly lower than in the other groups. In diabetes the differences were not significant and in "hypercholesterolaemia of other origin" they were at the borderline of significance (S = 28).

Sialic acid. While in the diabetics mean values were in the control range, in the atherosclerotics they were significantly lower. In the group with "hypercholesterolaemia of other origin" significance was less distinct (S = 16).

WINZLER-*mucoid.* The lowest mean was found in the group with atherosclerosis, whereas in the diabetes group and in "hypercholesterolaemia of other origin" reduction was not significant (S = 19). The figures of *fucose* and *hexuronic acid* are not represented in Table II, having revealed no change in any of the groups.

3. MPS values in gastric juice and serum

The hexosamine content showed a linear correlation in the hypercholesterolaemic and in the control groups (Fig. 4). Regression of the serum hexosamine values in relation to gastric juice was linear in both groups. The coefficients of regression were statistically significant.

Table II

(a) *Mean values of MPS components in gastric juice*
[mg/g protein]

	Hexose	Hexosamine	Sialic acid	Fucose	Mucoid	Hexuronic acid
Controls (150 cases)	143.2	76.4	37.7	2.23	42.0	3.35
Atherosclerosis (24 cases)	111.9	47.0	23.2	2.34	23.1	2.86
Diabetes (10 cases)	157.1	71.3	39.0	2.51	30.6	3.57
Hypercholesterolaemia of other origin (31 cases)	114.9	62.9	27.6	2.50	36.0	2.62

(b) *Statistical significance of differences of mean values of the hypercholesterolaemic group from the control values*

		Hexose	Hexosamine	Sialic acid	Fucose	Mucoid	Hexuronic acid
Atherosclerosis	t	2.0	4.8	3.9	not	4.0	not
	p%	<5.0*	<0.1***	<0.1***	signif.	<0.1***	signif.
Diabetes	t	0.6	0.7	0.2	not	1.7	not
	p%	>50	>40	>80	signif.	>5	signif.
Hypercholesterolaemia of other origin	t	2.1	2.6	3.1	not	1.4	not
	p%	<5*	<5*	<1**	signif.	>10	signif.

* = significant
** = markedly significant
*** = highly significant

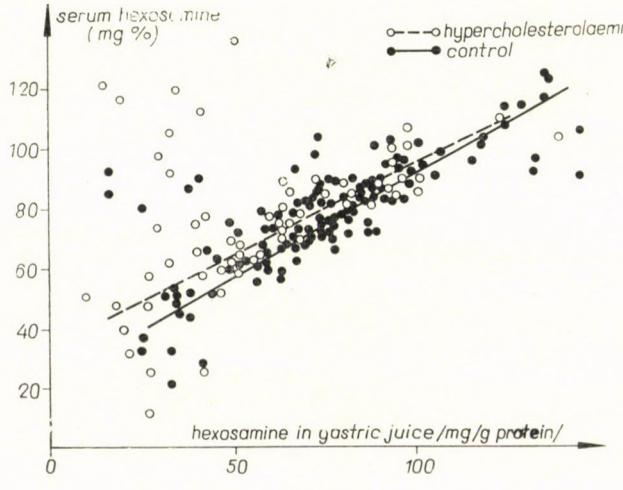

Fig. 4

The concentration of WINZLER-mucoid in gastric juice in relation to those in serum yielded a paraboloid curve. This unusual shape was due to the fact that medium concentrations of seromucoid were practically never associated with low gastric mucoid values, in opposition to low or high serum concentrations where such an association was not rare. Such relationships were not demonstrable in the group with hypercholesterolaemia. The other MPS components of gastric juice and of serum failed to reveal any definite relationship.

4. Gastric acidity and MPS composition of gastric juice

The mean values for the gastric MPS components in comparison to cases where the fasting juice contained no free acid did not diverge significantly from those presented in Tables I and II, i.e. the degree of acidity and the MPS pattern of gastric juice collected without stimulation of acid output revealed no correlations.

5. Gastric acidity and blood groups

In 150 control subjects, as shown in Table III, the incidence of anacidity varied with the individual blood groups; it was lowest with blood group 0. In the hypercholesterolaemic group the number of the cases tested was not sufficient for evaluation.

Table III

Distribution of subjects with anacidity according to blood groups
(150 controls)

Blood group	Anacidity of fraction collected after an overnight's fast (in per cent of examined cases)
A	52.5
B	62.7
AB	85.5
0	41.8

6. MPS pattern of human gastric wall

As shown in Table IV, the mean values for the individual components isolated from the gastric wall of normal and of atherosclerotic subjects displayed no significant difference.

Table IV

Mean concentrations of individual MPS components in normal and in atherosclerotic gastric wall
(mg/100 mg)

	Hexose	Hexosamine	Sialic acid	Mucoid	Hexuronic acid
No structural change (20 cases)	27.2	3.0	5.0	12.5	2.0
Evidence of atherosclerosis (20 cases)	25.0	2.6	4.4	10.0	3.0

Discussion

The above results were suggestive of a relationship between the MPS pattern of gastric juice and the serum cholesterol level. It seems that, if for reasons still unknown, the concentrations of the individual MPS components of gastric secretion are decreased, the serum cholesterol level is increased.

No definite correlation was found between the MPS components of serum and those of gastric juice.

GLASS et al. [59, 60] demonstrated chemical and electrophoretic differences between the mucoproteins in serum and those in gastric secretion. The present study failed to reveal any relationship between the degree of acidity and the MPS pattern of gastric juice. SCHRAGER [61] found reduced hexosamine concentrations in gastric juice after stimulating acid output.

It is known that the significance of gastric mucoids extends beyond the gastrointestinal tract. Outside their role as concerns intrinsic factor, blood groups, blood lipids, they have a diversity of other functions such as an inhibitory effect on bacterial virulence, connected with a growth-promoting activity on *Lactobacillus bifidus*, inhibition of virus haemagglutination, growth-promoting effect on young rats, enhancing nitrogen metabolism in the rat and haemopoietic activity in rats with induced anaemia, histamine-binding effect, influence on gastric motility etc. [18].

The present results point to a possible involvement of gastric MPS in the regulation of the serum cholesterol level and suggest that changes in gastric MPS concentration may also be responsible for certain disorders of lipid metabolism.

*

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REFERENCES

1. MORETON, J. R.: J. Lab. clin. Med. **35,** 373 (1950).
2. BARRITT, D. W.: Brit. med. J. **2,** 640 (1956).
3. BRONTE-STEWART, B., BLACKBURN, H. J.: Essential Fatty Acids. Ed. SINCLAIR, H. M. Butterworth, London 1957, P. 180.
4. OLIVER, M. F., BOYD, G. S.: Clin. Sci. **12,** 293 (1953).
5. BLOCK, W. J., BARKER, N. W., MANN, F. D.: Circulation **4,** 674 (1951).
6. HERZSTEIN, J., WANG-CHUN, I., ADLERSBERG, D.: Ann. intern. Med. **40,** 290 (1954).
7. MITCHELL, J. R. A., BRONTE-STEWART, B.: Lancet **1,** 167 (1959).
8. HOOD, B., ANGERWALL, G., ISAKSSON, B., WELIN, G.: Scand. J. clin. Lab. Invest. **6,** 1 (1954).
9. KAUFMANN, H.: Presse méd. **63,** 1045 (1955).
10. BAKER, S. P.: Circulation **15,** 889 (1957).
11. BOUCHIER, I. A. D., BRONTE-STEWART, B.: Lancet **1,** 363 (1961).
12. NESTEL, P. J., DENBOROUGH, M. A.: Lancet **2,** 828 (1961).
13. BRONTE-STEWART, B., KRUT, L. H.: J. Atheroscler. Res. **2,** 317 (1962).
14. MARKS, I. N., BANK, S., KRUT, L. H., BRONTE-STEWART, B.: Lancet **2,** 1068. (1962).
15. BRONTE-STEWART, B., BOTHA, M. C., KRUT, L. H.: Brit. med. J. **1,** 1646 (1962).
16. Editorial: Lancet **2,** 1232 (1966).
17. FRAZER-ROBERTS, J. A.: Brit. J. prev. soc. Med. **11,** 107 (1957).
18. GLASS, G. B. J.: Ann N. Y. Acad. Sci. **106,** 775 (1963).
19. CANTONE, A., RULLI, V., ROSSI, B.: Circulat. Res. **7,** 291 (1959).
20. ROSSI, B., RULLI, B.: Circulation **18,** 397 (1958).
21. CAPRARO, V., CANTONE, A., CRESSERI, A.: Naturwissenschaften **43,** 36 (1956).
22. CAPRARO, V., CANTONE, A.: Arch. ital. Biol. **96,** 187 (1958).
23. CUCURACHI, L., DETTORI, A. G.: Min. med **48,** 4503 (1957).
24. CASTELLANI, L., BERTOLA, G.: Min. med. **49,** 3453 (1958).
25. ANDREINI, A.: G. Geront. **8,** 559 (1960).
26. GOLDECK, H.: Haematol. lat. (Milano) **2,** 253 (1959).
27. PERESSINI, A., BOTTACIN, L., VOLPATO, S.: Acta paediat. lat. **11,** 693 (1958).
28. BIANCHINI, P., OSIMA, B.: Boll. Soc. ital. Biol. sper. **34,** 693 (1958).
29. GERŐ, S., RÉTSÁGI, GY., GERGELY, J.: Orv. Hetil. **103,** 1938 (1962).
30. KELLER, L., RÉTSÁGI, GY., BÁRY, L., GERGELY, J., GERŐ, S.: Orv. Hetil. **104,** 1024 (1963).
31. KABAT, E. A.: Blood Group Substances. Their Chemistry and Immunochemistry. Academic Press, New York 1956.
32. MORGAN, W. T. J.: Naturwissenschaften **46,** 181 (1929).
33. GLASS, G. B. J., BOYD, L. J., RUBINSTEIN, M. A., SVIGALS, C. S.: Science **115,** 101 (1952).
34. GLASS, G. B. J.: Acta haemat. jap. Suppl. **23,** 52 (1960).
35. MITCHELL, J. A., MITCHELL, S. C.: Amer. J. med. Sci. **247,** 36 (1964).
36. KIRKEBY, K.: Acta med. Scand. **178,** 433 (1965).
37. WALKER, R. S., WATSON, W. C., WATT, J. K.: Brit. med. J. **2,** 1438 (1958).
38. STEPHENSON, S. E. jr., YOUNGER, R. K., SCOTT, H. W.: Amer. J. Surg. **111,** 101 (1966).
39. BERKOWITZ, D.: Circulation **34,** Suppl. 3, 2 (1966).
40. VÉGH, M., BIHARI-VARGA, M.: Clin. chim. Acta **16,** 371 (1967).
41. JAKAB, L.: Orv. Hetil. **105,** 17 (1964).
42. SCHWARTZ, C. F., GILMORE, H. R.: Circulation **18,** 191 (1958).
43. MOGA, A., COUCOUIANU, M.: Rev. roum. Med. intern. **1,** 403 (1964).
44. GERŐ, S., BIHARI-VARGA, M., SZÉKELY, J., ROMICS, L., STEINGASZNER, O.: Orv. Hetil. **106,** 178 (1965).
45. SZÉKELY, J., BIHARI-VARGA, M., GERŐ, S.: Orv. Hetil. **108,** 927 (1967).
46. GOSSBERG, A. L., KOMAROV, S. A., SHAY, H.: Amer. J. Physiol. **162,** 136 (1950).
47. GLASS, G. B. J., BOYD, L., DREKKER, I. J., HEISLER, A.: Gastroenterology **20,** 430 (1952).
48. STARY, U., BURSA, F., KALEOGLÜ, O., BILEN, A.: Istanbul Univ. Tip. Fak. Mec. **13,** 243 (1950).
49. WEIMER, H. E., REDLICH-MOSHIN, J.: Amer. Rev. Tuberc. **68,** 594 (1952).
50. AYALA, W., MOORE, L. V., HESS, E. L.: J. clin. Invest. **37,** 81 (1951).
51. JACUBEIT, M., BRÜNGER, P., KNEDEL, M.: Klin. Wschr. **37,** 460 (1959).
52. DISCHE, Z.: J. biol. Chem. **167,** 189 (1947).

53. Rimington, C.: Biochem. J. **34,** 931 (1940).
54. Kunkel, H. G., Ahrens, E. A., Eisenmayer, E. L.: Gastroenterology **11,** 499 (1948).
55. Rappaport, F., Eichhorn, J.: Clin. chim. Acta **5,** 161 (1960).
56. Boyle, E., Moore, R. V.: J. Lab. clin. Med **53,** 272 (1959).
57. Dévényi, T., Bihari-Varga, M., Jakab, L., Gergely, J.: Kísérl. Orvostud. **15,** 326 (1963).
58. Dyrbye, M., Kirk, J. F.: J. Geront. **12,** 20 (1957).
59. Glass, G. B. J., Rich, M., Stephanson, L.: Gastroenterology **34,** 598 (1958).
60. Glass, G. B. J., Rich, M., Stephanson, L.: Fed. Proc. **16,** 46 (1957).
61. Schrager, J.: Nature (Lond.) **201,** 702 (1964).

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EFFECT OF OBSTRUCTION OF THE HEPATIC ARTERY ON THE COMPOSITION OF HEPATIC LYMPH

By

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After ligation of the hepatic artery in dogs the protein content of hepatic lymph remained lower, its relative albumin content higher than in blood plasma. This invalidates the claim that the lymph while still of the same protein composition as blood plasma when leaving the sinusoids, might become diluted by a filtrate produced in the peribiliary capillary system of lower permeability.

Electron microscopic studies have revealed the presence of large intercellular gaps between the endothelial cells of the sinusoids, the capillaries of the portal system, allowing the blood plasma to pass from the vascular lumen directly into Disse's spaces (FAWCETT [2], HAMPTON [3]). The size of these intercellular pores attains several hundred mμ which would be compatible with an entirely unrestricted passage of protein molecules through the sinusoid endothelial membrane without necessarily involving a process of molecular sieving (PAPPENHEIMER [4]). In this manner the extracellular fluid of the liver as well as the lymph draining from this organ would have to be of the same protein concentration as the blood plasma. Hepatic lymph has indeed a high protein content amounting to 80 to 90, but not to 100% of that of blood plasma (RUSZNYÁK et al. [5], YOFFEY and COURTICE [6]).

The fact that the liver receives blood not only from the portal vein but also from the hepatic artery led to the assumption (BRAUER [1]) that in the peribiliary capillary system which carries arterial blood, hydrostatic pressure must be considerably higher than in the sinusoids. These capillaries differ from the sinusoids in both structure and permeability, and the difference in protein concentration between hepatic lymph and blood plasma has been ascribed to admixture of less concentrated filtrate from these arterial capillaries. If this is so, after occlusion of the hepatic artery, the hepatic lymph must attain the composition of blood plasma. This possibility has been studied in the present experiments.

Material and method

The studies have been carried out in mongrel dogs of both sexes under intravenous chloralose anaesthesia (0.10 g per kg body weight). The abdomen was opened by midline incision, the main lymphatic trunk draining the liver was exposed at the hilum and a polythene catheter

was inserted into it with its tip directed distally. A broad band was placed loosely around the hepatic artery. Lymph was collected from the opened lymphatic for 20 to 30 minutes, then the band was tightened to occlude the artery. Thereafter, lymph was collected for further two periods of half an hour each.

Pressures were recorded by means of a Siemens electromanometer with a Statham strain gauge in the brachial artery, in the hepatic vein by means of a catheter via the right atrium, and in the portal vein by a plastic cannula introduced through one of the minor branches of the splenic vein. Infusions or blood transfusions were avoided during the experiment so as to keep the composition of plasma constant.

The protein content of plasma and lymph samples was determined after sulphuric acid digestion by the WAGNER—PARNASS microdestillation method. The protein fractions were estimated by paper electrophoresis (pH = 8.6; barbiturate buffer; acid fuchsin staining).

Results

A total of 18 experiments was carried out. The animals, with their abdomen open during the whole course of the study, exhibited a successive decline of arterial blood pressure, the values being 104 ± 5.8 mm Hg at the start and 82 ± 6.5 mm Hg at the end of the experiment. Pressures in the portal and hepatic veins also showed a slight reduction (Table I).

Table I

Mean blood pressure (mm Hg) during collection of hepatic lymph in 18 animals

	Femoral artery	Portal vein	Hepatic vein
Prior to experiment	104±5.8	6.8±0.4	5.6±0.6
At 30 minutes	96±6.2	5.6±0.3	4.8±0.5
At 60 minutes	82±6.5	4.5±0.3	4.0±0.3

Mean protein concentration in hepatic lymph, which was 5.02 g per 100 ml, i.e. 87.7% of that of the blood plasma prior to ligation of the hepatic artery, was little affected by ligation of the artery, since the values registered after the procedure did not amount to more than 89.8% of the protein concentration of blood plasma. No significant change was demonstrable during the subsequent 30 min period of study either (Table II).

Table II

Protein content (g/ 100 ml) of blood serum and hepatic lymph before and after ligation of hepatic artery

	Before n = 18	30 min n = 18	60 min n = 16
Serum	5.72±0.13	5.76±0.08	5.62±0.17
Lymph	5.02±0.16	5.06±0.16	5.08±0.18
Ly/Se×100	87.7±3.5	89.8±3.0	90.1 ±2.4

Table III

Protein fractions in serum and hepatic lymph before and after ligation of hepatic artery

	n	Albumin	α_1	α_2	β	λ
Before Se	18	33.5±1.3	5.3±0.4	14.5±0.9	26.5±1.6	19.9±1.1
Ly	18	36.5±2.0	5.5±0.4	12.8±1.0	23.3±1.0	21.4±1.6
30 min Se	18	34.1±1.2	6.1±0.3	14.2±0.6	25.1±1.4	20.4±1.3
Ly	18	35.2±1.8	5.4±0.5	13.7±0.9	25.2±1.6	21.7±1.9
60 min Se	16	34.3±1.6	7.2±0.8	14.6±1.3	23.6±1.3	20.1±1.3
Ly	16	37.7±1.3	5.7±0.4	12.4±0.8	24.3±1.6	19.9±1.1

Paper electrophoresis revealed the albumin concentration in hepatic lymph to be slightly higher, its globulin concentration slightly lower than in the blood plasma. Ligation of the hepatic artery did not affect significantly these relationships. Correlation of the values derived from 52 parallel electrophoretic studies of blood plasma and of hepatic lymph revealed the mean albumin concentration of blood plasma to be 34%, against 36.4% of hepatic lymph, the difference between the two values being significant (one-sample "t" test, $p < 0.01$).

Discussion

On the evidence of the present results, the protein content of hepatic lymph corresponds to nearly 90% of that of blood plasma while the albumin content slightly (but statistically significantly) exceeds that of blood plasma. Ligation of the hepatic artery though involving a reduction in arterial and portal pressures, in other words in the filtration pressure, caused no significant elevation in the protein content of hepatic lymph and left its relative albumin concentration unaffected.

The reduced absolute protein content combined with an increased relative albumin concentration points to a filtration through a membrane of restricted permeability. Would the peribiliary capillary system of the hepatic artery provide a membrane of this description, then on occlusion of the hepatic artery blood flow in this system would have to cease or at least the hydrostatic pressure would have to fall as low as to make filtration impossible. Or, in other words, on ligation of the hepatic artery, hepatic lymph would have to attain the protein content of blood plasma. Failure of this to happen in the present study pleads against the existence of an independent hepatic arterial capillary system combining a relatively high hydrostatic pressure with a greatly reduced

membrane permeability, and suggests that, despite its porous structure, the sinusoid membrane might be able to restrict the passage of large protein molecules. Thus, the differences in protein content and composition between blood plasma and hepatic lymph cannot be explained by the presence of two distinct capillary beds with different permeabilities.

REFERENCES

1. BRAUER, R. W.: Physiol. Rev. **43,** 115 (1963).
2. FAWCETT, D. W.: In: The Microcirculation. REYNOLDS, S. R. M., ZWEIFACH, B. W. eds. Univ. Illinois Press, Urbana, Ill. 1959. p. 1.
3. HAMPTON, J. C. (1958) Acta anat. (Basel) **32,** 262 (1958).
4. PAPPENHEIMER, J. R.: Physiol. Rev. **33,** 387 (1953).
5. RUSZNYÁK, I., FÖLDI, M., SZABÓ, G.: Lymphatics and Lymph Circulation. Pergamon Press, London 1960.
6. YOFFEY, J. M., COURTICE, F. C.: Lymphatics, Lymph and Lymphoid Tissue. Arnold, London 1956.

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BLOOD COAGULATION IN POLYCYTHAEMIA VERA

By

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Coagulograms of patients with polycythaemia vera have been studied during remissions and exacerbations of the disease. The majority of the complex changes demonstrable during exacerbations had a tendency to disappear in the course of remissions.

Polycythaemia vera is inseparable from hyperactivity of erythropoiesis, as reflected by an increase in the volume of circulating blood as also by excessive haematocrit (hcr) values going hand in hand with hyperviscosity of the blood. All these factors, joined sooner or later by circulatory failure, greatly prolong the circulation time. The platelet count is usually high. Alterations of the vascular wall are common, owing to the prevalence of the disease beyond the fourth or fifth decade. All this would sufficiently explain the high incidence of thromboembolic complications in polycythaemia vera. There are, however, still other causes. There is ample evidence [3, 4, 10, 20, 21] that abnormal plasma factors are common in the disease which thus may give rise to thromboembolic and haemorrhagic episodes in one and the same case [15, 17]. This indicates the presence of profound disturbances in blood coagulation.

After earlier studies [5, 6, 16, 18, 19], now the scarcely clarified problems of individual coagulation factors in the various stages of the disease have been investigated, with especial attention to the questions, (i) which of the current tests are the most reliable indicators of coagulation defects, and (ii) in what manner are these affected by exacerbations and remissions of the disease.

Material and method

Coagulation studies were carried out in 30 cases of polycythaemia vera, in 12 male and 18 female patients ranging in age from 33 to 71 (mean, 55) years. The tests were performed in 20 patients during exacerbation, in 8 during remission, in 2 each during both exacerbation and remission. In this manner we had a total of 32 examinations as a basis for evaluation. In all of these cases the tests included a coagulogram [8] and blood clot retraction. In addition, partial thromboplastin time (PTT) was examined in 5 cases during exacerbation. Blood counts, haemoglobin (Hgb), myelogram, haematocrit were routinely checked. These also guided us in determining whether the prevailing stage at the time of the coagulation studies was that of remission or of exacerbation. The criteria of exacerbation included an erythrocyte count of more than 5.5

Table I

Coagulation factors in

Stage	Total number of cases	Retraction		Platelet count		Thrombin inactivation		Toluidine blue time	
		N	R	N	I	N	I	N	I
Exacerbation	22	8	14	6	16	5	17	10	12
Remission	10	7	3	10	—	10	—	10	—

N = normal
I = increased
R = reduced

million, a haematocrit of more than 50%, haemoglobin above 16 g per 100 ml; hypercellular bone marrow, hepatosplenomegaly, dizziness, headaches, itching, haemorrhagic phenomena. During remission, blood counts, haemoglobin and haematocrit were normal, the liver and spleen were slightly enlarged or of normal size and the symptoms had generally disappeared.

The coagulogram was determined according to GERENDÁS [8], the platelet count by the direct method of FEISSLY and LÜDIN as modified by FISCHER and GREMER [7].

Statistical significance was determined by Student's t test.

Results

The factors of blood coagulation presented in Table I comprise bleeding time, recalcification time, Quick's prothrombin index, coagulation-accelerating effect of blood serum, prothrombin utilization, thrombin time, toluidine blue time, thrombin inactivation, platelet count and blood clot retraction, during both remission and exacerbation.

All coagulation factors showed some abnormality during exacerbation, whereas the values were entirely or almost entirely normal during remission.

The most distinct changes were revealed by the prothrombin index, prothrombin utilization, thrombin inactivation and platelet count.

The prothrombin index was low in 18 out of 22 cases during exacerbation and in 3 out of 10 during remission (mean, $55.6 \pm 22\%$ and $86.0 \pm 18\%$, respectively).

Prothrombin utilization (normal range: 40 to 70%) was reduced in 19 out of 22 examinations during exacerbation and in 6 out of 10 during remission (mean, $16.5 \pm 14.1\%$ and $37.9 \pm 12.8\%$, respectively). With both prothrombin index and prothrombin utilization the deviation was significant ($p = 0.1$ to 1.0%).

Thrombin inactivation (normal range: $k = 0.25$ to 0.35) was increased in 17 out of 22 cases during exacerbation and normal in all of the 10 examined cases during remission.

The platelet count exceeded 400,000 in 16 out of 22 instances during exacerbation, being above 700,000 in 9 cases. During remission, the platelet count ranged between 200,000 and 250,000.

polycythaemia vera

Thrombin time		Prothrombin utilization		Serum effect		Prothrombin index		Recalcification time		Bleeding time	
N	I	N	R	N	R	N	R	N	I	N	I
7	15	3	19	10	12	4	18	6	16	3	19
10	—	4	6	9	1	7	3	8	2	10	—

Retraction was slow in 14 out of 22 instances during exacerbation and in 3 out of 10, during remission. In some of the cases, bleeding, recalcification, thrombin and toluidine blue times were also abnormal.

The fibrinogen level was normal during remission and slightly, not significantly, reduced in a few cases during exacerbation. We failed to demonstrate labile fibrinogen or any significant alteration of fibrinolytic activity in 32 instances. (These three factors have not been included in Table I.) Partial thromboplastin time (normal value: 75″) was examined in 5 cases during exacerbation. It was increased in three (120″, 130″, 165″), normal in two cases

Discussion

Earlier, thromboembolic and haemorrhagic phenomena were the most common and often lethal complications of polycythaemia vera. As a result of ^{32}P treatment, these complications have become scarcer, but they nevertheless do occur [10, 12, 24]. The present authors have for instance witnessed thrombosis and haemorrhages during remission [15, 17]. The actual situation is, therefore, that the complex coagulation defects, though generally subsiding during remission, persist to some degree in a number of cases.

BURGER et al. [4], later NAGY and BURGER [20] emphasized that despite the normal fibrinogen level, correction of the fibrinogen demand according to the increased haematocrit value will not fail to reveal a relative fibrinogen deficiency. They found an increased fibrinolytic activity, protracted retraction and clotting times as well as a reduction in maximum elasticity, and stressed the significance of hypocirculation associated with the disease.

The fibrinogen level was normal in the present cases and exacerbations did not effect it significantly. (We usually did not adjust the values to the haematocrit; in the few cases where we did, lower values were found.) It must be mentioned that in the literature there is no agreement on this issue [1, 3, 10, 12, 23, 24].

The most distinct alterations were shown by the prothrombin index, prothrombin utilization, thrombin inactivation and the platelet count.

Reduction of the prothrombin index and of prothrombin utilization was noted by other authors too [4, 10, 11, 12]. The former is indicative of liver impairment, the latter of a derangement in the pro-phase of blood clotting.

The platelet count was high in 16 out of 22 cases during exacerbation. Numerous authors [2, 9, 13] regard thrombocytosis as one of the major factors of thromboembolism in polycythaemia vera. An increased tendency of the platelets to agglutinate has also been observed [14, 23]. During remission all of the patients had a normal platelet count. Retraction was slow in 14 out of 22 cases during exacerbation and in 3 out of 10 during remission, in agreement with the observations of REISSMANN [22] and of LAVSKY [12].

In some bleeding, recalcification, thrombin and toluidine blue times were also abnormal. Deficiency in the two last-named factors suggested a possible accumulation of circulating anticoagulant factors. It is of interest that KOLUTOVA [10], LASCH and LINKE [11] have demonstrated increased plasma heparin levels in polycythaemia vera, a finding connected by these authors with the susceptibility to haemorrhage typical of polycythaemia.

REFERENCES

1. ARLETTI, O., BALLERINI, G.: Arch. path. Clin. med. **33,** 223 (1957).
2. BARTA, I.: Orv. Hetil. **70,** 230 (1929).
3. BJÖRKMAN, S. E., LAURELL, C. B., NILSEN, I. M.: Scand. J. clin. Lab. Invest. **8,** 304 (1956).
4. BURGER, T., NAGY, I., KESZTHELYI, B., PEER, B.: Orv. Hetil. **103,** 15 (1962).
5. FAZEKAS, S., NAGY, GY., PETRÁNYI, GY.: Orv. Hetil. **102,** 596 (1961).
6. FAZEKAS, S., NAGY, GY., PETRÁNYI, GY.: Orv. Hetil. **104,** 214 (1963).
7. FISCHER, W., GREMER, W. D.: Röntgen- u. Lab.-Prax. **10,** 49 (1957).
8. GERENDÁS, M.: Véralvadás és vérzéscsillapítás. Medicina, Budapest 1960.
9. JURGENS, R., BACH, K.: Dtsch. Arch. klin. Med. **176,** 626 (1934).
10. KOLUTOVA, N. N.: Полицитэмия. Медгиз. Киев 1960.
11. LASCH, H. G., LINKE, A.: Dtsch. Arch. klin. Med. **200,** 290 (1953).
12. LAVSKY, G. K.: Эритремия. Медгиз. Москва 1959.
13. LUDECKE, H.: Virchows Arch. path. Anat. **293,** 218 (1934).
14. MERAWITZ, P., RÖNNER, W.: Dtsch. Arch. klin. Med. **94,** 529 (1908).
15. NAGY, GY.: Magy. Radiol. **6,** 345 (1964).
16. NAGY, GY., PETRÁNYI, GY.: Magy. Radiol. **7,** 208 (1965).
17. NAGY, GY.: Magy. Belorv. Arch. **19,** 272 (1966).
18. NAGY, GY., SZILÁGYI, J., OSVÁTH, S., MARCZ, I.: Acta med. Acad. Sci. hung. **23,** 139 (1967).
19. NAGY, GY., DESEŐ, GY.: Kísérl. Orvostud. (in press).
20. NAGY, I., BURGER, T.: Orv. Hetil. **105,** 1501 (1964).
21. OLESEN, K. H., POULSEN, L.: Acta med. scand. **139,** 145 (1951).
22. REISSMANN, F.: Istanbul Üniv. Tip-Fak. Mec. 18, **418** (1955).
23. ROSENTHAL, R. L.: J. Lab. clin. Med. **34,** 1321 (1949).
24. VARELA, J. E. et al.: Nucl.-Med. (Stuttg.) **3,** 1 (1962).

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TRANSITIONAL ELECTROENCEPHALOGRAPHIC MANIFESTATIONS BETWEEN ICTAL GENERALIZED SPIKE-AND-WAVE PATTERN AND GENERALIZED REPETITIVE FAST DISCHARGE OF THE GRAND MAL TYPE

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In an attempt to throw light on the pathophysiologic factors accounting for the difference between the petit mal and the grand mal mechanisms, four cases have been presented where the generalized spike-and-wave pattern was found in combination with generalized repetitive fast discharge. In the first two cases, originally of a 3 c/sec. spike-and-wave pattern, during the same seizure spike-and-wave and repetitive fast activity appeared alternatingly parallel with grand mal and petit mal paroxysms, whereas the other two patients showing petit mal variant activity had alternatingly seizures either of petit mal type with spike-and-wave pattern or tonic fits with repetitive fast discharge.

The observations allowed the following conclusions.

(i) There are demonstrable transitional EEG signs between petit mal seizures of the spike-and-wave pattern and grand mal paroxysms with repetitive fast discharge.

(ii) In epileptic encephalopathy with spike-and-wave variant, tonic seizures of the repetitive fast discharge type, with slight motor and marked vegetative manifestations are relatively frequent during sleep, a finding consistent with the observations of Gastaut et al. (1966).

(iii) Natural or hexobarbital induced sleep, as well as diazepam favour the production of the transitional signs.

In the light of recent knowledge regarding the neuronal mechanism of the generalized spike-and-wave and repetitive fast discharge type of electric seizures (Pollen 1964, Matsumoto and Ajmone Marsan 1964) and of the decisive role of interneuronal recurrent collateral inhibition in the rhythmic EEG phenomena (Eccles 1965), the present observations were tried to interpret in favour of a unitary mechanism of the two seizure patterns accounting for the difference and combination as well in the electric and clinical manifestations.

In the light of accumulating evidence on the pathomechanism of epilepsy, the petit mal seizures have come to be regarded as being distinct, beside the special clinical seizure types, in certain respects from other epileptic manifestations. This view is based on (i) the EEG morphology, namely the synchronous bilateral spike-and-wave pattern, (ii) the relative absence in the 3 c/sec.-type of cerebral damage marked by neurologic symptoms, PEG-changes or focal interictal EEG abnormalities; (iii) The signs pointing to hereditary factors; (iv) the presumed part played by subcortical structures, particularly by the diencephalic reticular formation in the production of the seizures.

It is, on the other hand, well known that petit mal epilepsy of the spike-and-wave pattern may combine with generalized motor seizures of tonic or tonic-clonic type, suggesting that in petit mal epilepsy there is also a susceptibility to grand mal seizures. Little is known, however, about the pathophysiologic elements connecting or dividing the two seizure mechanisms.

It seemed of interest to present an analysis of four cases combining generalized repetitive fast discharges* with a spike-and-wave mechanism.

In many cases, however, the repetitive bursts mark motor seizures of only tonic character and therefore GASTAUT et al. (1966) justly criticized the comprehensive term of grand mal discharge. In the clonic phase of grand mal seizures the repetitive fast bursts become gradually associated with slower waves; tbe electric seizures are developed gradually (by successively increasing amplitudes), they are of fairly long duration and are usually followed by depression. In opposition to this, the repetitive fast bursts accompanying tonic seizures spring abruptly from the ground activity, they are short in duration; slow-wave activity is confined to the just terminal phase and features of postparoxysmal-depression are usually absent. The present authors would suggest the comprehensive term "generalized repetitive fast discharge" applying to the ictal EEG signs of tonic, as well as of tonic-clonic, seizures.

Case reports

I. Combination of a 3 c/sec. generalized spike-and-wave mechanism with repetitive fast discharge

Case 1. V. J., an 11-year-old boy had had his first grand mal seizure in febrile state at the age of 3 months. Subsequently he had had recurrent convulsions, invariably with pyrexia. Since the age of 6 years he had been experiencing repeated daily spells of clouding associated with eyelid-flutter and with conjugated deviation to the left, with no loss of tone, occasionally with psychomotor features (oral and limb automatisms). Antiepileptic treatment with trimethodione, hydantoin, primidone and acetazolamide was of no benefit. His mental capacity verged on imbecility. The EEG tracings consistently showed a generalized 3 c/sec. spike-and-wave mechanism activated by hyperventilation. On readmission neurologic signs of focal lesion were absent as they had been before. The PEG pointed to slight ventricular dilatation. Serial EEG tracings revealed a generalized 3 c/sec. spike-and-wave mechanism activated by hyperventilation and by hexobarbital. In hexobarbital sleep and on diazepam administration an occasional seizure pattern of oculo-clonic absence with generalized spike-and-wave pattern introduced by repetitive fast discharge could be observed (Fig. 1); on other occasions, the generalized spike-and-wave pattern alternated with generalized repetitive fast discharge within the same paroxysm, clearly reflecting the competition between the two mechanisms (Fig. 2). On one occasion we were able to record the electric phenomena accompanying the production of a grand mal seizure. The spike-and-wave activity introducing the oculo-clonic seizure gradually yielded to repetitive fast discharge, then it was again interrupted by large slow waves showing, however, multiple spikes on the ascending limbs, and lastly after a brief period of desynchronization, the repetitive fast discharge built up gradually, parallel with the manifestation of the tonic phase of the grand mal seizure. The subsequent clonic phase was marked by a gradually slowing rhythmic activity of gross spikes with slow terminal components. This phase was followed by postparoxysmal depression (Fig. 3).

Treatment with diazepam and primidone proved of sufficient benefit.

Case 2. T. M., a 20-year-old female patient had been born with blue asphyxia and 2200 g body-weight. Since her twelfth year she had had epileptic seizures, prevalently of the grand mal type introduced by adversion to the left, occasionally by vocalization. There had also been psychomotor and generalized tonic seizures and oculo-clonic absences. She had been on regular

* The term grand mal discharge has been applied by GIBBS and GIBBS (1952) to 10 c/sec or faster generalized repetitive bursts believed typical of the ictal EEG morphology of tonic-clonic grand mal seizures.

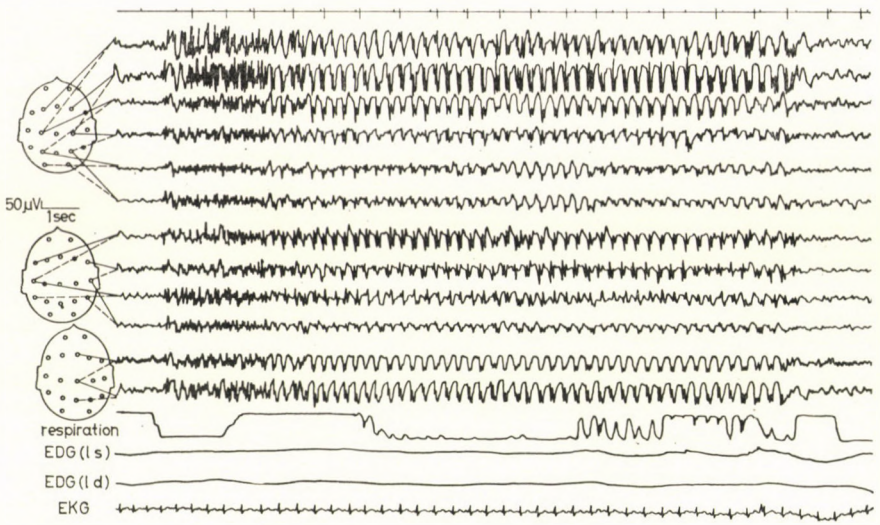


Fig. 1. V. J., 11-year-old boy. 3 c/sec. spike-and-wave seizure introduced by repetitive fast discharge

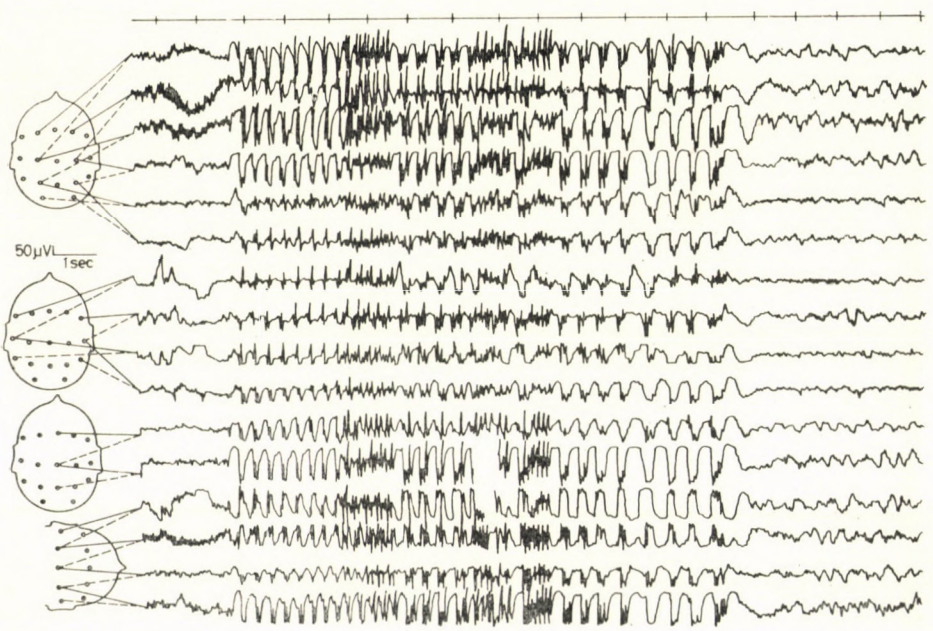
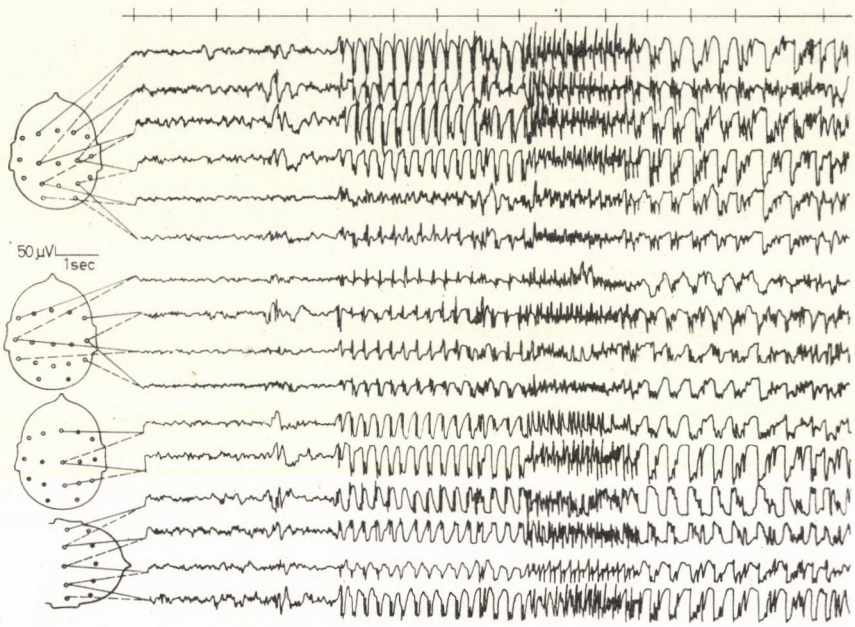


Fig. 2. V. J., 11-year-old boy. Still awake after injection of 0.25 g of hexobarbital. Competition of spike-and-wave mechanism and repetitive fast discharge in the course of a petit mal seizure



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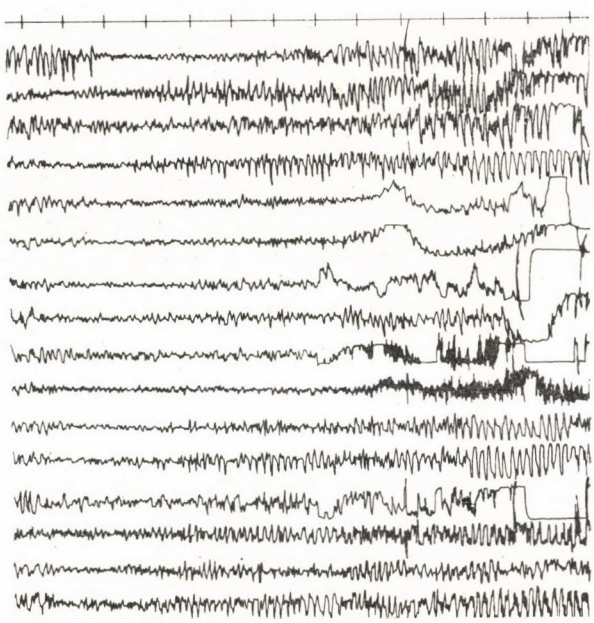

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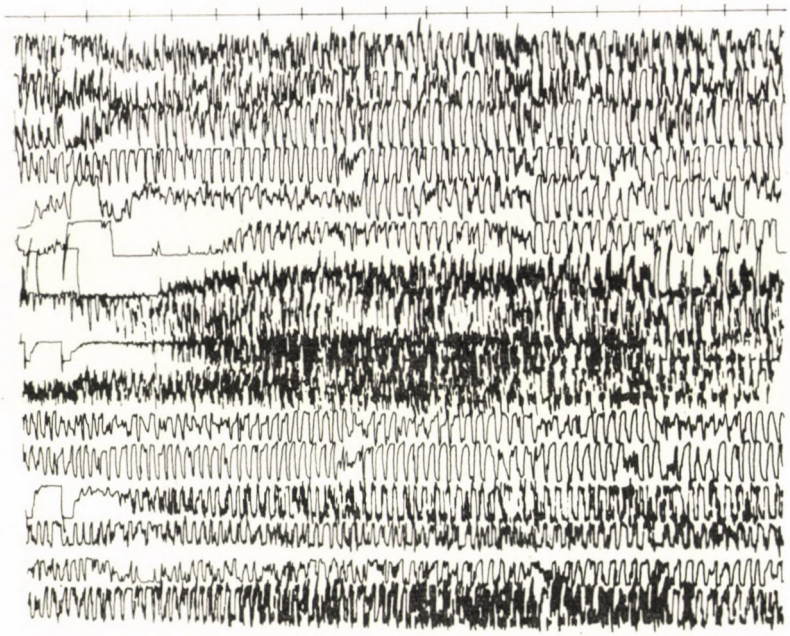

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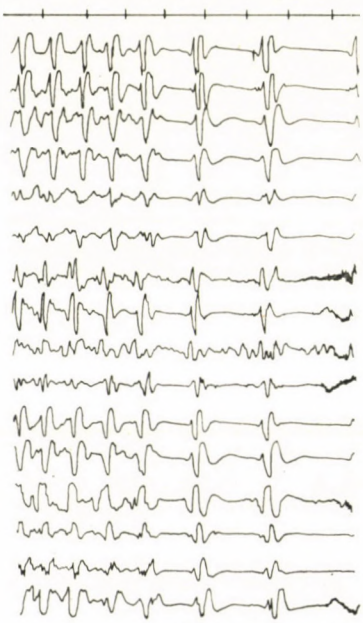

e)

Fig. 3. V. J., 11-year-old boy. Still awake after injection of 0.2 g of hexobarbital. Conversion of spike-and-wave petit mal seizure into grand mal paroxysm. (Continuous recording.)

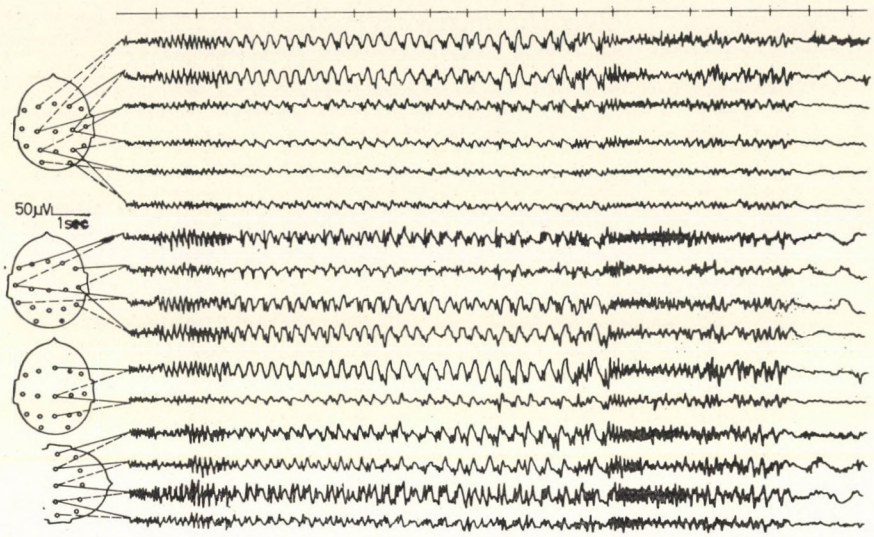

Fig. 4. T. M., 20-year-old female. Still awake after injection of 0.3 g of hexobarbital. Conversion of spike-and-wave pattern into repetitive fast discharge followed by postictal depression. (Paper speed at the beginning of the record and in the first 3 seconds of the seizure, 15 mm/sec.)

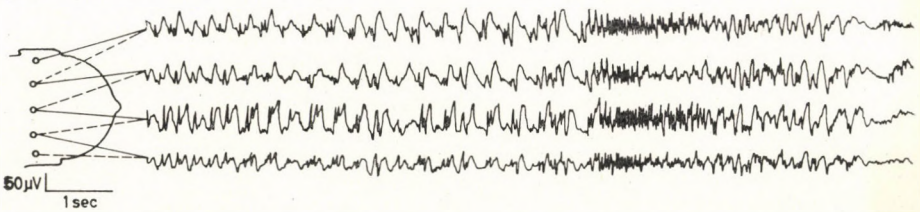

Fig. 5. Enlarged detail of Fig. 4. Gradual accumulation of spike components in the course of the conversion of the spike-and-wave activity into repetitive fast discharge

antiepileptic treatment for eight years, including hydantoin, trimethadione and primidone, without any apparent benefit. Her intellectual capacity was fully preserved, but there were epileptic features in her personality. Neurologic examination revealed no abnormality. PEG showed a normal ventricular system. Serial EEG confirmed oculo-clonic absences with a 3 c/sec. spike-and-wave pattern, reproducible by hyperventilation. In hexobarbital slumber prolonged generalized spike-and-wave activity appeared without any noticeable clinical manifestation. Generalized 3 c/sec. spike-and-wave paroxysms turning into generalized repetitive fast discharges occurred in natural as well as in hexobarbital induced sleep. In these cases it could be readily discerned how the spike components of the spike-and-wave pattern became more and more numerous, while the slow-wave components were vanishing. Seizures of this kind were invariably followed by postictal depression (Figs 4, 5).

Diazepam and ethosuccimide proved of sufficient benefit.

II. Association of a spike-and-wave variant mechanism with repetitive fast discharges accompanying generalized tonic seizures

Case 1. R. M., a 12-year-old girl had been backward in her motor and mental development. Since the age of 3 she had been experiencing repeated daily myoclonic seizures, alternating with (or joined by) spells of absence with loss of tone, and since her ninth year she had had

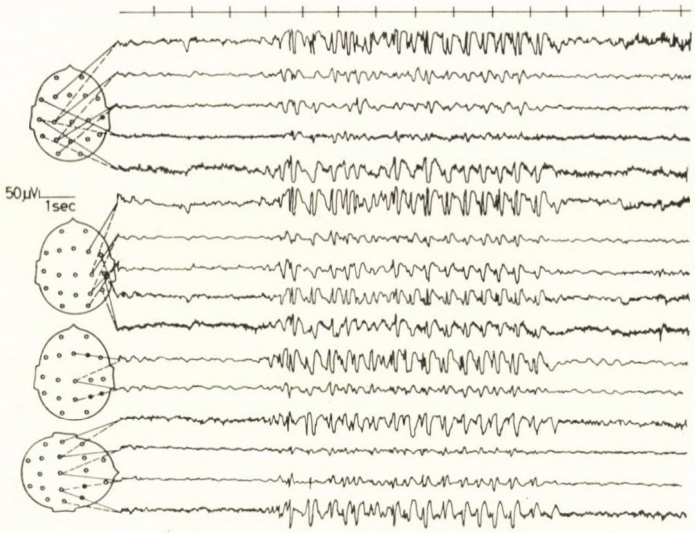

Fig. 6. R. M., 12-year-old girl. Spike-and-wave variant paroxysm in awake state

generalized tonic and occasional grand mal seizures. Antiepileptic treatment with trimethadione and primidone since the age of 6, produced no benefit. On repeated investigations the PEG had been found normal and the EEG revealed a generalized spike-and-wave activity.

She was admitted in a status epilepticus made up of myoclonic absences. There was no focal symptom by neurological examination. EEG revealed spike-and-wave bursts at 2 or 3-minute intervals synchronously with the clinical myoclonic manifestations. Even between the bursts there was a delta-activity, partly rhythmic, partly polymorphous in character.

After relief of the status epilepticus still remained a great number of generalized bursts of a spike-and-wave variant (Fig. 6). In hexobarbital slumber as well as during deeper sleep, generalized repetitive fast discharges of 2 to 4 sec. duration appeared; the simultaneous clinical manifestations were confined to a slight tonic extension of the trunk. The discharges usually terminated by slow-wave activity and were followed by depression (Fig. 7). Tonic seizures of similar duration associated with repetitive fast discharges occurred with great frequency during natural sleep. On the evidence of polygraphic recordings, these discharges were associated with significant vegetative phenomena including apnoea and bradycardia (Fig. 8). The myoclonic absences and the pathological activity dominated by the spike-and-wave variant responded to diazepam, and later to combined treatment with diazepam, oxazolidine and hydantoin, in contrast to the tonic seizures of repetitive fast discharges which even increased in frequency. After the status epilepticus had ceased, the I. Q. corresponded to idiocy.

Case 2. F. L., a 14-year-old boy suffered asphyxia at birth. Since the age of 6 months he had had repeated daily spells of absence, joined later by generalized tonic fits and grand mal seizures. Antiepileptic drugs including phenytoin, phenacetamide and primidone had produced no benefit. Owing to gross mental deficiency he had been kept in a special home. The generalized motor convulsive seizures had a tendency to develop into a status epilepticus. This had been actually the cause of his admission. His neurologic state was marked by extremital rigidity and by ataxia, the PEG showed a significant dilatation of the lateral ventricles and gross dilatation of the third ventricle.

The resting EEG revealed generalized slow spike-and-wave paroxysms (Fig. 9). During hexobarbital sleep including light sleep as well as the slow-wave phase, generalized repetitive fast discharges 3 or 4 sec. in duration were registered, parallel with apnoea, bradycardia and a weak, barely noticeable axial tonic fit (Fig. 10).

Apart from carbamazepine or a combination of primidone and diazepam, no other antiepileptic drug proved of any value.

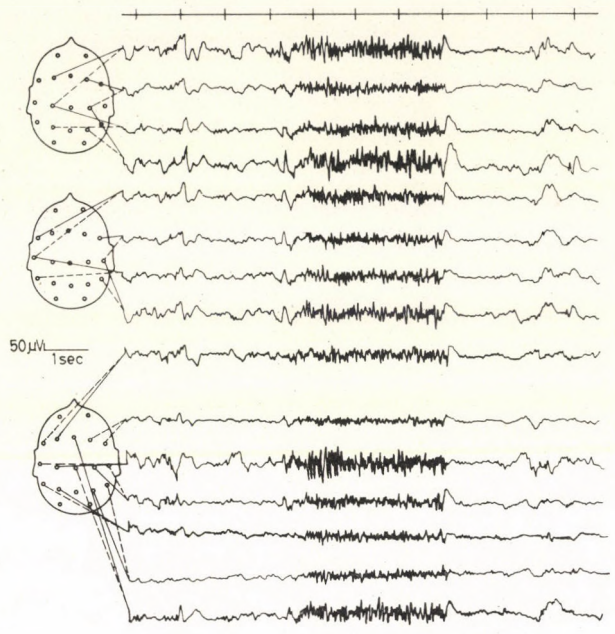

Fig. 7. R. M., 12-year-old girl. Hexobarbital sleep. Generalized repetitive fast discharges followed by postictal depression

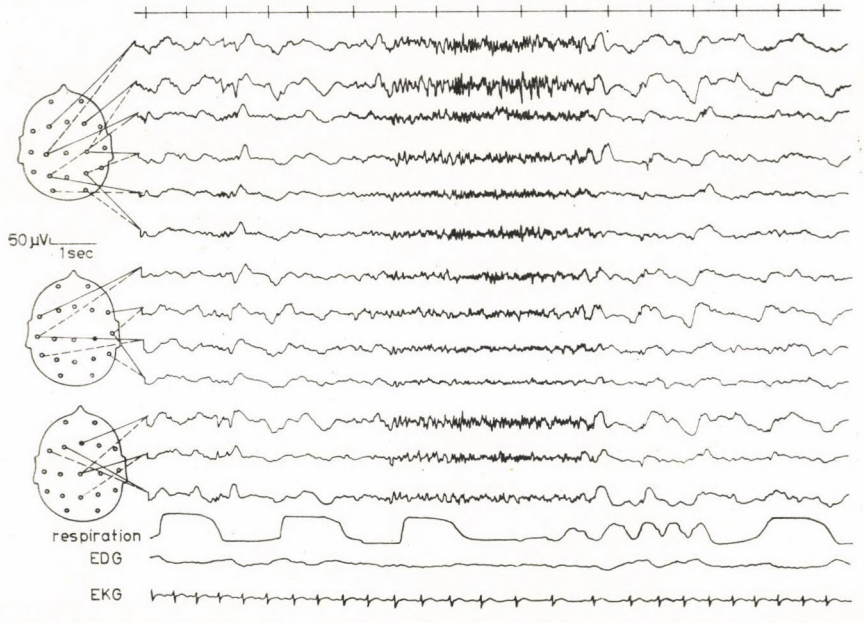

Fig. 8. R. M., 12-year-old girl. Natural sleep. Tonic seizure with apnoea, bradycardia and repetitive fast discharge

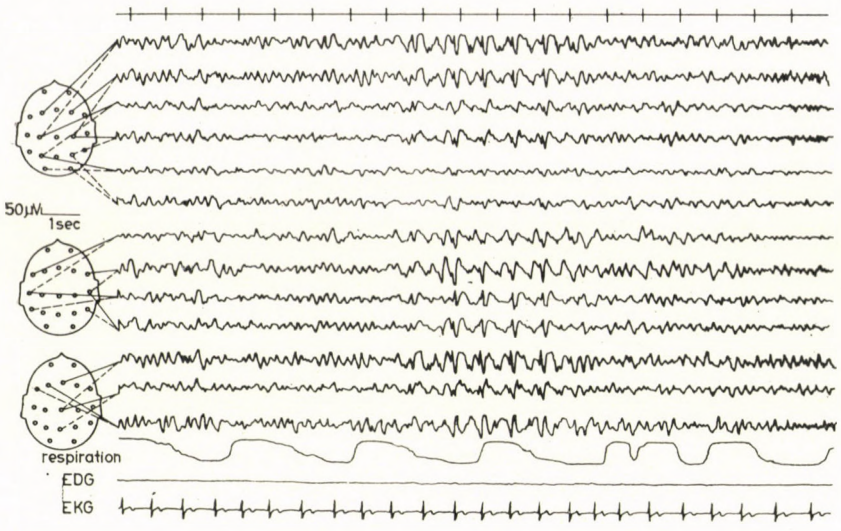

Fig. 9. F. L., 14-year-old boy. Spike-and-wave variant paroxysm in awake state

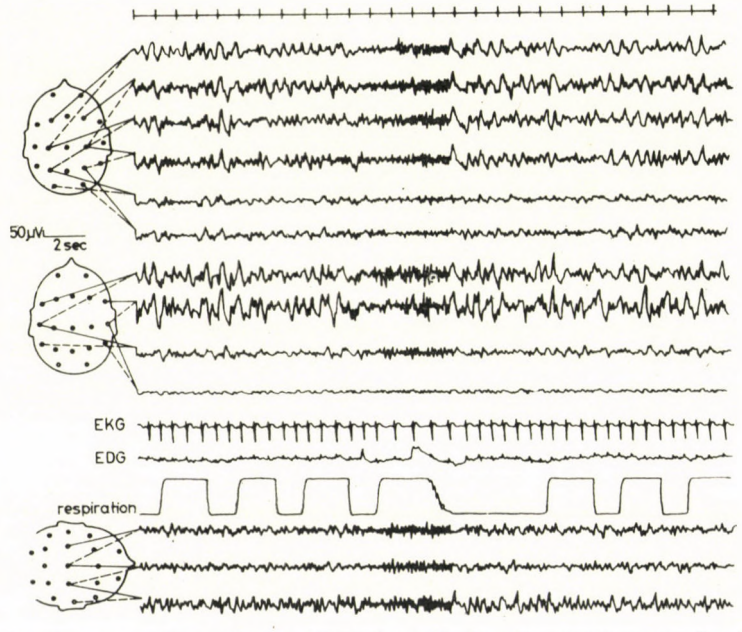

Fig. 10. F. L., 14-year-old boy. Hexobarbital sleep. Tonic seizure with apnoe, bradycardia and repetitive fast discharge

Discussion

Association of the spike-and-wave pattern with generalized repetitive fast discharges is relative rarely demonstrable. This may be partly due to the circumstance that there is little opportunity for ictal EEG recordings. Nevertheless, the atlas of GIBBS and GIBBS (1952) contains numerous examples of this combination, even during the same seizure in patients with petit mal and grand mal seizures. HEDENSTRÖM and DREYER (1967) described a case with alternating seizure-types of spike-and-wave pattern during an oculo-clonic petit mal, and of repetitive fast discharge with psychomotor paroxysms. GASTAUT et al. (1966) classified LENNOX' syndrome as a particular type of epileptic encephalopathy marked by slow spike-and-wave activity. The generalized tonic seizures prevalent in this syndrome are associated with repetitive fast discharges.

WEIR and SIE (1966) demonstrated experimentally the conversion of a metrazol-induced spike-and-wave pattern into grand mal discharges in cats with experimental lesion of the rostral pontine structures.

In the first two cases the same electric paroxysm combined alternating patterns of a 3 c/sec. spike-and-wave activity and of repetitive fast discharges, either through conversion of the repetitive fast discharge into a spike-and-wave pattern, or conversely, thus reflecting a competitive mechanism. Evidence of postictal depression was only seen after sustained intensive repetitive fast discharge.

In the second two cases presented in this study the mechanism of spike-and-wave variant was associated with tonic seizures carrying repetitive fast discharges.

Clinically an alternation of petit mal seizures with generalized tonic or grand mal paroxysms was observed in all four cases. In the two cases illustrating the first type of combination it was possible to record the spike-and-wave paroxysm associated with the oculo-clonic absences, and in one of them also the electric activity during the grand mal seizure. In the other two cases representing the second combination we were able to record the electric phenomena during both the petit mal and the tonic seizures.

All the patients proved resistant to conventional antiepileptic drugs and in all of them there was evidence of cerebral damage.

Sleep whether natural or barbiturate-induced was found to favour the transitional phenomena, in other words, it promotes the combination of the two patterns. Drugs of the diazepam type produced a similar effect which was probably the reason why transitional phenomena were demonstrable in our cases.

From the analysis of the electric phenomena recorded in the present cases it clearly emerges that the two different mechanisms of generalized epileptic seizures may alternate or merge into one another in one and the same

subject. This pleads against the general belief that the difference between the two mechanisms is fundamental.

Comparison of the already explored pathophysiological features of the two mechanisms might lead to a more comprehensive view of them.

Both mechanisms are characterized by rhythmic, self-sustaining repetitive discharge appearing over the entire brain. Of their distinctive features the rhythmically recurring slow-wave component of the spike-and-wave mechanism is the most important. POLLEN (1964) has shown that the slow-wave component represents a superficial reflection of hyperpolarization generated in deeper cortical layers, in other words, it is an expression of an inhibitory mechanism. Each spike component is followed by inhibition, obviously as a rebound phenomenon. In accordance with this interpretation, the clinical manifestations of the spike-and-wave mechanism are mostly inhibitory in character; motor convulsions, if present at all, are confined to myoclonic twitchings and even these invariably coincide with the spike components.

As concerns the ictal phenomena of the repetitive fast discharge pattern, it is known from intracellular microelectrode studies (MATSUMOTO and AJMONE MARSAN 1964) that the discharge starts when it comes to paroxysmal depolarization shift in the cell, and that during the repetitive fast discharge coinciding with the tonic motor phenomena rhythmic oscillations of the membrane potential are demonstrable. Then, the membrane becomes gradually repolarized, the oscillations slow down and increase in amplitude. This phase corresponds to the appearance of rhythmic slow waves in the EEG, and clinically to the jerks of the clonic phase. This is followed by the postictal depression in which factors of exhaustion as well as of overlapping hyperpolarization are probably involved. If the clinical seizure is tonic, the electric pattern is confined to fast repetitive discharge and, according to GASTAUT et al. (1963) there is no postictal depression either. In the present cases, however, the repetitive fast discharges accompanying the tonic seizures observed during sleep, ended by a large slow wave, and postictal depression was also demonstrable. In the case of spike-and-wave seizures, too, the rhythmic spike-and-wave activity had a higher frequency at the onset, to slow down successively toward the end. Here, however, postictal depression was never observed. If, on the other hand, the spike-and-wave mechanism turns into repetitive fast discharge, the seizure is invariably followed by depression. It seems that postictal depression is prone to occur if the depolarization was excessive, whereas an intensive, slow-wave-generating inhibitory hyperpolarization mechanism does not favour its production. It is therefore highly doubtful whether the presence or the absence of postictal depression may be interpreted in favour of a possible cortical or subcortical origin of a seizure.

The investigations of ECCLES (1965) connected the rhythmic wave phenomena of the EEG with an interneuronal recurrent collateral inhibition.

There are many indications (JASPER 1966) that the primary cause of epilepsy lies in a weakness of cortical inhibition, and that it is a temporary breakdown of the cortical inhibitory mechanism which clears the way for the ictal phenomena. The mechanism underlying the spike-and-wave seizures has presumably the particular feature of being still compatible with a rhythmic recurrent inhibition. Presumably in the spike-and-wave variant mechanism the inhibitory reverberation is less organized than in the 3 c/sec. spike-and-wave pattern, probably in consequence of the cortical damage usually present in these cases, which would account for the greater susceptibility of this type to ictal phenomena accompanied by repetitive fast discharges.

The present cases clearly show that the classical spike-and-wave mechanism may be converted into repetitive fast discharge and that, often enough, the spike-and-wave mechanism results from a seizure the onset of which is marked by repetitive fast discharges. It is in all probability the factor of recurrent interneuronal inhibition which determines the frequency of bursts and the proportion of the slow-wave components in both types, i.e. in the spike-and-wave pattern as well as in the repetitive fast discharge type of seizure. If this inhibitory mechanism fails to assert itself, a spike-and-wave mechanism yields to repetitive fast discharges, while if it does, a seizure originally of the repetitive fast discharge type is converted into the spike-and-wave pattern. In the light of this interpretation the repetitive fast discharges represent that type of ictal seizure mechanism where cortical inhibition is at its minimum and it is only a gradual increase in membrane repolarization and lastly a hyperpolarization which bring the seizure to an end parallelly with a gradual interpolation of slow waves. In the spike-and-wavetype it is, on the contrary, the rhythmic recurrent interneuronal inhibition which gains prevalence until full suppression of the seizure has been achieved.

It is mainly in the spike-and-wave mechanism where the role of the non-specific system seems to be essential. On the grounds of an earlier study (HALÁSZ and HIDASI 1967) production of the spike-and-wave mechanism is ascribed to a functional derangement of the reticular activating system decreasing tonic facilitation of the cortical neurons.

The weakening of the facilitating influence of the reticular activating system favours the release and autonomy of reverberation of collateral recurrent inhibition. A mechanism of this kind may also favour the development of grand mal seizures, sleep having been found to promote the production of repetitive fast discharges in patients with spike-and-wave variant activity. In this case, however, the recurrent collateral inhibitory mechanism fails to assert itself owing to cortical damage.

The working hypothesis outlined in the foregoing connects the two seemingly distinct seizure patterns and also accounts for their possible combinations. It may furthermore help to understand the differences in the clinical types.

The same recurrent rhythmic inhibitory mechanism which determines the spike-and-wave pattern, may well account for the possible absence of gross motor convulsions in petit mal epilepsy. This may be due either to a direct inhibition of the motor neurons or an inhibition of the facilitating influence of the reticular activating system on the motor system. These conjectures, however, are awaiting further investigation.

REFERENCES

GIBBS, F. A., GIBBS, E. L.: Atlas of Electroencephalography. Wesley Press, Cambridge, Mass. 1952. Vol. 2.
ECCLES, J. C.: Epilepsia **6,** 89—115 (1965).
GASTAUT, H., ROGER, J., OUAHCHL, S., TIMSIT, M., BROUGHTON, R.: Epilepsia **4,** 15—44 (1963).
GASTAUT, H., ROGER, J., SOULAYROL, R., TASSINARI, C. A., REGIS, E., DRAVET, C.: Epilepsia **7,** 139—179 (1966).
HALÁSZ, P., HIDASI, J.: Ideggyógy. Szle **9,** 467—474 (1967).
HEDENSTRÖM, I. V., DREYER, R.: Nervenarzt **38,** 68—72 (1967).
JASPER, H.: Proc. XIV. European EEG Meeting. Marseille, 1966.
MATSUMOTO, H., AJMONE MARSAN, C.: Exp. Neurol. **9,** 305—326 (1964).
POLLEN, D. A.: Electroenceph. clin. Neurophysiol. **17,** 398—404 (1964).
WEIR, B., SIE, P. G.: Epilepsia **7,** 30—43 (1966).

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О ВОЗМОЖНОСТИ РАЗВИТИЯ ТОКСИЧЕСКОЙ ГЛАУКОМЫ ПОД ВЛИЯНИЕМ ЭТИЛИРОВАННОГО БЕНЗИНА И ТЕТРАЭТИЛСВИНЦА

З. М. СКРИПНИЧЕНКО

ОДЕССКИЙ НАУЧНО-ИССЛЕДОВАТЕЛЬСКИЙ ИНСТИТУТ ГЛАЗНЫХ БОЛЕЗНЕЙ И ТКАНЕВОЙ ТЕРАПИИ ИМЕНИ АКАД. В. П. ФИЛАТОВА

(Поступила 19 декабря 1966 г.)

1. Авторами впервые установлено, что под влиянием ТЭС и этилированного бензина, развивается своеобразная форма токсической гиперсекреционной глаукомы.

2. Лабильность состояния внутриглазного давления, повышенное количество водянистой влаги при отсутствии изменений в путях её оттока и увеличение концентрации белка в ней, сосудистые нарушения в глазу, жалобы больных, наличие вегетативных расстройств и др., указывают на застойный характер установленной нами гиперсекреционной токсической ТЭС глаукомы.

3. Токсическая ТЭС глаукома является вторым примером известной до настоящего времени глаукомы с выявленной этиологией. В отличие от бенгальской, токсическая ТЭС глаукома развивается при наличии общих вегетативно-сосудистых нарушений в организме и при поражении нейрососудистого рефлекторного аппарата, регулирующего офтальмотонус.

Тетраэтилсвинец (ТЭС) широко применяется на транспорте. Объясняется это тем, что до настоящего времени ТЭС является почти не превзойденным антидетонатором. Будучи добавлен в небольших количествах (0,16%) к бензину, ТЭС резко повышает его октановые числа.

Тетраэтилсвинец — вегетативнососудистый яд. Он быстро и легко проникает через гематоэнцефалический барьер. В клинике ТЭС-интоксикации преобладают симптомы, определяющие нарушение функций центральных отделов нервной системы.

Нами, начиная с 1945 года, наблюдались молодые, до работы с этилированным бензином совершенно здоровые люди, у которых на фоне хронического поражения появились изменения в органе зрения. Характерным при этом явились; нарушения регуляции внутриглазного давления и изменения диска зрительного нерва.

Установленные изменения в органе зрения у молодых людей, соприкасающихся с этилированным бензином, могли быть обусловлены наличием в нём тетраэтилсвинца. Широкое применение в народном хозяйстве тетраэтилсвинца и этилированного бензина, возможность поражения при этом органа зрения и развитие глаукомы, обусловили необходимость проведения нами дальнейших исследований.

Для выяснения характера влияния тетраэтилсвинца на орган зрения, на протяжении пяти лет (1955—1960) нами проводились профилактические обследования более пятисот человек, занимающихся его производством.

Почти половина наблюдаемых были молодые люди в возрасте до 30 лет. Благоприятные условия труда способствовали тому, что лица, занимающиеся производством ТЭС, находились на предприятии длительное время, 6—20 лет и более.

Совместно с врачами различных специальностей нами изучалось общее состояние здоровья лиц, бывших в контакте с ТЭС. По данным 6217 исследований артериального давления, у значительного числа обследованных лиц были выявлены различные сосудистые нарушения: гипотония, наклонность к гипертензии, ангиоспазмы, сосудистые кризы и др. Под влиянием ТЭС согласно данным электрокардиограмм наблюдались изменения в интрамуральной проводниковой системе сердца. Изменения в кислотности желудочного содержимого определялись в начале значительным повышением, в дальнейшем снижением её вплоть до ахилии. При нормальном уровне сахара в крови у большого числа лиц гликемические кривые были с высоким подъёмом и замедлённой гипогликемической фазой. Изменения в периферической крови встречались не часто и были не стойкие. Содержание свинца в моче не превышало показателя его у здоровых людей. Выявленные изменения в общем состоянии здоровья явились характерными для ТЭС-интоксикации.

Лица, находившиеся в контакте с ТЭС, не часто предъявляли жалобы на орган зрения. В сравнении с мужчинами, женщины чаще жаловались на беспокоившую их боль в области головы, которая сопровождалась тошнотой, рвотой и кратковременной потерей зрения. Под влиянием ТЭС отмечалось быстрое и легко наступающее раздражение коньюнктивы. При отсутствии воспалительных явлений имели место расширение сосудов в лимбе и прорастание их в поверхностные слои по периферии роговицы. Диаметр сосудов был не одинаков, местами встречались ампулообразные их расширения.

У лиц, преимущественно с большим сроком пребывания в контакте с ТЭС, наступало сужение и даже спазм сосудов сетчатки. У ряда лиц встречался отёк сетчатки на диске зрительного нерва. Наблюдалось расширение физиологической экскавации и перемещение сосудистого пучка в темпоральную сторону.

В сравнении с показателями здоровых людей, у лиц, подвергавшихся действию ТЭС, давление в центральной артерии сетчатки (ЦАС) было повышено. Гипертензия в ЦАС проявлялась на фоне общей гипотонии и сочеталась с повышением офтальмотонуса. Однако, разница давления в ЦАС и внутри глаза была достаточной, чтобы сохранялся нормальный баланс питания сетчатки. Именно этим можно объяснить относительно небольшие изменения остроты и поля зрения у лиц даже с нарушенной регуляцией офтальмотонуса.

Данные ангиоскотометрии и исследования по Бьерруму также свидетельствуют, что ТЭС, как ангиотропный яд, наряду с общим характером действия, оказывает также влияние и на сосуды глаза.

Регуляция офтальмотонуса исследовалась методом эластотонометрии (ЭК) по Филатова—Кальфа. Контролем явились показатели здоровых людей соответствующего возраста.

У обследованных лиц были сняты 3930 ЭК. Изменения в характере ЭК у части лиц определялись высоким уровнем офтальмотонуса, повышенным началом и большим подъёмом кривых.

В сравнении с мужчинами, у женщин значительно чаще наблюдался патологический характер ЭК. Установленный факт наличия у женщин, в сравнении с мужчинами, более частого изменения ЭК согласуется с известным положением, что женщины легче и быстрее поражаются ТЭС, к тому же, как известно, женщины чаще болеют глаукомой.

Показатели ЭК в зависимости от возраста обследованных лиц намного отличились от данных здоровых людей. Число лиц с патологическим характером ЭК росло также в соответствии с увеличением срока пребывания в контакте с ТЭС.

Нарушение регуляции офтальмотонуса определяет наличие глаукоматозного процесса. Лица с патологическими типами ЭК обследовались нами в условиях стационара. У большого их числа уровень офтальмотонуса превышал норму. В соответствии с уровнем внутриглазного давления увеличивалась амплитуда суточных его колебаний. Изменения в уровне офтальмотонуса происходили в различное время и не одинаково на обоих глазах. Для лиц, подвергавшихся действию ТЭС, характерным является смешанный тип суточных кривых внутриглазного давления.

Необходимо указать, что глаукома развивалась у лиц молодого возраста. При повышении офтальмотонуса некоторые больные жаловались на боль в области головы, ощущение тумана, кратковременную потерю зрения и снижение зрения в сумерки. У ряда лиц снижалась острота зрения, развивалась гиперметропия, определялось концентрическое сужение границ поля зрения и значительно расширялись ангиоскотомы. Примером могут явиться: Б-й М. А. К-в, 27 лет, стаж работы 5 лет, срок наблюдения 5 лет; И. И. С-в, 37 лет, стаж работы 7 лет, срок наблюдения 5 лет; А. Т. Б-в, 49 лет, стаж работы 17 лет, срок наблюдения 5 лет. (Рис. 1, 2, 3.)

Изменения в органе зрения дополняли клинику общей ТЭС-интоксикации. Несмотря на молодой возраст, у таких больных наблюдалась эмоциональная неустойчивость, повышенная раздражительность, ослабление памяти, тремор пальцев вытянутых рук и др. Имели место также повышенная салливация и потливость, экзофтальм, снижение температуры тела, понижение артериального давления, нарушение кислотности желудочного содержимого и характера гликемических кривых. У ряда больных проявлялась наклонность к гипертензии, ангиоспазмам и др.

Согласно принятой на Всесоюзном совещании (1952) классификации первичной глаукомы, у наблюдаемых нами лиц была выявлена

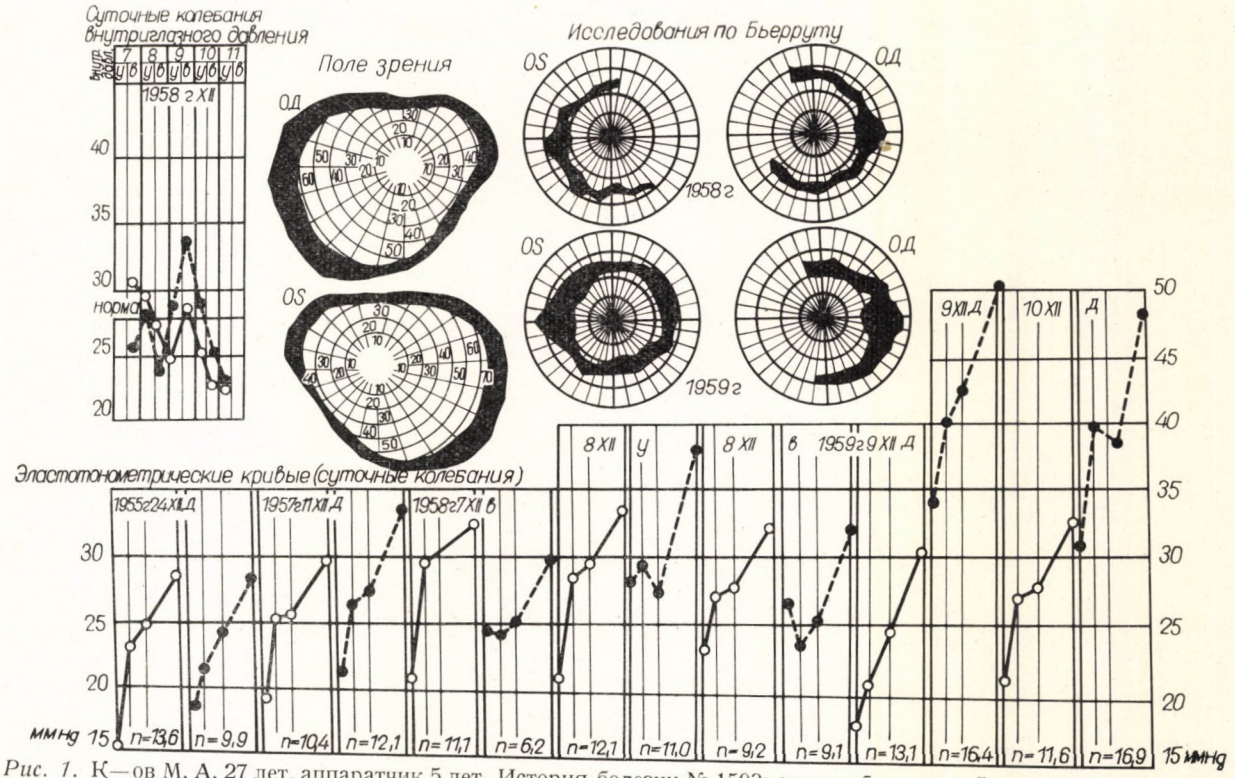

Рис. 1. К—ов М. А. 27 лет, аппаратчик 5 лет. История болезни № 1593: срок наблюдения 5 лет. Острота зрения:

оба глаза = 1,5 (1955 г)
правый глаз = 0,9—1,0
левый глаз = 0,5 с корр. + 1,0 д = 0,6—0,7 (1959 г)

Условные обозначения: ОД ———, OS — — — —, день — д, утро — у, вечер — в, подъем — п

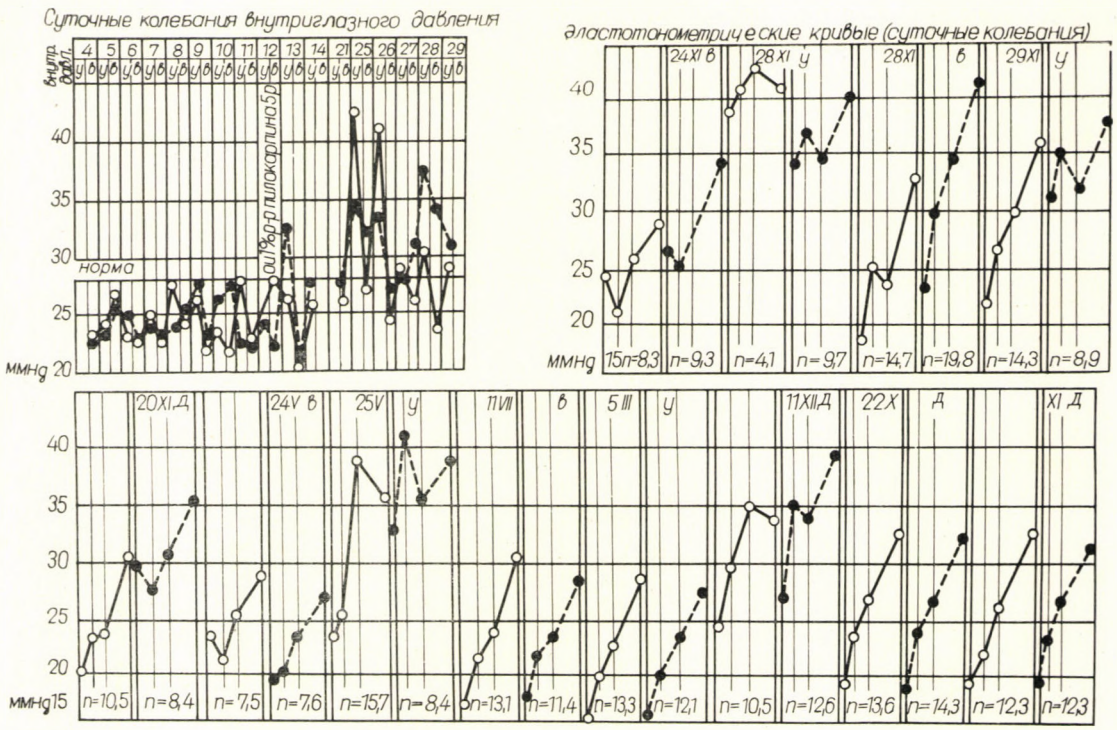

Рис. 2. С—ов И. И. 37 лет, аппаратчик 7 лет. История болезни № 1504: срок наблюдения 5 лет. Острота зрения:

правый глаз = 1,2 (1955 г)
левый глаз = 1,4
правый глаз = 0,7 сс + 0,5 д = 0,9 (1959 г)
левый глаз = 0.9 сс + 0,5 д = 1,0

Условные обозначения: ОД ———, OS — — — —, день — д, утро — у, вечер — в, подъем — п

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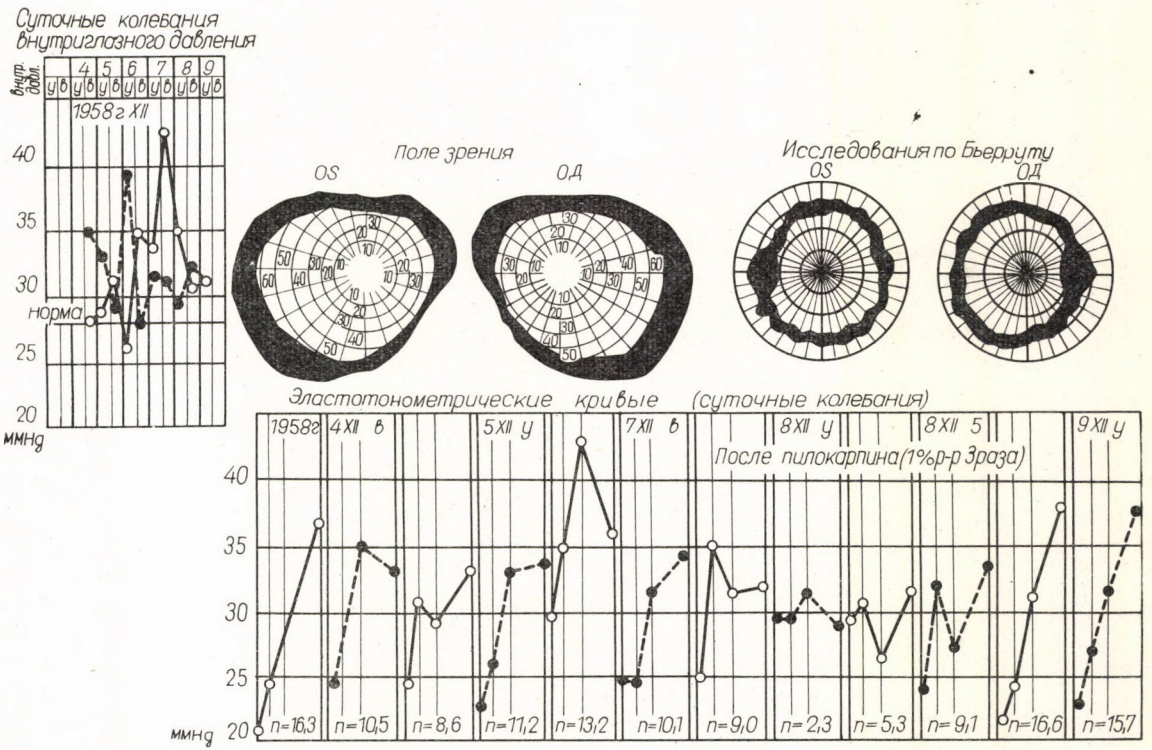

Рис. 3. Б—ов А. Т. 49 лет, аппаратчик 17 лет. История болезни № 1586: срок наблюдения 5 лет. Острота зрения:
оба глаза = 1,0 (1955 г)
оба глаза = 1,0 с корр.+ 1,0 д (1958 г)
Условные обозначения: ОД ———, OS — — — —, у — утро, в — вечер, п — подъем

застойная, начальная, компенсированная, либо субкомпенсированная глаукома.

Данные отдаленных наблюдений (3—5 лет) позволили установить стойкий характер нарушения регуляции внутриглазного давления под влиянием ТЭС-интоксикации.

Лица, занимающиеся производством ТЭС, находятся под постоянным надзором врачей. Ежемесячно подвергаются профилактическим осмотрам. Без разрешения врача работать с ТЭС не разрешается. Рабочие с большим сроком пребывания в контакте с ТЭС ежегодно на 2 месяца переводятся на работу, исключающую возможность влияния токсических веществ. Всем рабочим предоставляется усиленное питание и они пользуются 2-х месячным отпуском, в период которого широко представляются путевки в санатории и дома отдыха.

Для изучения некоторых сторон механизма нарушения регуляции офтальмотонуса при ТЭС-интоксикации были проведены экспериментальные исследования.

На протяжении шести лет (1954—1959) в опыте находилось 257 животных. Для получения модели хронической ТЭС-интоксикации нами был разработан метод затравки животных возрастающими количествами ТЭС. Большая часть кроликов подвергалась затравке повторно и находилась в опыте 2 и более года.

После длительных исследований нам представилось возможным в опыте на кроликах получить модель токсической ТЭС глаукомы. Подъём и большие колебания офтальмотонуса, наблюдаемые в период опыта, удерживались на протяжении 5—6 месяцев после его прекращения (Рис. 4, 5).

Угнетенине холинэстеразы является характерным для поражения ТЭС. Проведенные исследования на 53 кроликах позволили установить, что под влиянием ТЭС снижается активность холинэстеразы и нарушается регуляция офтальмотонуса. Однако, анализ полученных данных показал, что характер расстройства регуляции внутриглазного давления не находился в прямой связи от степени снижения активности холинэстеразы.

Для выяснения характера обмена камерной влаги в глазу были проведены тонографические исследования под контролем ЭК по С. Ф. Кальфа (1962, 1964). В сравнении со здоровыми, у пораженных ТЭС животных истинный уровень офтальмотонуса (Ро) повышался в среднем от 10,7 ($\pm$3,0) в контроле, до 12,2 ($\pm$2,53) мм в опыте. Повышение истинного уровня офтальмотонуса можно объяснить увеличением минутного объёма камерной влаги (F), который от 0,94 ($\pm$0,63) в контроле, повышался до 2,74 ($\pm$1,37) в опыте. При большом минутном объёме камерной влаги легкость оттока (С) также повышалась в среднем от 0,13 ($\pm$0,08) до 0,37 ($\pm$0,19). Проведенные исследования показали, что под влиянием ТЭС повышалась секреция камерной влаги, при этом пути её оттока не нарушались. Зависимость уровня истинного оф-

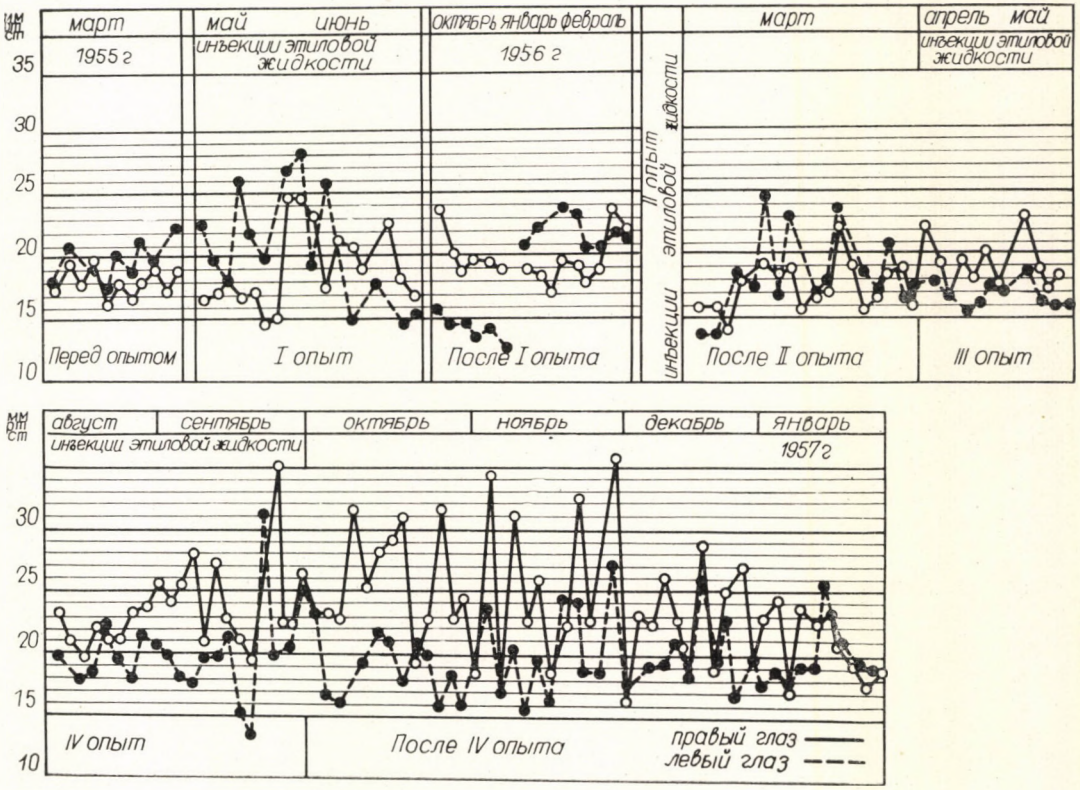

Рис. 4. Кривая внутриглазного давления кролика N 767

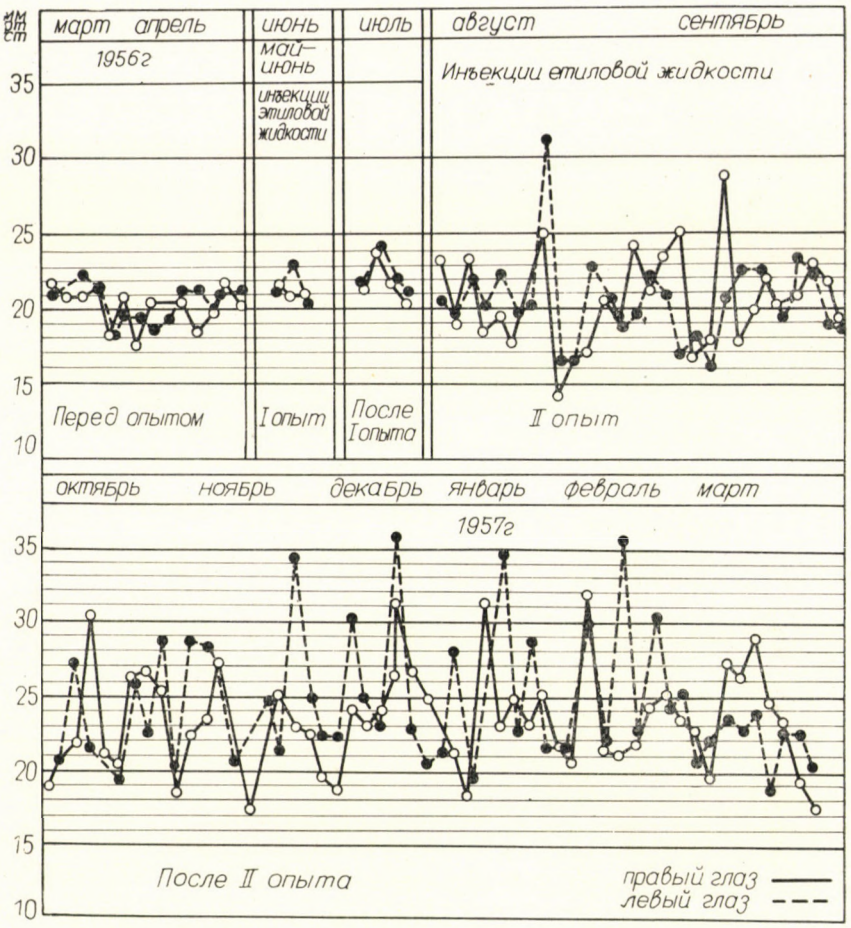

Рис. 5. Кривая внутриглазного давления кролика N 410

тальмотонуса (Ро) от минутного объёма камерной влаги (F) доказана методом вариационной статистики. Коэффициент корреляции этих показателей был высокий — 0,67 в контроле и 0,59 в опыте.

Повышение объёма влаги под влиянием ТЭС могло быть обусловлено нарушением проницаемости мембран глаза. Для выяснения этого был применён биохимический метод нефелометрического исследования по Францешетти и Вилянду (1928). Наблюдения велись на 43 кроликах (86 глаз). Результаты исследования здоровых кроликов согласуются с данными литературы. Если количество белка во влаге глаза контрольных животных принять за 100%, то у опытных групп концентрация его, в среднем, повышалась до 80%.

Реактивность нейрорефлекторного аппарата, регулирующего офтальмотонус, изучалась путём определения его чувствительности к адреналину,

атропину и пилокарпину. Нагрузочно-разгрузочные пробы с вегетативными ядами, проведенные на 103 животных помогли установить характер изменений функциональных расстройств в нервно-сосудистом аппарате, регулирующем офтальмотонус. Нарушенная под влиянием ТЭС деятельность этого аппарата протекала по типу повышенной чувствительности денервированных структур, для которых характерным является, наряду с большой степенью проявления, также и неадекватный характер реакции. (Кеннон и Розенблют, 1961). Функциональную диссоциацию в деятельности нейрососудистого аппарата, регулирующего офтальмотонус, можно объяснить нарушением нормальных взаимоотношений корковых и подкорковых вегетативных центров, что является характерным для ТЭС-интоксикации.

TOXIC GLAUCOMA INDUCED BY ETHYLATED BENZENE AND TETRAETHYL LEAD

By

Z. M. SKRIPNYCHENKO

(1) It has been shown that ethylated benzene and tetraethyl lead give rise to a special form of toxic hypersecretory glaucoma.

(2) Lability of intra-ocular tension, increased amount of aqueous humour, lack of intra-ocular fluid drainage, high protein content of the aqueous humour, vascular lesions in the eyes, complaints pointing vegetative disturbances, etc. indicate the congestive nature of the glaucoma induced by tetraethyl lead.

(3) The one induced by tetraethyl lead is the second kind of glaucoma of known aetiology. Its development is accompanied by changes in the vegetative nervous system and an impairment of neuro-vascular reflexes regulating intra-ocular pressure.

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ТКАНЕВАЯ ТЕРАПИЯ ПО В. П. ФИЛАТОВУ

Н. А. ПУЧКОВСКАЯ, Т. У. ГОРГИЛАДЗЕ и Е. А. БУДИЛОВА

ИНСТИТУТ ГЛАЗНЫХ БОЛЕЗНЕЙ И ТКАНЕВОЙ ТЕРАПИИ ИМЕНИ АКАДЕМИКА В. П. ФИЛАТОВА, Г. ОДЕССА, СССР

(Поступила 19. декабря 1966 г.)

Научная деятельность выдающегося советского ученого Владимира Петровича Филатова богата замечательными открытиями. Творческая биография ученого отмечена такими значительными вехами, как предложенный им новый метод пластической хирургии, как разработанная и широко внедренная им в практику операция пересадки роговой оболочки.

Впервые широко применив роговицу трупных глаз как материал для пересадки роговой оболочки, В. П. Филатов отметил, что просветление бельма вокруг пересаженной роговицы бывает выражено значительно чаще после трансплантации трупной консервированной роговицы, чем после пересадки свежей роговой оболочки, взятой от только что удаленного по той или иной причине глаза. Это наблюдение, а также более высокий процент успеха, отмечавшийся после кератопластики с использованием консервированной роговицы, а также другие факты, навели В. П. Филатова на мысль о том, что в роговичной ткани в процессе консервации накапливаются какие-то новые вещества, обладающие высокой биологической активностью.

Поскольку эти вещества образуются не в мертвых, а в еще живых «переживающих» тканях, академик В. П. Филатов назвал их, по происхождению, биогенными стимуляторами.

Так начал развиваться метод тканевой терапии.

С начала возникновения метода было накоплено много ценных экспериментальных и клинических данных, с очевидностью показавших большую биологическую активность консервированных тканей, а также были проведены исследования, которые дали основание для последующих теоретических обобщений.

В. П. Филатов считал, что отделенные от организма животные или растительные ткани при воздействии на них таких факторов среды, которые затрудняют их жизнь, подвергаются биохимической перестройке. При этом в тканях вырабатываются вещества, стимулирующие биохимические процессы в них. Указанные вещества, способствующие тканям сохранять жизнь в неблагоприятных условиях, названы «стимуляторами биологического происхождения» или короче «биогенными стимуляторами».

В. П. Филатов рассматривает образование биогенных стимуляторов как способ приспособления обмена веществ организма к действию условий среды, выработанной эволюционным путем. Он подчеркивает также, что биогенные стимуляторы образуются в тканях, пока они еще живы, в результате биохимической их перестройки и, таким образом, они являются продуктами деятельности живых клеток.

Биогенные стимуляторы, будучи введены в какой-либо организм тем или иным путем (имплантация обогащенной ими ткани или инъекции экстракта из нее), активируют в нем жизненные процессы. Усиливая обмен веществ в организме, они тем самым повышают физиологические функции организма. Этим они увеличивают сопротивляемость к патогенетическим факторам и усиливают его регенеративные свойства, что и способствует выздоровлению. Это положение подтверждается огромным количеством клинических и экспериментальных данных, а также исследованиями гигантского масштаба, проведенными в настоящее время в ветеринарии и животноводстве.

В настоящее время широко применяются тканевые экстракты, взвеси, мази, порошки и другие виды тканевых препаратов.

Фармакологическим Комитетом при МЗ СССР утверждены следующие тканевые препараты для медицинской практики: экстракт алоэ для инъекций, экстракт плаценты, пелоидодистиллат, ФИБС, экстракт алоэ внутрь, взвесь плаценты, торфот и др.

В Одессе имеется завод, вырабатывающий тканевые препараты. Если в 1959 г. завод выпускал 19,5 миллион ампул, то в 1965 г. — 54 милл. ампул. Приведенные цифры показывают, как увеличилась выработка и спрос тканевых препаратов.

В последние годы тканевая терапия широко стала применяться более чем в 20 странах мира.

Прекрасные лечебные качества и широкой диапазон действия, которыми обладают тканевые препараты, позволили советским медикам широко рекомендовать их в медицинскую практику с профилактической и лечебной целью.

Остановимся теперь на областях применения тканевой терапии. Общеизвестно ее применение в офтальмологии. Родившись в этой области, она нашла свое постоянное место.

Накоплен обширный клинический материал, свидетельствующий об эффективности тканевых препаратов при ряде глазных заболеваний. Она успешно используется при пигментной дегенерации сетчатки, при атрофиях зрительных нервов, особенно токсического происхождения, при глаукоме, тромбозе сосудов сетчатки, при начальной катаракте, при симпатическом воспалении. Весьма перспективным является применение тканевой терапии с профилактической целью для предупреждения прогрессирования близорукости, а также для лечения осложненной близорукости. В этих случаях ис-

пользуется свойство тканевых препаратов стимулировать функцию соединительной ткани организма. Тканевая терапия в комбинации со специфическими средствами эффективно используется при токсоплазмозе глаза. Подконъюнктивальные инъекции препарата торфота оказались весьма эффективными при лечении помутнения роговицы. Применение тканевых препаратов значительно улучшает процесс приживления трансплантата при кератопластике.

Помимо глазных заболеваний, тканевые препараты нашли широкое применение в хирургии как средство, усиливающее регенерацию костной ткани, при спаечной болезни, при язвенной болезни, при вяло текущих ранах, при эндартериозе и атеросклерозе сосудов конечностей, при каузальгиах и фантомных болях, для ускорения рассасывания келоидных рубцов, при контрактурах Дюпюитрена, при вяло текущих ранах и язвах тканевая терапия способствует появлению сочных грануляций, очищению раны, уплощению ее краев и энергичной эпителизации гранулирующей поверхности. Это сопровождается уменьшением болей в области рубцов и язвенных поверхностей и значительным улучшением самочувствия больных. Применение тканевых препаратов оказывает большую услугу гинекологам и др.

Тканевая терапия сыграла большую роль в годы отечественной войны и в послевоенном периоде в деле лечения военных травм и их последствий.

Особо важно использование тканевых препаратов в геронтологии. Из многих работ известно, что тканевые препараты повышают функции эндокринных и в том числе половых желез, регулируют обменные процессы, повышают окислительные процессы в тканях и т. д. Теперь, когда борьба со старением становится реальной практической задачей, очень большое значение в ее разрешении может иметь тканевая терапия.

Основанием для применения тканевых препаратов в гериатрической практике с целью лечения и предупреждения болезненных явлений, которые отягощают преждевременное старение организма, послужили многочисленные клинические и экспериментальные наблюдения, которые показали, что тканевая терапия, являясь одним из методов неспецифического воздействия на организм, способствует нормализации обменных процессов и активизации его компенсаторных механизмов, повышению регенераторных свойств и общей его сопротивляемости патогенным влияниям.

Этим свойством тканевых препаратов можно объяснить хорошее их воздействие на возрастные изменения, такие как: пресбиопия (повышается острота зрения, повышается аккомодация, увеличение поля зрения, стабилизация развития возрастного помутнения хрусталика, а также возрастных изменений глазного дна у людей, прошедших у нас курсы тканевой терапии (гетерокожа, взвесь плацентарной ткани и другие тканевые препараты, предложенные В. П. Филатовым).

Как уже выше сказано, фармакологическим Комитетом СССР утверждено для применения в клинической практике 7 тканевых препаратов. Это: экстракт алоэ, плаценты, ФИБС, пелоидодистиллат, пеллоидин, торфот, взвесь плаценты.

Лечение проводится обычно по следующей схеме: инъекции под кожу или внутримышечно по 1,0 мл ежедневно в течение 30—45 дней, курсы лечения повторяются 2—3—4 раза в году в зависимости от течения и характера процесса. Как мы уже говорили, целесообразно сочетать тканевые препараты с другими лекарственными веществами. Такие лекарственные средства как антибиотики, кортикостероиды или витамины можно вводить одновременно с тканевым препаратом. Также тканевые препараты могут быть введены в организм в виде подсадок автоклавированных тканей, взвесей, ионофореза, приема внутрь и в виде примочек и др. Хотя мы не можем говорить о строгой специфичности отдельных тканевых препаратов, но на основании многолетних клинических наблюдений мы считаем, что ФИБС предпочтительно применять при туберкулезных процессах, при сосудистых нарушениях, у пожилых людей с хроническими глазными заболеваниями, у больных глаукомой. При ревматических заболеваниях мы назначаем пелоидодистиллат. При помутнении роговицы наиболее полезным оказывается торфот, экстракт плаценты и экстракт алоэ имеют более широкий диапазон применения — это высокая близорукость, атрофия зрительных нервов, пигментная дегенерация сетчатки, неспецифические хронические воспалительные и дегенеративные процессы в сосудистом и зрительно-нервном аппарате глаза и многие другие хронические заболевания.

Природа биогенных стимуляторов, их химический состав до этого времени продолжают живо интересовать исследователей, т. к. углубленное изучение этих вопросов позволит еще больше повысить эффективность тканевой терапии и шире внедрять ее в практику.

Ряд биохимических исследований свидетельствует о том, что под влиянием тканевых препаратов происходят весьма важные изменения в структуре тканей. Белки тканей становятся более лабильными, способность их расщепляться трипсином заметно усиливается. Улучшается биосинтез их.

В опытах на кроликах было показано, что у животных, предварительно обработанных тканевыми препаратами, последующее заражение глаза герпетическим вирусом вызывало значительно менее тяжелые изменения, чем у контрольных, не подготовленных животных. Наблюдалось также повышение устойчивости животных, обработанных тканевыми препаратами к стрихнинному отравлению, к анилиновым поражениям, к анемизирующим факторам, к явлениям токсикоза, возникающим после подкожного введения раствора марганцевокислого калия.

Весьма убедительные данные о профилактическом и лечебном действии тканевых препаратов получены физиологами нашего института. На большом

экспериментальном материале было показано, что тканевая терапия оказывает выраженное благоприятное влияние на течение лучевой болезни. Важно при этом подчеркнуть, что устойчивость животных к действию ионизирующей радиации повышалась после предварительной обработки животных тканевыми перпаратами. Такая обработка оказывалась даже более эффективной, чем лечебное введение препаратов после уже развившегося поражения. Так, при профилактическом введении тканевых препаратов после острого облучения дозой в 600 рентгенов выживало 96% животных, при лечебном применении этих препаратов 69% животных, между тем как в контроле выживаемость животных равна была 50%. Эти данные находят подтверждение и в работах других авторов (К. Н. Чочия, Д. Балдандорж).

Таким образом, ряд экспериментальных и клинических наблюдений свидетельствует о том, что тканевые препараты активируют защитные системы организма и этим благоприятствуют его большей устойчивости и выздоровлению.

В последнее время опубликованы весьма интересные данные о комплексном применении тканевых препаратов совместно с физическими методами лечения при полиомиэлите в восстановительном периоде заболевания и в периоде остаточных явлений. Наблюдения над значительным количеством больных показали, что лечение с применением общеизвестных медикаментозных средств в сочетании с тканевой терапией приводило к быстрому восстановлению функций пораженных конечностей.

Весьма актуальное значение имеет вопрос о химическом составе биогенных стимуляторов. В исследованиях А. Ф. Сысоева, изучавшего динамику изменений количества органических кислот в консервируемых на холоде тканях, было установлено, что органические кислоты накапливаются до определенного срока консервации, после чего количество их понижается. Кривая накопления кислот приближается к кривой повышения биологической активности тканевых препаратов. Исследования химического состава биогенных стимуляторов требуют еще дальнейшего продолжения. Этому вопросу следует уделить большое внимание.

Каждый новый тканевой препарат, предложенный в медицинскую практику для профилактических и лечебных целей, предварительно проходит длительный путь различных экспериментальных исследований на животных по установлению лечебной эффективности, оптимальных доз и полной безвредности для организма.

Для каждого нового тканевого препарата разрабатывается индивидуальный режим технических условий приготовления, который обеспечивает наибольший выход биологически активных веществ, высокую лечебную эффективность, стандартность препарата и безвредность для организма. После проверки препарата на токсичность путем однократных и повторных введений различных доз, включая специальные исследования реакции, реак-

ции на введение чужеробного белка у разнообразных животных, установления возможности производить стандартизацию по характерным химическим показателям, препарат изучается с точки зрения его биологической активности на специально разработанных тестах.

Наиболее простыми и доступными тестами являются так называемые «дрожжевой», «роговичный», и др. тесты.

Помимо того, профилактическая и лечебная эффективность тканевых препаратов подвергается обязательной проверке при различных заболеваниях, экспериментально вызванных у животных.

Модели заболевания:

различные формы анемии:

а) выживаемость белых крыс после спленэктомии

б) после отравления фенил-гидразином

в) после отравления уксусно-кислым свинцом;

экспериментальный аллоксановый диабет

экспериментальный герпетический кератит;

экспериментальный гепатит с переходом в цирроз;

экспериментальный миокардит;

экспериментальный атеросклероз.

Таким образом, при проверке профилактической и лечебной эффективности тканевых препаратов по В. П. Филатову на моделях болезни отчетливо проявляется поливалентность действия их и определенное преимущество их применения с профилактической целью.

Институт глазных болезней и союзные лечебные учреждения СССР располагают огромным клиническим материалом по применению тканевой терапии. При таких заболеваниях, как осложненная миопия, атрофия зрительных нервов, пигментная дегенерация сетчатки, наблюдения насчитывают тысячи больных, в течение длительных сроков наблюдения, поэтому в настоящее время мы имеем основания рекомендовать широко использовать этот метод лечения в клинической практике.

Акад. В. П. Филатов был большим оптимистом в науке, он учил не опускать рук у постели больного, не говорить больному «нет».

Все его научные открытия и в большей мере тканевая терапия являются могучим лечебным методом борьбы с безнадежными заболеваниями, которые ранее считались неизлечимыми.

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ELECTROENCEPHALOGRAPHIC, ELECTRODERMOGRAPHIC AND PUPILLOMOTOR RESPONSES UNDER HEXOBARBITAL EFFECT IN MAN*

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The patterns of EEG, EDG, and pupillomotor responses to sensory stimuli have been studied from the aspects of their interrelations and their dependence on the successive levels of hexobarbital effect in 17 subjects.

It has been possible to identify well-defined types in the morphology and amplitudes of evoked EEG activity, each predominating at different levels of anaesthesia. These are as follows. (1) "Pure desynchronization" (arousal response) at the earliest or at the final barbiturate effect; (2) "after-synchronization" in deepening anaesthesia; (3) "pure synchronization" in medium deep anaesthesia; (4) "transitional type", also in deepening anaesthesia; (5) gross depression and, eventually, total absence of responsiveness, in deep anaesthesia.

The same response patterns occur in reversed order with diminishing depth of anaesthesia.

The magnitude of EDG amplitudes and the intensity of pupillomotor responses reveal specific relationships to evoked EEG modifications.

Interruption of sensory excitation during diminishing barbiturate effect may precipitate the "paradoxical" phase of sleep which corresponds to a kind of deep anaesthesia though in some cases it may be compatible with a considerable degree of responsiveness.

Each of these response patterns are assumed to represent hierarchically organized non-specific structures in the brain stem each carrying and utilizing individual non-specific informations, and each having different barbiturate-sensitivity. It is suggested that a possible "energization" of these arousal structures by external or internal stimuli generates an energizing interaction between the structures. Excitation of respiration, cardiovascular and motor functions involved by these reactions are of significance in the complex management of barbiturate coma.

It has been shown that hexobarbital anaesthesia is able to "activate" epileptic spike discharges in the interictal period, but spikes of different patterns and localizations are favoured by different levels of anaesthesia (KAJTOR 1964).

Appearance or a definite increase of generalized 3/sec. spike-and-wave activity is confined to the initial and terminal phases of intravenous hexobarbital effect. On the other hand, spike bursts of temporal lobe epileptic foci are associated with a medium depth of anaesthesia. The spikes recorded from the lateral gyri of the temporal lobe exposed for surgical removal of an epileptic focus, and those picked up from the ventricular surface of the hippocampus of the same lobe were found in different phases of anaesthesia. Certain types

* Based on a lecture presented at the 5th International Congress of Electroencephalography and Clinical Neurophysiology, Vienna 1965.

of basotemporal spike discharges can only be studied in connection with the isoelectric phases, under extremely deep anaesthesia. Certain individuals develop two clinically and electrically entire distinct seizure mechanisms. There appear for instance "psychomotor" seizures at first, joined later by myoclonic jerks unrelated to the first type. The frontocentral spikes and spike-and-wave variants are favoured by superficial sleep, temporal spikes by deep anaesthesia.

Our observations suggest that the cerebral systems inhibiting discharges of epileptogenic foci of different types and localizations show a different responsiveness to barbiturates.

Severe protracted barbiturate coma is characterized by a flat, irregular EEG. Intensive and long-continued peripheral sensory (faradic) stimuli increase, however, the amplitudes of the EEG and definitely reduce its frequency, as a typical response of human cerebral bioelectrical activity in this condition. Induced synchronization of EEG may be associated with an amelioration of cardiovascular, respiratory and pupillodilator functions, as well as with weak incoordinated automatic movements indicating a tendency to restoration of biologic functions. In superficial anaesthesia, the same stimuli result in lower amplitudes and higher frequencies.

It has been inferred from these observations that the morphologic response pattern of the EEG to the same stimuli varies with the depth of barbiturate-induced sleep (KAJTOR and OSZLÁNSZKY 1963).

The present study has been concerned with the effects of sensory stimuli on the morphology, amplitudes and time relations of EEG and EDG response patterns, furthermore with the relationships of these responses to pupillomotor activity at sequential levels of hexobarbital anaesthesia. It was also examined whether the evoked bioelectric changes were indicative of any connection between the successive levels of barbiturate anaesthesia and certain non-specific structures of the brain.

Material and methods

The study involved 17 male and female subjects, aged between 15 and 48, without any indication or evidence of cerebral injury or neuropsychiatric abnormality and having had no drug treatment for at least seven days. The resting records in the awake state revealed no abnormality. With exception of two healthy adult volunteers, recordings in induced sleep were made because of suspected paroxysmal activity.

Hexobarbital was administered by the intravenous route, with the patient in the recumbent position. The dose varied between 475 and 1000 mg, according to individual tolerance reflected by the EEG. The injection was continued until the examiner had ascertained that the generalization of delta-activity had reached its maximum corresponding to age. Establishment of full delta-activity was considered the criterium of medium depth anaesthesia.

EEG recording was continued until the patient had opened his eyes or uttered a few words in response to a simple question addressed to him in a loud voice.

The recordings were made with an 8- or 16-channel ink-writer machine at a paper speed of 30 mm/sec. The time constants were 0.1 to 0.3 sec. for the EEG, and 0.3 to 1 sec. for the EDG, adapted to the special requirements of the case, and were maintained throughout. Lon-

gitudinal chains of six electrodes with the same interelectrode distances were placed to symmetrical points of the hemispheres, the leads being frontocentral, centroparietal, anterotemporal—mediotemporal, mediotemporal—posterotemporal. By varying the modality, duration and intensity of the sensory stimuli it was expected to gain richer information on the dynamics of the EEG and EDG responses than it would be possible by stimuli of one single modality and a fixed duration or of rhythmic recurrence, as for instance by single or paired click stimuli.

During the actual injection of barbiturate, the stimuli were applied sparsely. As soon as the injection was completed, all through the entire course of the recording, sensory stimuli of various modalities were applied at close but varying intervals without interfering in any serious degree with the process of recovery from barbiturate sleep. The very EEG changes resulting from occasional automatic voluntary movements of the subject were generally suited for evaluation in the context of response patterns.

The auditory stimuli comprised the sound of an electric buzzer, clapping of hands, calling the patient by name in a loud voice, posing him a simple question, and a command to open his eyes. Tactile stimuli were retraction of the eyelid, touching the cornea with cotton-wool. As nociceptive stimuli there were repeated pinpricks and pinching applied to the skin of the arm; kinesthetic and proprioceptive stimuli were evoked by passive and automatic voluntary movements; thermic stimuli: suprapubic cooling by local application of ether in the case of enuretics; vagal interoceptive stimuli were arisen by voluntary deep inspiration or coughing.

Sensory stimuli were applied at 1 to 3 min. intervals during 2 to 5 sec. or longer, in groups, particularly when the responses to the first stimuli had been indistinct or when the aspect of habituation was being studied. The buzzer stimulation was modified in pattern but not in intensity during the experiment. Clapping of hands, verbal stimuli and pinpricks also were kept, as far as possible, at a constant intensity.

The evoked EEG pattern was evaluated by comparison of two segments of 6 sec. duration each, the first preceding the onset of stimulation, the second following its cessation immediately. EEG changes were evaluated as responses when appearing inside the first 5 seconds and when extending over at least one third of the period of evaluation. The EEG changes could best be studied, in the frontocentral and centroparietal recordings, since the most distinct responses were recorded over the anterior half of the skull, though occasionally the maximum responses appeared over the temporoparietal areas. The EEG responses appeared simultaneously on both sides.

Pupillary diameter was estimated after gentle retraction of the eyelid by one of the examiners who had trained this beforehand on model circles of various sizes. Estimation was made easier by the subdued light in which the tests were carried out and by the sluggishness of pupillary reactions under the influence of barbiturate.

For testing the galvanic skin response (EDG) a bipolar palm to forearm lead was used with a time constant of 0.2 to 1 sec. being kept unchanged in the same experiment. Biphasic EDG was measured from tip to tip, monophasic curves from the baseline to the peak of the deflection.

All manipulations and events to be regarded as stimuli were registered against time within one second by means of the time marker. The observations were carried out in a practically soundproof room with reduced light allowing the correct estimation of pupillary diameter.

Results

Despite the variable duration of anaesthesia, the bioelectric patterns of response were consistent throughout. These observations have been reported in a preliminary communication (KAJTOR 1965).

I. Morphology of bioelectric responses

The typical changes evoked in EEG potentials registered from the earliest signs of barbiturate effect to the somnolent state before awakening, fall into four main patterns, apart from various intermediate types which are, however, relatively rare.

1. "*Pure desynchronization*" (PD) is an obvious though not fully complete desynchronization of background activity, identical with the "arousal

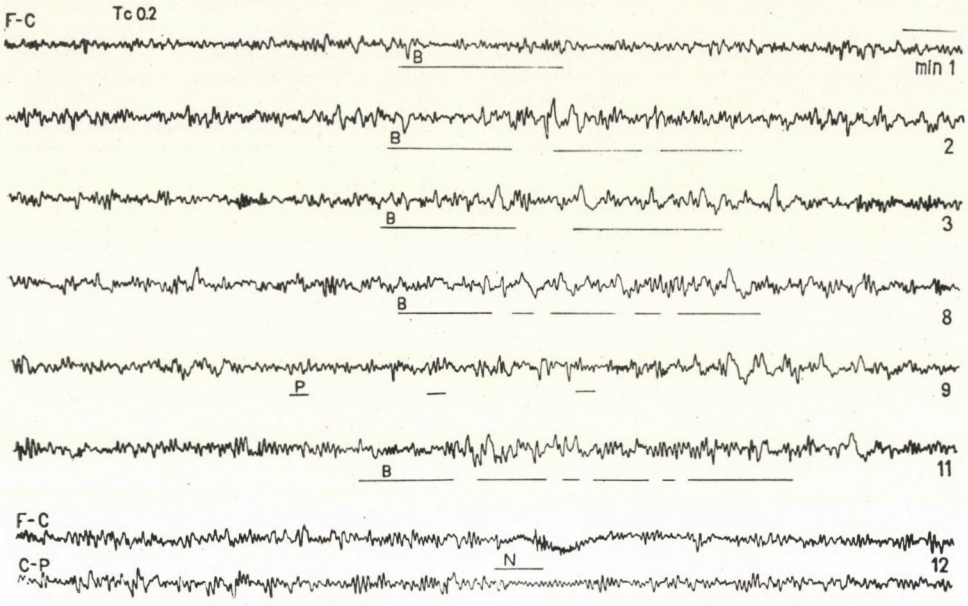


Fig. 1. Detail of tracing recorded in A-type anaesthesia. Min. = minute of anaesthesia from the beginning of hexobarbital injection; TC = time constant; calibration = 50 μv and 1 sec.; F—C = anterior frontal-central lead; B = electric buzzer; P = pinprick; N = calling by name

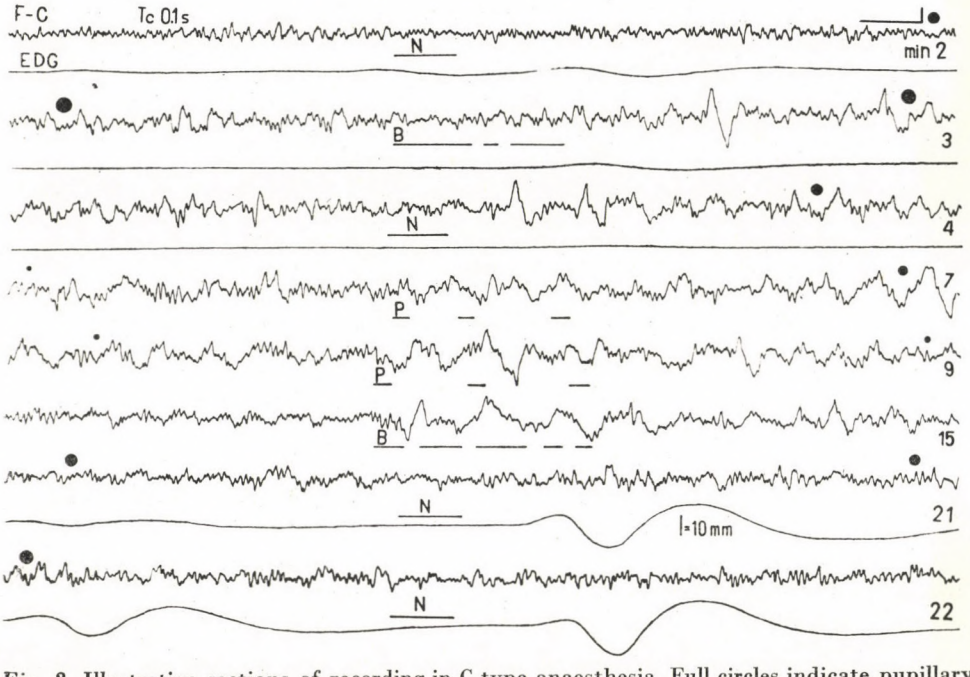


Fig. 2. Illustrative sections of recording in C-type anaesthesia. Full circles indicate pupillary diameters

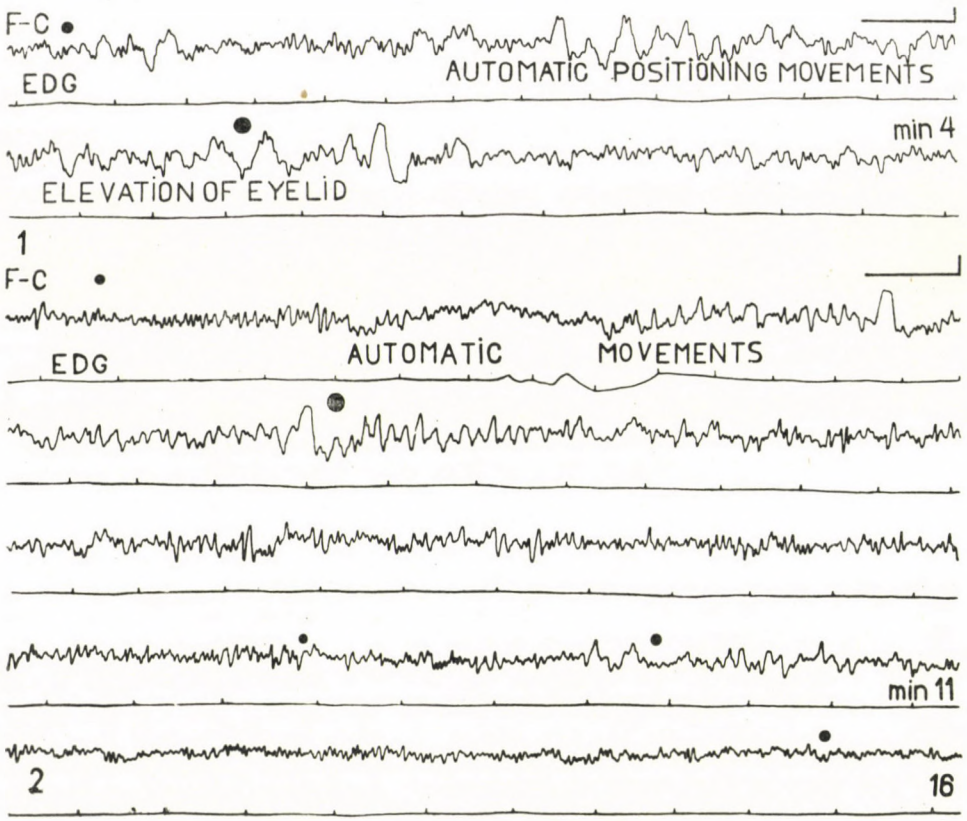


Fig. 3. B-type anaesthesia. Section 2 shows gradual return of the "paradoxical phase" under the influence of massive sensory stimuli (motion)

response" (Fig. 1, min. 1 and 12; Fig. 2, min. 2 and 22). Gradual blurring and eventual disappearance of this pattern coincide with impairment and loss of consciousness, while its gradual return marks the terminal phase of barbiturate effect. Near the end of the hexobarbital injection, the tracings of five subjects revealed periods of generalized low-voltage and increased frequency, coinciding in three cases with brief tonic seizures of extension. The state of deep coma obviously excludes any relationship between generalized desynchronization and "arousal" reaction in these cases.

2. "*After-synchronization*" (AS) is made up of two phases. The prevalent high-amplitude component in the theta-delta background activity is preceded by a desynchronization period of variable degree but none the less distinct (Fig. 1, min. 2, 9, 11; Fig 2, min. 3, 4, 21). Occasionally, the stimulus has sufficient effect to evoke a series of 2 or 3 coupled responses of this kind (Fig. 1, min. 11); it seems as if the run of 7—3 c/sec. waves is induced by the desynchronization component. Though AS may be encountered during progression

of sleep, it prevalently coincides with the phase of decreasing anaesthesia being gradually replaced by superficial sleep.

3. "*Pure synchronization*" (PS) is clearly set off against the background activity as a run of 1 to 5 c/sec. high-amplitude waves without any introductory or subsequent desynchronization period (Fig. 1, min. 3, 8; Fig. 2, min. 7, 9, 15; Fig. 3, min. 4). The background activity of higher frequency is often superimposed on the evoked high-amplitude waves.

The PS response pattern is in fact identical with those runs of slow, high amplitude waves evoked by FISCHGOLD and MATHIS (1959), CHATRIAN et al. (1963) and KAJTOR and OSZLÁNSZKY (1963) by means of peripheral sensory stimuli in various states of unconsciousness.

4. "*Transitional*" *responses* (TR) were termed those relatively rare intermediate types which differ from the foregoing patterns by the following features: (a) slowing with no increase in amplitude; (b) runs of high slow-waves joined by a low-amplitude, high-frequency period (K-complex); (c) a short period of low-amplitude, 10—14 c/sec. activity is interpolated between two high delta-waves.

5. Response was considered as "*undefinable*" (UR) when the sensory stimulus was insufficient to precipitate distinctly identifiable EEG patterns (lack of EEG-response).

II. Quantitative study of EEG responses

EEG tracings recorded during hexobarbital sleep in 17 subjects were selected for quantitative analysis. The test period extended from the moment when the first barbiturate fraction was injected, until the appearance of the first typical electric and biologic manifestations of recovery from the drug effect. This last phase was marked by a definite preponderance in the number of "arousal" reactions and by spontaneous orientation movements. The recordings were terminated at this point.

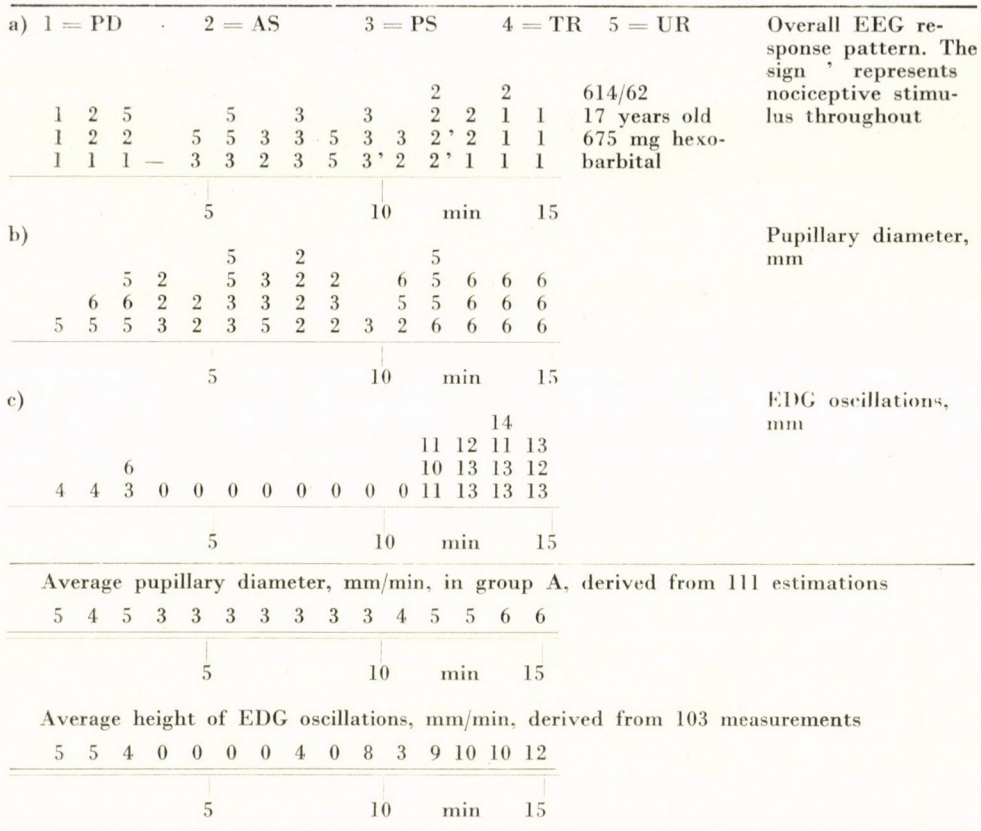
Each response registered from the start of the injection until completion of recording was identified on the grounds of configuration and amplitude. Morphology and other characteristics of the responses as well as the ineffective stimuli were marked separately by Arabic figures (examples in Tables I, II and III).

1. Viewed in their individual context including distribution and time relations in respect to the stages of barbiturate anaesthesia the evoked patterns allowed to divide the subjects under study into the following main groups.

A) 7 subjects, aged between 17 and 48 years (mean, 28.7). The dose of hexobarbital ranged between 600 and 750 mg (mean, 696 mg). The time of injection averaged 278 sec. The terminal PD responses were found to accumulate between the 11th and 15th minute of global barbiturate effect (Table I A).

Table I

Responses of one of the representatives of group A



B) 4 subjects aged between 15 and 35 (mean, 23.7) years. Barbiturate doses between 600 and 900 mg (mean, 732 mg), average duration of injection, 293 sec. Bioelectric and other arousal manifestations appeared between the 19th and 22nd min. of the global record (Table II A).

C) 6 subjects aged between 15 and 35 years (mean, 20.3). Barbiturate doses between 475 and 1000 mg (mean, 777 mg); average duration of injection, 310 sec. The manifestations of the terminal phase of anaesthesia were found to occur between the 23rd and 25th minutes of the total record (Table III A).

2. The stimuli totalled 597 in number; 193 in group A, 170 in group B and 234 in group C. The stimuli delivered to each person during the injection of hexobarbital averaged 1.5/min. in group A, 1.6/min. in group B, and 1.2/min. in group C. On termination of the injection, the rates of stimuli were increased to an average of 2/min. in group A, 1.9/min. in group B, and 1.6/min. in group C.

Table IA

Percentage distribution of response variables in EEG segments of identical length in the successive phases of anaesthesia of type A
(Group A)

Phases of anaesthesia, min.	1—2	3—4	5—6	7—8	9—10	11—12	13—14	15	
EEG response pattern									
UR (5)	0	1	9	4	7	1	0	0	
	0%	6%	*37%*	*16%*	*25%*	2%	0%	0%	
TR (4)	0	2	0	0	1	2	0	0	
	0%	11%	0%	0%	4%	5%	0%	0%	
PS (3)	0	3	10	10	6	2	0	0	
	0%	16%	*42%*	*42%*	*21%*	5%	0%	0%	
AS (2)	5	11	4	9	12	19	7	1	
	22%	*61%*	17%	*38%*	*43%*	*49%*	23%	13%	
PD (1)	18	1	1	1	2	15	22	7	
	78%	6%	4%	4%	7%	*39%*	*76%*	*88%*	
Number of stimuli =100%	23	18	24	24	28	39	29	8	Total *193*

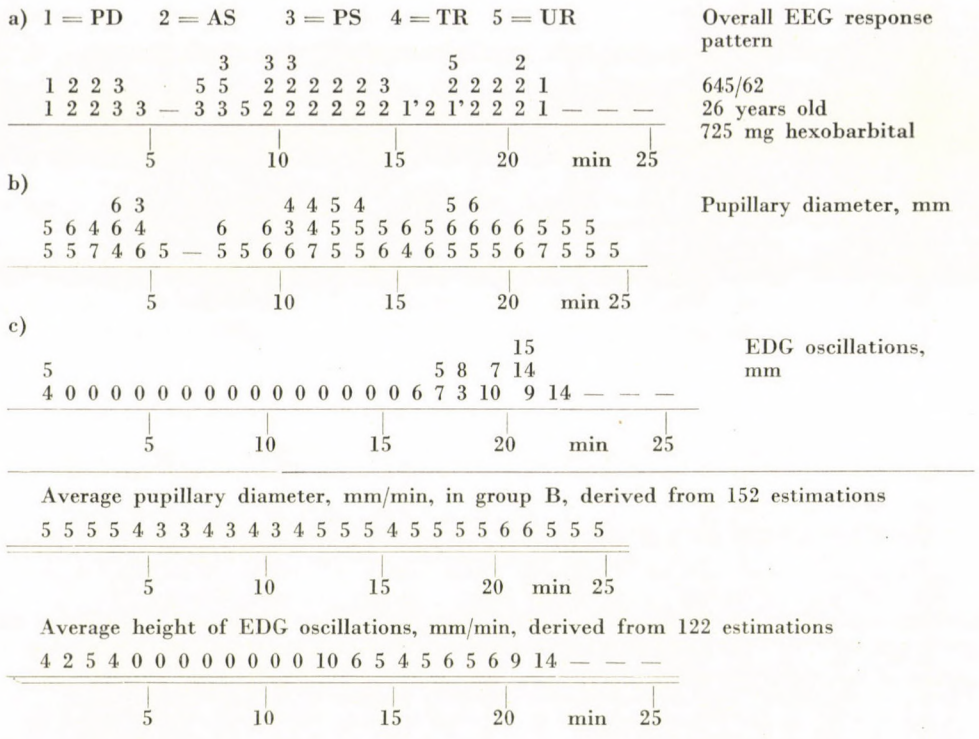
In group A, the highest mean age, i.e. the maximum drug tolerance, together with the highest average number of stimuli and the minimum of drug doses, were responsible for the short duration of anaesthesia. In group C, on the other hand, the lowest mean age, highest mean dose, lowest mean number of stimuli resulted in the longest duration of anaesthesia. Group B represents an intermediate type between the two others.

After separation of the three types of anaesthesia, the next step was to find a statistical approach to the quantitative and time relationships of the response variables to the successive phases of barbiturate effect. It was examined in each narcosis group separately whether the individual response patterns were scattered at random over the entire length of anaesthesia or whether each of the response variables showed any significant tendency to accumulate in one period of both the progressing and regressing sleep.

With this objective in view, the overall response patterns of each subject belonging to the same group were summarized. This procedure was simplified by the arrangement of Tables I to III; here the identical minutes of the overall individual response profile were placed in the same vertical row. The task was thus simply to count each of the five response types occurring in each successive minute from the beginning of hexobarbital administration until the end of its effect. This resulted in a global pattern of responses reflecting the number of PD, AS, PS, TR or UR responses recorded in each particular minute over

Table II

Responses of one of the representatives of group B



the global test period. It was soon observed that one-minute intervals were quite unnecessary. In group A characterized by short anaesthesia 2-minute, and in groups B and C 3-minute periods have proved adequate for statistical evaluation. Since the overall profil of responses in minutes happened to correspond to odd numbers, the last (15th) minute was taken as a separate period in group A and the first in groups B and C.

This done, the task was to calculate the percentage distribution of the response patterns for each successive 2- and 3-min. segment. The results of these calculations are shown in Tables I A, II A and III A.

Tables I A, II A, III A show clearly that in all three types of barbiturate effect, accumulation of response patterns PS and UR is confined to the sole phase following immediately upon the termination of barbiturate supply, whereas that of PD coincides with two opposite periods, of the initial, i.e. progressive, and of the terminal, i.e. regressive, barbiturate effect. The most propitious factors for PD ("pure desynchronization") responses are provided by inebriety and drowsiness marking the earliest and the passing barbiturate

Table IIA

Percentage distribution of response variables in EEG segments of identical length in the successive phases of anaesthesia of type B
(Group B)

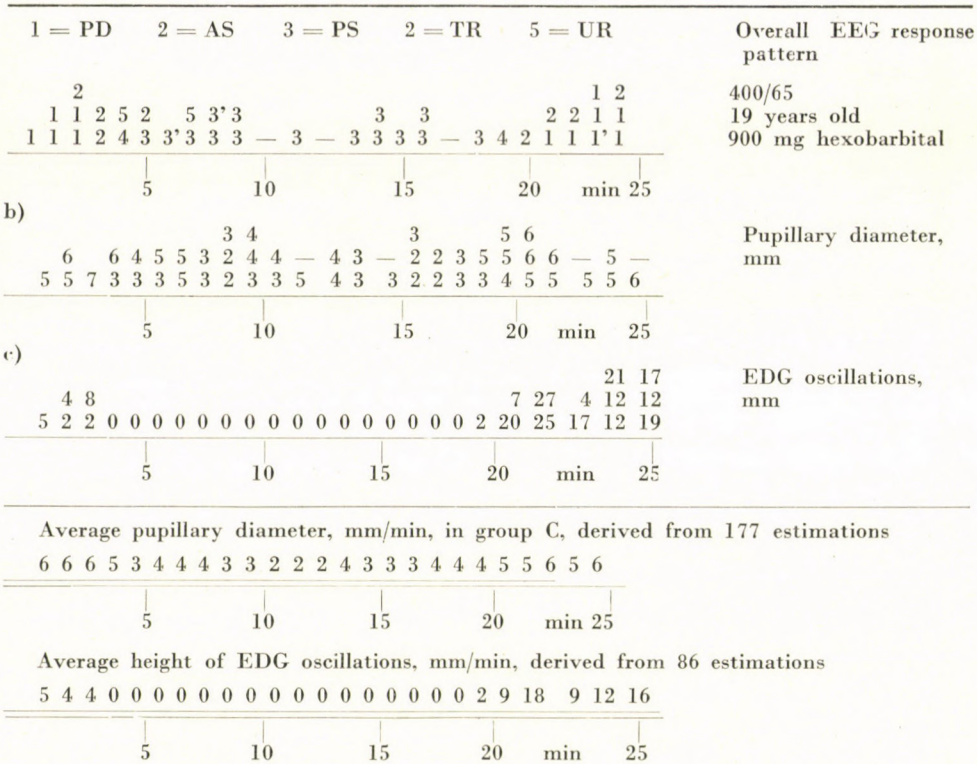
Phases of anaesthesia, min.	1	2—4	5—7	8—10	11—13	14—16	17—19	20—22	23—25	
EEG response pattern										
UR (5)	0	3	5	8	3	0	3	1	0	
	0%	12%	*24%*	*35%*	14%	0%	10%	6%	0%	
TR (4)	0	0	5	1	0	0	0	0	0	
	0%	0%	*23%*	4%	0%	0%	0%	0%	0%	
PS (3)	0	2	9	11	9	3	0	0	0	
	0%	9%	*43%*	*48%*	*41%*	13%	0%	0%	0%	
AS (2)	0	16	1	3	10	19	19	2	0	
	0%	*70%*	5%	13%	*45%*	*83%*	*66%*	13%	0%	
PD (1)	5	2	1	0	0	1	7	12	9	
	100%	9%	5%	0%	0%	4%	24%	*80%*	*100%*	
Number of stimuli = 100%	5	23	21	23	22	23	29	15	9	Total *170*

effect. With increasing depth of narcosis, that is from the 2nd to 4th minutes in the course of the injection, these responses disappear very fast though gradually, over varying lengths of time in the three groups and entirely cease to become again prevalent in the last minutes of anaesthesia. Parallel with the fast disappearance of the PD response, the AS ("after-synchronization") responses gain prevalence, indicating that increasing barbiturate concentrations in the blood favour the responsiveness of some other cerebral structure too. The prevalence of AS responses is also biphasic. In this manner, the UR + PS period is interpolated between two AS phases. This is particularly clear in groups B and C.

A further deepening of the barbiturate effect involves depression of those structures, too, whose response to peripheral stimuli results in the AS pattern, though this functional impairment is less severe and less persistent than that of the PD-producing population of neurons. As the barbiturate effect attains its maximal depth, the AS responses are overwhelmed by the PS ("pure synchronization") responses which become predominant in 2 to 3 minutes. During approximately the same period the number of "unidentified responses" (UR) suddenly increases. The appropriate level of anaesthesia, nearly coinciding with the termination of hexobarbital administration, is marked by a full

Table III

Responses of one of the representatives of group C



delta-activity with some interspersed short flat periods indicating the maximal depth of anaesthesia. There are TR and AS responses to be seen, in addition to the PS + UR patterns, as a sign of the temporary amplifying of the stimuli.

Transitional responses (TR) were never demonstrable during the prevalence of the PD pattern and tended to appear when AS responses were gaining predominance. In two representatives of group B, the number of TR was conspicuously high, particularly in the UR + PS periods. However, in relation to the global an ount of responses, the ratio of TR was 3% in all three groups.

A further analysis concerned the percentage distribution of the individual response patterns in each of the three types of anaesthesia with reference to the summarized response scheme of these three types (Table IV).

The V shows the per cent deviation between the maximum rates of the individual response variables reached in definite periods and their rates of occurrence outside these periods.

Table IIIA

Percentage distribution of response variables in EEG segments of identical length in the successive phases of anaesthesia of type C
(Group C)

Phases of anaesthesia, min.	1	2—4	5—7	8—10	11—13	14—16	17—19	20—22	23—25	
EEG response pattern										
UR (5)	0	1	8	14	10	12	7	4	0	
	0%	5%	*32%*	*45%*	*38%*	*37%*	*23%*	14%	0%	
TR (4)	0	1	1	1	1	1	0	2	0	
	0%	5%	4%	2%	4%	3%	0%	7%	0%	
PS (3)	0	1	11	12	14	11	11	0	0	
	0%	5%	*44%*	*40%*	*54%*	*33%*	*35%*	0%	0%	
AS (2)	0	10	5	4	1	9	12	16	8	
	0%	*48%*	20%	12%	4%	27%	*39%*	*57%*	24%	
PD (1)	6	8	0	0	0	0	1	6	25	
	100%	*38%*	0%	0%	0%	0%	3%	21%	*76%*	
Number of stimuli = 100%	6	21	25	31	26	33	31	28	33	Total *234*

Table IV

	PD	AS	PA	UR	TR	Total
Anaesthesia, type A	67	68	31	22	5	193
	35%	*35%*	16%	11%	3%	
Anaesthesia, type B	33	74	34	23	6	170
	19%	*44%*	20%	14%	3%	
Anaesthesia, type C	46	65	60	56	7	234
	20%	27%	*26%*	*24%*	3%	
						597

The values indicate, by how many per cent the rate at which the individual response pattern occurs during its period of prevalence (expressed in minute of global anaesthesia) exceeds the rate at which the same pattern is identifiable during the other phases of anaesthesia (expressed in their total duration in minutes).

Table V

1. Per minute rate of EEG response variables
(in the period of prevalence)

	PD	AS	PS	UR
Anaesthesia A	min. 1—2, 11—15 9/min.	3—4, 9—12 7/min.	5—10 4.3/min.	5—10 3.3/min.
Anaesthesia B	min. 1, 20—25 3.7/min.	2—4, 11—19 5.3/min.	5—13 3.2/min.	5—10 2.2/min.
Anaesthesia C	min. 1—4, 20—25 4.5/min.	2—4, 17—22 4.2/min.	5—13 11/min.	5—16 3.7/min.

2. Per minute rate of EEG response variables
(in other periods of the same recording)

	PD	AS	PS	UR
Anaesthesia A	0.7/min.	3/min.	0.5/min.	0.3/min.
Anaesthesia B	0.6/min.	0.5/min.	0.3/min.	0.3/min.
Anaesthesia C	0.07/min.	1.7/min.	1.4/min.	0.9/min.

3. Difference between the rates of response of the two groups

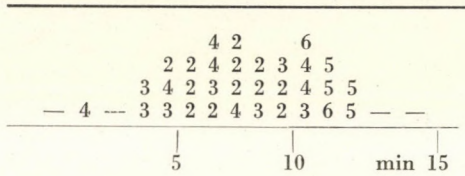
	PD	AS	PS	UR
Anaesthesia A	1885%	133%	860%	633%
Anaesthesia B	517%	960%	967%	633%
Anaesthesia C	5420%	147%	685%	311%

For the independence analysis of the results we used the chi-square test on the ground of contingency tables. At the conventional fiducial limit of 5 per cent the data presented in Tables I to III reveal definite correlations between the sequential accumulations of evoked EEG response patterns and the individual phases of anaesthesia, the two parts of barbiturate effect having been evaluated separately. (From Table I $\chi^2 = 128.217623 + 84.2437604$; from Table II $\chi^2 = 119.852607 + 68.672922$; from Table III $\chi^2 = 130.009271 + + 130.782139$.)

In evaluating the correlations between the magnitudes of EDG-oscillations and the level of anaesthesia derived from Tables VI to VIII we have divided the duration of anaesthesia as well as the magnitudes of oscillations into two, more accurately into three, categories, taking also the O-values into

Table VI

Changes in the pupillary diameter and in the EDG oscillations in A-type anaesthesia



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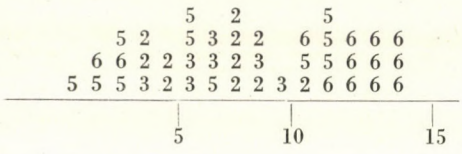
Pupillary diameter mm



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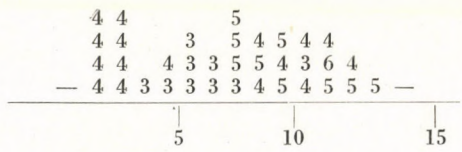

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Total

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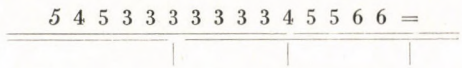
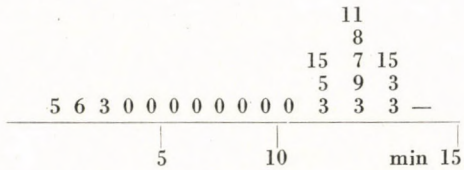


Average, min



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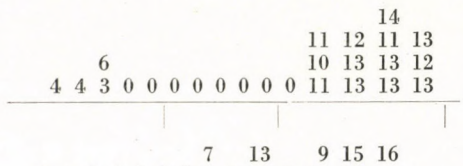
EDG oscillations mm



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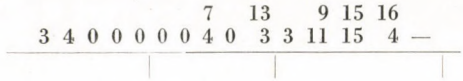

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Total

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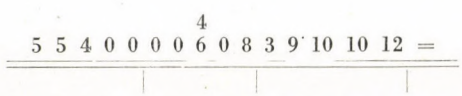
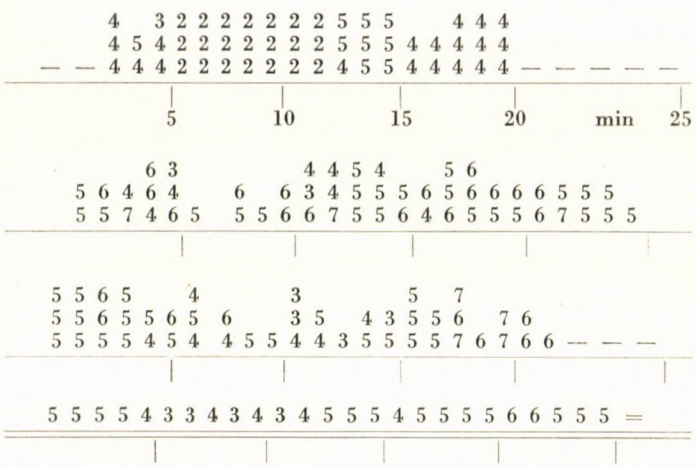


Average, min

account. On the evidence of this test the EDG-oscillations increase excessively in amplitude during the last third phase of anaesthesia. Here too the correlations become demonstrable at a fiducial limit of 5 per cent. (From Table VI $\chi^2 = 13.431957$; from Table VII $\chi^2 = 41.2438599$; from Table VIII $\chi^2 = 31.7626521$.)

Table VII

Changes in the pupillary diameter and in the EDG oscillations in B-type anaesthesia

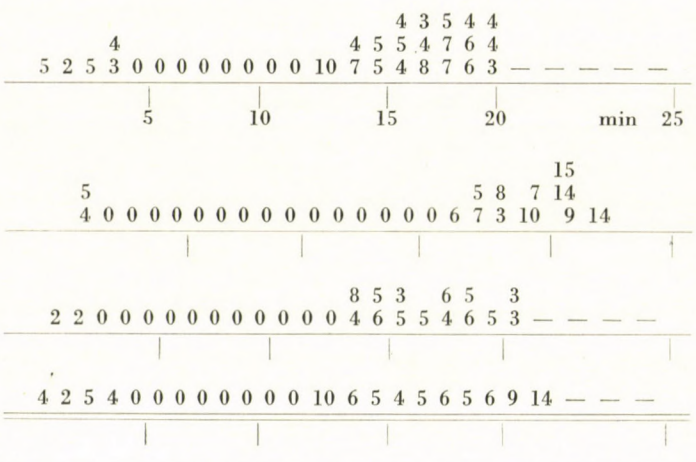


Pupillary diameter mm 613/62

645/62

629/62 Total *152*

Average, min



EDG oscillations mm 613/62

645/62

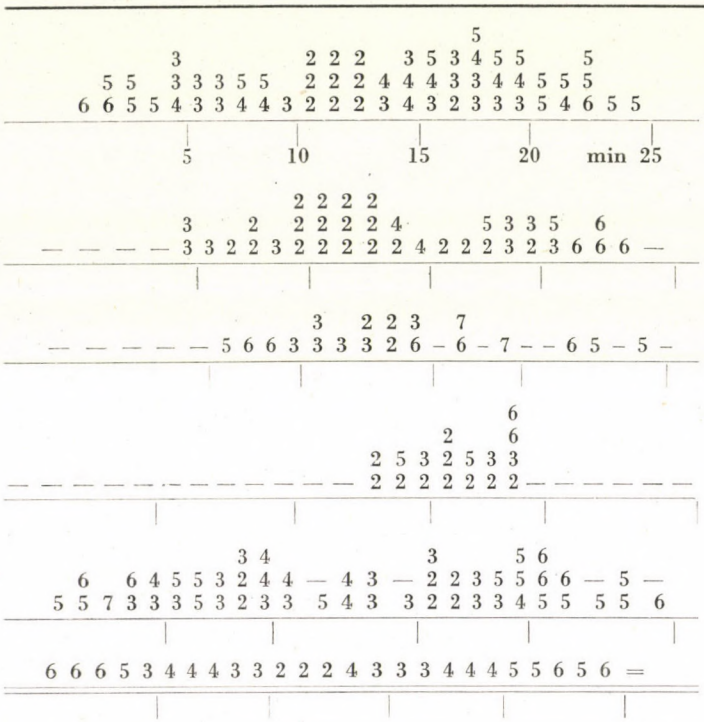
629/62 Total *34*

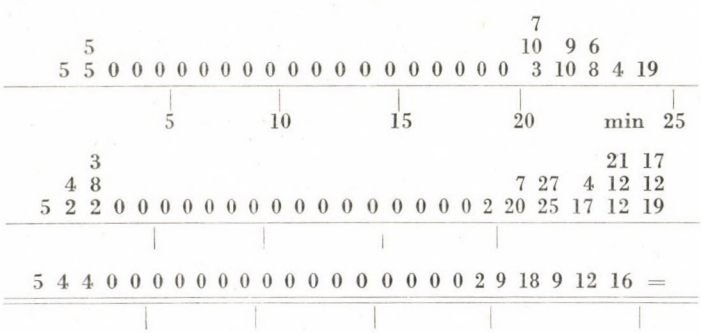
Average, min

In analysing the significance of the changes in pupillar diameter the data presented on each side of Table VI to VIII have also been evaluated separately. The data in Tables VI and VIII reveal definite correlations between the individual phases of anaesthesia, pupillar diameter and EEG-response pattern at the conventional fiducial level of 5 per cent (Table VI $\chi^2 = = 52.1289291 + 54.8126891$; as regards Table VII, here similar correlations, if any, are not demonstrable below the 10 per cent level: $\chi^2 = 89.6512468 + + 59.9351157$; Table VIII $\chi^2 = 116.367429 + 105.954868$).

Table VIII

Changes in the pupillary diameter and in the EDG oscillations in C-type anaesthesia





III. Pupillomotor and electrodermographic changes at different levels of hexobarbital anaesthesia

The foregoing observations were regarded as consistent with our claim that the individual EEG response patterns represent reactions of distinct non-specific cerebral structures, functionally separable on the very ground of their

different tolerance to barbiturate. In order to support this assumption the pupillomotor and electrodermographic responses were recorded parallel with the changes in the level of barbiturate effect, these having proved, in our experience, reliable indicators of the depth of barbiturate anaesthesia.

Eleven persons were selected from the three foregoing groups for these studies. Pupillary diameter was generally checked during the 2 to 3 sec. period prior to sensory stimulation and during the 3 to 8 sec. period subsequent to it, since under the effect of barbiturate the pupillomotor response takes at least 2 sec. but mostly more time to become manifest.

Estimations of pupillary diameter yielded 111 data in group A (3 subjects) 152 in group B (3 subjects) and 177 in group C (5 subjects). EDG oscillations were recorded 6 times in group A (3 subjects), 54 times in group B (3 subjects) and 30 times in group C (2 subjects) though 103 stimulations had been carried out in group A, 122 in group B and 86 in group C. This added up to 440 values for pupillary size and 181 for the EDG reaction.

The pupillary diameter generally showed distinct changes parallel with the successive levels of barbiturate effect, particularly in groups A and C. The early phase of barbiturate effect was accompanied by mydriasis in approximately one third of the cases. In the subsequent phase of anaesthesia there was an obvious tendency to miosis, whereas in the terminal phase again a tendency to mydriasis, at this time more distinct and more persistent than in the early phase of drug action.

Peripheral sensory stimulation evoking a transitory mydriasis in deep anaesthesia resulted in a definite reduction of spontaneous dilatation of the pupil as an effect of a weak pupillary constriction at more superficial levels. All these signs required many times 5 to 10 sec. to appear or to disappear.

The variations in pupillary diameter often showed conspicuous relationships with the appearance or absence of EDG responses including the amplitudes of oscillations, furthermore with the prevalence or fading of certain EEG response patterns. The phases of barbiturate anaesthesia marked by responses of the PS and UR types were usually associated with miosis but the EDG was sometimes inhibited at levels associated with mydriasis.

The EDG proved highly sensitive to barbiturates as illustrated by Tables I to III. By the end of the 4th minute after the injection of hexobarbital, the EDG was abolished and its earliest reappearance was noted at about the beginning of the third section of anaesthesia, and even this was exceptional. On the other hand, its amplitudes during the last third of anaesthesia in groups A and B and during its last fifth in group C were twice or three times as high as those found in the alert state without any drug effect. By the present stimulation procedure it was rarely possible to elicit an unbroken succession of EDG responses for longer than one half of the total length of anaesthesia in any of the three groups.

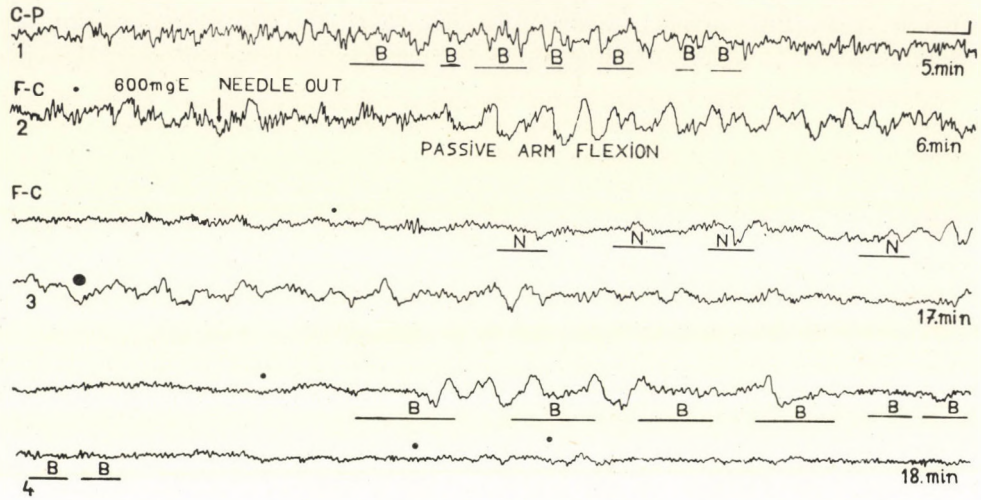


Fig. 4. Segment 1: B-type anaesthesia. Segments 2 to 4 have been recorded from another subject. No. 2 is from a C-type, Nos 3 and 4 are from a B-type hexobarbital tracing

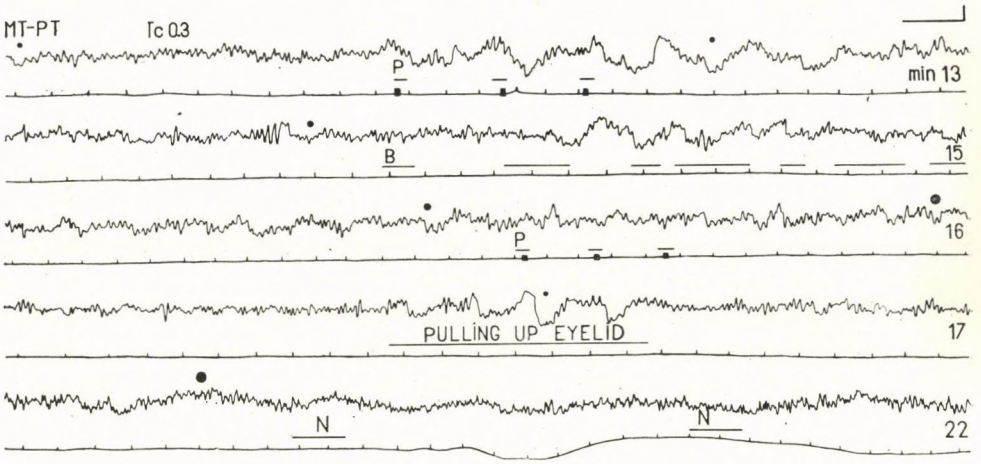


Fig. 5. Details of a tracing recorded during C-type anaesthesia. MT—PT = mediotemporal—posteriotemporal pair

The relationships between the appearance of EEG, EDG and pupillomotor responses and the levels of anaesthesia have been presented graphically in Figs 6, 7, and 8. It clearly emerges from these complex graphs that numerical prevalence of any of the EEG response patterns I to IV marking well-defined phases of anaesthesia is closely associated with definite changes in EDG and pupillomotor activity.

At the levels of barbiturate anaesthesia where PS and UR responses were predominant modifications, miosis occurred practically always, and stimulation

resulting in PS responses was hardly ever followed by definite mydriasis. Spontaneous automatisms of posture, as well as evoked incoordinated movements of adversion (Fig. 3, 1 and 2) or "réponses de sommation" (Fig. 4, 3) were accompanied by a slowly developing mydriasis. This was, however, in no relation to the impressive EEG responses.

(a) In group A of short anaesthesias there was an unmistakable tendency to miosis between the 5th and 10th minutes of anaesthesia; the number of pupillary diameters of about 2 mm size occurred in 37% of the total against a 5.4% rate during the first and last thirds of anaesthesia.

Disappearance as well as reappearance of the EDG response occurred at similar levels of anaesthesia. Exceptionally it was possible to elicit a transient EDG response by pinpricks even at superficial sleep level (Table VI, Fig. 6).

(b) In group B the phase of miosis was confined to one subject. It was however, unusually long, persisting until the end of the 5th to 15th minute of anaesthesia and being resistant to all kinds of sensory stimuli. Here too, the EDG response was abolished during the period of miosis (Table II, Fig. 7).

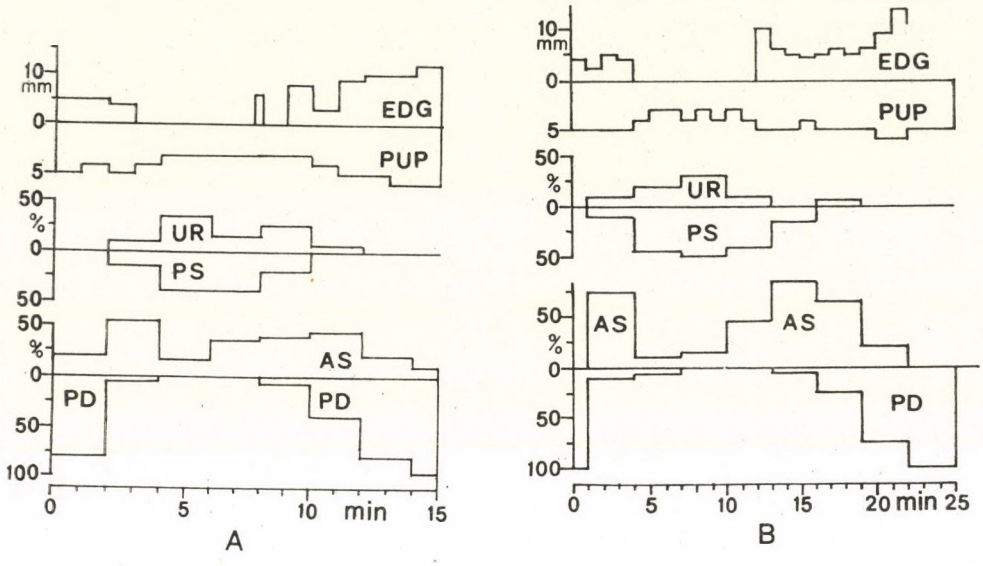
(c) Type C, i.e. protracted anaesthesia, proved more favourable to miosis than did type B; the ratio of miotic pupils attaining 68.5% in the period between the 5th and 9th minutes as against 19.2% in the previous and subsequent phases. This type of anaesthesia extremely inhibited the EDG-generating neural structures. Between the 3rd and 20th minutes, that is during more than two thirds of the total period of anaesthesia, even repeated nociceptive stimuli failed to elicit EDG responses.

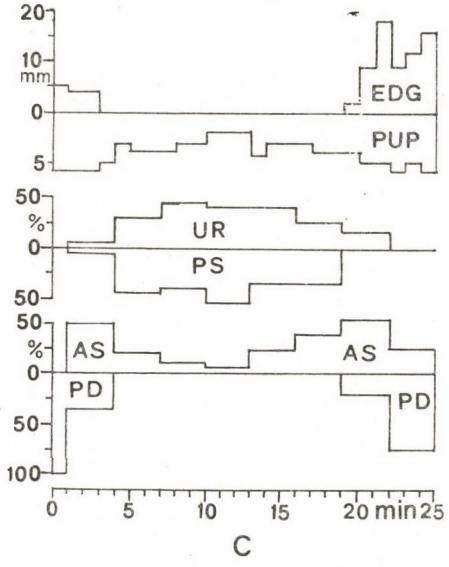
(d) The phases of sleep associated with miosis were marked by a high incidence of PS as well as of UR responses. Their joint occurrence totalled 51.6% in group A, 41.6% in group B and 76.6% in group C. During the phase of miosis the EDG was entirely paralyzed. It was never associated with PS or UR response but invariably with PD responses and occasionally with AS responses. During slumber in the terminal phase of barbiturate effect there was a surprising increase in the EDG amplitudes with simultaneous mydriasis (Fig. 2, min. 21—22).

IV. Presumable factors affecting the type of EEG response

(a) Though we had to work without an automatic analyser for the study of EEG frequencies, close visual study yielded some informative evidence as regards the influence of pre-stimulatory frequency on the evoked EEG responses and on other reactions.

In certain phases of hexobarbital anaesthesia the basic EEG frequency tended to increase and its amplitudes to diminish. This process began a few minutes after completion of the hexobarbital injection with a basic activity made up of delta-theta waves attaining 80 to 200 μv and resulted gradually





Figs 6—8. The prevalence of percentage rates of the individual response patterns and the joint changes of pupillomotor and EDG activities reflect the order of succession of the individual phases of anaesthesia. The average values of pupillary diameters and of EDG amplitudes are given in mm and referred to minutes. The average rates of response variables are referred to 3-minute intervals, with the exception of the last minute in group A, and the first in groups B and C. The divisions of the vertical scale represent 25% changes

in a moderately irregular, 10 to 16 c/sec. pattern of 20 to 40 μv amplitudes over the entire brain (Fig. 2, min. 15; Fig. 4, min. 17—18; Fig. 5, min. 13—17). This phase of induced sleep is reminiscent in both electromorphological and biological respects of that phase of natural sleep which has been described as the "paradoxical phase" (PP) of sleep by JOUVET et al. (1960) and as its "IVth stage" by DELANGE et al. (1962) and which is marked by the appearance of rapid eye movements (REM). The corresponding phase of barbiturate-induced sleep lacks REM but it does share other features of natural sleep, namely, a considerable reduction of bioelectric and other responsiveness.

(b) Certain sections of the tracings revealed a divergence between electric excitability of the brain and background activity. Different degrees of cerebral responsiveness were associated with the same background morphology; stimuli of similar intensity resulted in dissimilar modifications of the EEG. In a mixture of 12—16 c/sec. low voltage EEG (Fig. 4, min. 18), or an alpha dominance (Fig. 5, min. 13, 15) in the waking state were sometimes associated with PS and UR responses together with miosis, furthermore with an absence of EDG and of automatic movements, and with a relaxation of the striated muscles those of eyelids too, resulting in spontaneous opening of the eyes. In a background activity of similar type PD responses were prevalent in the terminal phase whereas AS responses were exceptional, and the appearance of high EDG amplitudes was fairly common.

(c) Duration and intensity of stimulation are of obvious significance, particularly with regard to the PS containing phases of anaesthesia. In some instances, stimulation had to be continued over 2 to 4 minutes to evoke PS responses (Fig. 4, min. 17; Fig. 5, min. 15). The EEG modifications often exceeded in duration the inductive stimuli (Fig. 1, min. 3, 8, 9) or, on the contrary, the original background activity returned before the end of stimulation (Fig. 4, min. 18); again in other cases the EEG responses disappeared parallel with the stimulation (Fig. 4, min. 5; Fig. 5, min. 17).

Habituation to stimuli was rarely observed and never outside the phase of "paradoxical sleep" (Fig. 4, min. 18). This suggests the interpretation that during the "paradoxical" phase, some sleep-protective mechanism manifesting itself with a simultaneous inhibition of pupillomotor activity and EDG may become prevalent.

(d) The nature of stimulus is probably also of significance. During the peak blood barbiturate levels, i.e. immediately after the end of injection. nociceptive as well as vagal (coughing), proprioceptive, kinesthetic and thermic stimuli seemed to influence electric activity of the brain more profoundly than did tactile or simple auditive (buzzer) stimuli. This is indicated by the pin-prick-evoked PS responses (Fig. 2, min. 7) also seen in association with passive or automatic active movements (Fig. 3, 1; Fig. 4, 2). Moreover, the serial responses evoked by nociceptive stimuli were interspersed with electric manifesta-

tions typical of more superficial levels of sleep. For instance, there appeared one AS among the PS responses.

Auditory stimuli of a personal meaning such as calling by name were doubtlessly more efficient than, for instance, the sound of a buzzer. This is in agreement with the observations of OSWALD et al. (1956) in human natural sleep, indicating that discrimination of the meaning of words is not completely abolished at certain levels of unconsciousness.

Discussion

Inflow of peripheral stimuli into the brain during hexobarbital anaesthesia results in bilateral simultaneous EEG responses varying in type with the level of artificial sleep. Evoked modifications of human EEG, particularly the K-complex associated with barbiturate anaesthesia, have been closely studied by BRAZIER (1954), ROTH et al. (1956), FISCHGOLD and SCHWARTZ (1961). However, to our knowledge, the claim that along with the dominant EEG response patterns characterizing the successive levels of barbiturate anaesthesia, the pupillomotor responses and the EDG activity also vary in rate and intensity in a typical manner, and that these changes are closely co-ordinated, has not been substantiated thus far by statistical evidence.

The electric response variables specified by the present authors in barbiturate-induced sleep reveal consistent relationships with the successive phases of barbiturate effect. Each individual response variable has some particular EEG segment (maximum depth of sleep) or segments (increasing or diminishing depth of sleep) as its optimum. The very same level of anaesthesia which is optimal to a certain type of response may be less favourable to (or definitely incompatible with) some other. The response variables under review follow an unvarying chronologic order. Though scarcely affected by the duration of anaesthesia, they are influenced to some degree by the character and intensity of the stimulus as well as by the rate of intravenous administration of the barbiturate.

Massive manifestation of any of the main response variables marks particular level of barbiturate sleep, reflecting at the same time the responsiveness of cerebral electrogenesis and the prevailing level of anaesthesia. All the described bioelectric variables may be regarded as representatives of the activity of separate, non-specific information-carrying structures. Each determine the morphology of evoked electric modifications at different levels of anaesthesia. Their order of cumulative appearance is by this fact strictly depending on the successive phases of the latter. In addition to the time locked predominance of a specific pattern of EEG response it is also the degree and tendency of pupillomotor and EDG reactions which closely reflect the sequence of the phases of anaesthesia.

It seems that, with increasing barbiturate blood levels, the individual PD, AS and PS producing and facilitating non-specific structures are successively paralyzed in the order of their different hexobarbital sensitivity. The reticular structures of maximum barbiturate sensitivity make up the substrate of "arousal responses", whereas the most barbiturate-resistant non-specific structures emit PS responses. It is of interest to recall here that particular phase of anaesthesia which resembles the "paradoxical" phase of natural sleep is characterized not only by its electric pattern but also by its high threshold of reactivity. Further features adding to this similarity are the PS + UR type of response, distinct miosis and absence of EDG.

The biological significance of the PS and AS responses is not sufficiently understood. The PS responses are considered to represent a functional capacity of the brain which may be paralleled with an ontogenetic level of responsiveness corresponding to the first days of extrauterine life. At this early stage of development, peripheral stimuli produce in the human being (KELLAWAY 1957) as well as in animals (ANOKHIN 1964) high-amplitude, slow-wave PS responses, instead of the increased frequency and reduced amplitude, i.e. the PD (desynchronization) response characteristic of the adult and child.

It is, therefore, reasonable to assume that the PS response pattern expresses the activity of the primordial arousal system which in early extrauterine life is the sole representative of cerebral bioelectric responsiveness. In the course of ontogenesis, on this old system are gradually superimposed more differentiated other arousal structures which produce desynchronization responses (PD) when reaching functional maturity; nevertheless they may function also in adult life during certain phases of natural sleep in the K-complex made up as a high-amplitude, slow-wave component. In conditions affecting consciousness, for instance in deep barbiturate anaesthesia or in other comatose states, this primitive arousal system seems to be the only structure capable of responding to external stimuli with augmented cerebral reactivity and of signalling partial primitive arousal by emitting high-amplitude slow waves.

There is consistent evidence that internal as well as external sensory stimuli streaming into the brain may give rise to runs of slow-wave activity (GRASTYÁN et al. 1952, BONVALLET and SIGG 1958a, b, POMPEIANO and SWETT 1962). Electric stimulations at slow frequencies to various cerebral structures may induce EEG synchronization and recruiting responses in animals (HUNTER and JASPER 1949, HESS 1957, MAGNES et al. 1961, FAVALE et al. 1961, BUCHWALD et al. 1961, HÖSSLI and MONNIER 1962, MORUZZI 1964). These EEG responses are associated with a reduction of alertness and motor activity, even with behavioural patterns of sleep.

In opposition to these observations, PRINCE and SHANZER (1966) stated to have induced a high-amplitude synchronized cortical activity in the frontal

area by high frequency electrical stimulation of the pontomesencephalic reticular substance and the sciatic nerve in cats subjected to anaesthesia by 22 to 27 mg/kg of pentobarbital. The synchronization response was associated with biologic signs of arousal.

Inhibitory responses to excitation of the non-specific thalamic nuclei by low frequency electric impulses have been demonstrated in man, (JUNG and HASSLER 1960, SPIEGEL et al. 1960). Generalization of synchronization in the EEG was closely followed by a gradual impairment of concentration and of awareness until the total loss of consciousness.

It is certainly difficult but in our view not impossible to bridge over the gap between the foregoing observations and our interpretation of the PS responses. The difficulty of this task is well illustrated by the divergence of views in respect of the K-complex. To quote a few examples:

(a) The K-complex seems to act so as to muffle the arousal stimulus (WALTER 1953). Its major feature is that it occurs without arousing the subject (BRAZIER 1954). The K-complex seems to signal the intake of neutral afferent impulses by the brain (FISCHGOLD and SCHWARTZ 1961). (b) The K-complex represents a biological correlate of a primordial perceptual process, in other words, a primitive state of vigilance, triggering off the process of arousal (BANCAUD et al. 1953, ROTH et al. 1956,). (c) The high slow transient factor seems to represent the primary process activated by the impulse whereas the rhythmic fast component is in all likelihood the manifestation of the prevailing arousal process (KELLAWAY 1957, VETTER and BÖKER 1962).

In the present study, sensory stimulation during deep anaesthesia was inefficient or resulted in PS responses with occasional mydriasis. The pupillary reaction shows that the structures producing PS are capable of emitting arousal impulses in response to excitatory stimuli. The appearance of two wave components of opposite nature in the AS responses, particularly those forming short chains from alternating runs of frequent and slow wave responses during superficial sleep (Fig. 1, min. 3, 9, 11) may be signs of a phasic inhibition brought about by bulbar inhibitory impulses on the ARAS, as demonstrated by MAGNES et al. (1961) in animals. DELL et al. (1961) as well as BONVALLET and ALLEN (1963) suggest the possibility of a mesencephalo-bulbar-mesencephalic cycle. VETTER and BÖKER (1962) believe, as do the present authors, that the first desynchronized component of the AS responses may well represent the stimulatory (arousing) impulses of this cycle.

With gradual recovery from hexobarbital anaesthesia, the AS responses step by step disappear and the number of the PD modifications increases, parallel with extreme EDG oscillations during growing intensity of somatomotor and pupillomotor responses. Manifestations of returning consciousness and verbal reactivity may also be observed. These behavioural changes signify the rein-

tegration of the responsiveness and of the multiple interplay of the cerebrocortical structures of voluntary activity.

On the ground of the present observations the morphologic features of EEG responsiveness outlined in the foregoing may well represent different non-specific brain stem systems, each influencing the EEG, pupillomotor, and EDG activity in different phases of anaesthesia, most probably in relation to their individual barbiturate tolerance.

Evidence derived from studies on cats and monkeys permits to connect the mechanism of barbiturate effect with an inhibitory influence on the synaptic functions of certain mesencephalic reticular structures (FORBES and MORRISON 1939, KING 1956, FELDMAN and PORTER 1960, FELDMAN and WAGMAN 1963). It is reasonable to assume that certain hierarchic and some antagonistic tendencies existing between these structures are suppressed in the state of consciousness and successively released with deepening anaesthesia. BRAZIER (1954, 1961, 1963) finds sufficient support to the claim that deepening barbiturate anaesthesia gradually abolishes normal inhibitory mechanisms of the cerebral cortex, while releasing EEG rhythms suppressed in the normal state of consciousness.

From the present observations it emerges that the mechanism of arousal from deep hexobarbital anaesthesia is in no way confined to any single arousal structure. Instead, it may be attributed to an interaction of excitatory influences of different non-specific arousal structures, each of individual barbiturate-sensitivity but in functional hierarchy. Arousal is elicited by repeated sensory stimuli of adequate intensity, and triggered off, in all probability, by the most barbiturate-resistant, PS-producing structure assumed to represent the primordial arousal system in ontogenesis. Inflow of sensory stimuli has an "energizing" effect on the reactivity of this system, enabling it under favourable circumstances to emit, on its part, further "energizing" stimuli to the less barbiturate tolerant AS system which, in its turn, may promote the excitability of the PD system by a similar mechanism.

These effects of the peripheral impulses are coupled with their stimulating influence on cardiovascular functions which greatly enhances the process of detoxication by accelerating elimination of the drug from the organism.

REFERENCES

ANOKHIN, P. K.: Electroenceph. clin. Neurophysiol. **16,** 27—43 (1964).
BANCAUD et al.; Rev. neurol. **89**, 399—418 (1953)
BONVALLET, M., SIGG, B.: J. Physiol. (Paris) **50,** 63—74 (1958a).
BONVALLET, M , SIGG, B.: J. Physiol. (Paris) **50,** 175—205 (1958b).
BONVALLET, M., ALLEN, M. B. JR.: Electroenceph. clin. Neurophysiol. **15,** 969—988 (1963).
BRAZIER, M. A. B. in: Brain Mechanisms and Consciousness. Ed. DELAFRESNAYE, J. F. Blackwell, Oxford 1954, Pp. 163—199.
BRAZIER, M. A. B.: Brit. J. Anaesth. **33,** 194—203 (1961).

Brazier, M. A. B.: Electroenceph. clin. Neurophysiol. **15,** 287—298 (1963).
Buchwald, N. A., Wyers, E. J., Okuma, T., Hauser, G.: Electroenceph. clin. Neurophysiol. **13,** 509—518 (1961).
Chatrian, G. E., White, L. E. jr., Daly, D.: Electroenceph. clin. Neurophysiol. **15,** 272—280 (1963).
Dement, W.: Electroenceph. clin. Neurophysiol. **10,** 291—296 (1958).
Delange, M., Castan, Ph., Cadilhac, J., Passouant, P.: Rec. Neurol. **106,** 106—113 (1962).
Dell, P., Bonvallet, M., Hugelin, A. in: On the Nature of Sleep. Eds. Wolstenholme, G. E. W., O'Connor, M. Churchill, London 1961, Pp. 86—107.
Favale, E., Loeb, C., Rossi, G. F., Sacco, G.: Arch. ital. Biol. **99,** 1—22 (1961).
Feldman, Sh., Porter, R. W.: Electroenceph. clin. Neurophysiol. **12,** 111—118 (1960).
Feldman, S., Wagman, I. H.: Electroenceph. clin. Neurophysiol. **15,** 747—760 (1963).
Fischgold, H., Mathis, P.: Obnubilations, comas et stupeurs; Études electroencéphalographiques. Masson, Paris 1959, P. 126.
Fischgold, H., Schwarz, B. A. in: On the Nature of Sleep. Eds. Wolstenholme, G. E. W., O'Connor, M., Churchill, London 1961, Pp. 209—236.
Fois, A.: The Electroencephalogram of the Normal Child. Thomas, Springfield 1961, P. 124.
Forbes A., and Morrison, B. R.: J. Neurophysiol. **2,** 112—128 (1939).
Grastyán, E., Hasznos, T., Lissák, K., Molnár, L., Ruzsonyi, Z.: Acta physiol. Acad. Sci. hung. **3,** 103—122 (1952).
Hess, W. R.: Arch. Psychiat. Nervenkr. **196,** 329—336 (1957).
Hössli, L. and Monnier, M.: Pflüger's Arch. Ges. Physiol. **275,** 439—451 (1962).
Hunter J. and Jasper, H. H.: Elektroenceph. clin. Neurophysiol. **1,** 305—324 (1949).
Jouvet et al.: Rev. Neurol. **103,** 189—205 (1960).
Jung, R., Hassler, R. in: Handbook of Physiology. Eds. Field, J., Magoun H. W., Hall, V. E. Section American Physiological Society, Washington 1960. Vol. 2. Pp. 863—927.
Kajtor, F,: Arch. Psychiat. Nervenkr. **205,** 375—396 (1964).
Kajtor, F., Oszlánszky, O.: Arch Psychiat. Nervenkr. **204,** 39—57 (1963).
Kajtor, F.: 6th International Congress of EEG and Clinical Neurophysiology. Vienna 1965. Pp. 117—221.
Kellaway, P.: Premier Congrès International des Sciences Neurologiques. 4e Congrès International d'Électroencéphalographie et de Neurophysiologie Clinique. Bruxelles 1957, Pp. 141—154.
King, E. E.: J. Pharmacol. exp. Ther. **116,** 404—417 (1956).
Magnes, J., Moruzzi, G., Pompeiano, O.: Arch. ital. Biol. 99, 33—67 (1961).
Moruzzi, G.: Electroenceph. clin. Neurophysiol. **16,** 2—17 (1964).
Oswald, I., Taylor, A. M., Triesman, M.: Brain **83,** 440—453 (1956).
Pompeiano, O., Swett, J. E.: Arch. ital. Biol. **100,** 311—342 (1962).
Prince, D. A., Shanzer, S.: Electroenceph. clin. Neurophysiol. **21,** 578 (1966).
Roth, M., Shaw, J., Green, J.: Electroenceph. clin. Neurophysiol. **8,** 385—402 (1956).
Schwab, R. S., Passouant, P., Cadilhac, J.: Montpellier méd. **45,** 501—514 (1954).
Spiegel, E. A., Wycis, H. T., Baird, III, H. W., Szekely, E. G. in: Electrical Studies on the Unanesthetized Brain. Eds. Ramsay, E. R., O'Doherty, D. S. Hoeber, New York 1960. Pp. 192—213.
Vetter, K., Böker, W.: Nervenarzt **33,** 390—393 (1962).
Walter, W. G.: The Living Brain. Duckworth, London 1953, P. 167.

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EFFECT OF DIMERCAPROL IN ACUTE ORGANIC MERCURY POISONING OF THE RAT

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Dimercaprol in a subcutaneous dose of 20 mg per kg given three times in 24 hours exerted some antitoxic activity in rats poisoned with organic alkyl, alkoxy-alkyl, and aryl mercurials. As compared to mercury bichloride, the order of efficiency was as follows: mercury bichloride ($HgCl_2$) > phenyl mercury acetate (PMA) > ethyl mercury chloride (EMC) > diphenyl mercury (DPM) > phenyl mercury chloride (PMC) > methoxyethyl mercury chloride (MEMC). On protracted administration (6 times 10 mg per kg within 2 days, or 12 times 5 mg per kg within 3 days), dimercaprol exhibited a greater efficiency against MEMC, the mortality rate being reduced to values between those of DPM and PMC.

It is suggested that the protracted administration of dimercaprol may have a favourable effect in acute poisoning with one of the above mercurials.

According to experimental data (GILMAN et al. [1], BRAUN et al. [2], LONG and FARAH [3], NEUMAN and ALLEN [4], LEVY et al. [5]) and clinical observations (LONGCOPE and LUETSCHER [6]) dimercaprol is a potent antidote in acute poisoning with inorganic mercurial compounds, particularly mercury bichloride. Opinions differ, however, about the efficiency of dimercaprol in human acute poisoning with organic mercury compounds. Some authors (JALILI and ABBASI [7], JORON [8], KLIMMER [9]) believe that dimercaprol is an effective antidote and therefore they prescribe it with or without restrictions. Others (AHLBORG and AHLMARK [10], HÖÖK et al. [11], LUNDGREN and SWENSSON [12]) are of the view that dimercaprol is inefficient, or is efficient only in the late phase of acute poisoning, or in chronic intoxication. There are also authors (GLÖMME and GUSTAVSON [13], HAYES [14]) who consider dimercaprol to be harmful in such cases and therefore contraindicate its use in organic mercury poisoning.

To study the problem, experiments have been performed on rats poisoned with organic mercurials commonly used as fungicidal seed-dressers and industrial protectives, and the value of dimercaprol as an antidote has been tested.

Methods

Compounds tested. A list of the substances, with their chemical structures and melting points, is given below. All compounds are white, crystalline, volatile materials, which have a moderate tendency to sublimate, and are not, or weakly, soluble in water.

Name and abbreviation	Chemical structure	Melting point, °C
Ethyl mercury chloride, EMC	$C_2H_5-Hg-Cl$	249.5 to 253
Methoxyethyl mercury chloride, MEMC	$CH_3O-C_2H_4-Hg-Cl$	65 to 66
Phenyl mercury chloride, PMC	$C_6H_5-Hg-Cl$	189 to 194
Phenyl mercury acetate, PMA	$C_6H_5-Hg-OOC-CH_3$	149 to 151
Diphenyl mercury, DPM	$C_6H_5-Hg-C_6H_5$	121

Mercury bichloride, dimercaprol and sodium phenobarbital used in the experiments were of a purity meeting the requirements of the Hungarian pharmacopoeia.

Experimental animals. A total of 1188 male Wistar rats weighing 200 to 220 g was used in the study. The animals were quarantined for 2 weeks prior to the experiments, during which they were fed a standardized synthetic diet and allowed free access to water. Then the animals were put into cages, 10 rats in each, where they received the same diet, and water *ad libitum*. Body weight and general health were checked daily. After 14 days observation all the rats who had not died spontaneously were killed by decapitation. Routine necropsy, with special attention to the kidneys, was undertaken in each animal.

Method. In the initial phase of the experiments, based on the data of SWENSSON [15] as well as SWENSSON and ULFVARSON [16], the LD_{50} was estimated for mercury chloride and the organic mercurials administered by the intraperitoneal route, according to the technique of DEICHMANN and LEBLANC [17]. Then the intraperitoneal $LD_{50} \pm$ S.E. was estimated with 5 or 6 doses, each dose being tested in 10 animals, using the log-probit graphical method described by MILLER and TAINTER [18]. In the knowledge of the LD_{50} values it was established in groups of 40 rats each to what true LD values corresponded the calculated LD_{70} values.

Thus, the actual toxicity of each compound was determined. These compounds were given intraperitoneally to groups of 40 rats each, and dimercaprol was injected subcutaneously in doses of 20 mg per kg, three times daily. For prevention, dimercaprol was injected 5 min. before, and 60 and 180 min. after, the administration of the mercurial. For therapeutic purposes dimercaprol was injected 30, 60, and 210 min. after the mercurial (NEUMANN and ALLEN [4]). In the experiments where MEMC and phenobarbital were given concomitantly, the barbiturate was injected subcutaneously in doses of 40 mg per kg, simultaneously with the first injection of dimercaprol. For prolonged treatment, 6 times 10 mg per kg and 12 times 5 mg per kg of dimercaprol was administered in two and three days, respectively; the time of administration is indicated in Table III.

Results

Toxicity. As shown in Table I, mercury bichloride was the most toxic of the compounds. The alkyl-compound EMC, and the alkoxy-compound MEMC were 2 to 2 and a half times less toxic than mercury bichloride. The aryl-type compounds were the least toxic, among them, PMA exhibited a toxicity 4 times, PMC 6 and a half times, and DPM about 16 times, less than that of mercury bichloride. The LD_{50} for subcutaneous dimercaprol was 120 mg per kg, in agreement with data in the literature (DURLACHER et al. [19]).

Part of the poisoned animals died on the 4th to 6th day, in bad condition, with slight loss of weight, incontinency of urine, and finally tremor and ataxia. Necropsy revealed large white kidneys, with similar histological characteristics as seen in mercury bichloride poisoning. In the survivors the changes were less pronounced and resembled those in animals recovering from mercury bichloride poisoning.

Table I

Acute toxicity of mercury bichloride, MEMC, EMC, PMA, PMC and DPM in male albino rats weighing 200 to 220 g

Compound tested	LD_{50}	$LD_{50} \pm$ S.E.	Calculated LD_{70}
	mg per kg, intraperitoneally		
Mercury bichloride Cl—Hg—Cl	3.3	4.0±0.3	4.6 = LD_{90}
MEMC $CH_3O—C_2H_4—Hg—Cl$	16.0	9.4±0.9	12.0 = $LD_{67.5}$
EMC $C_2H_5—Hg—Cl$	16.0	9.9±0.4	12.0 = $LD_{92.5}$
PMA $C_6H_5—Hg—OOC—CH_3$	15.0	12.0±0.3	15.0 = $LD_{67.5}$
PMC $C_6H_5—Hg—Cl$	37.0	26.0±0.3	30.0 = LD_{80}
DPM $C_6H_5—Hg—C_6H_5$	72.0	63.0±4.0	72.0 = LD_{60}

Efficiency of dimercaprol. As shown in Table II, preventive dimercaprol treatment had a more favourable effect, except in MEMC poisoning. The order of dimercaprol efficiency was remarkably different from the order of toxicity. It potently antagonized the alkyl and aryl mercurials, while in the case of MEMC dimercaprol not only was inefficient, but in doses of 20 mg per kg

Table II

Effect of dimercaprol in mercury bichloride, MEMC, EMC, PMA, PMC and DPM poisoning of the rat

Compound tested	Preventive administration*			Therapeutic administration**		
	Organic mercury, mg/kg ip.	Death per total of animals	Mortality rate, %	Organic mercury, mg/kg ip.	Deaths per total of animals	Mortality rate, %
Mercury bichloride	4.6 = LD_{90}	0/20	0	4.6	1/20	5
MEMC	12.0 = $LD_{67.5}$	33/40	82.5	12.0	33/40	82.5
EMC	12.0 = $LD_{92.5}$	3/40	7.5	12.0	7/40	17.5
PMA	15.0 = $LD_{67.5}$	0/40	0	15.0	0/40	0
PMC	30.0 = LD_{80}	1/40	2.5	30.0	21/40	52.5
DPM	72.0 = LD_{60}	4/40	10.0	72.0	11/40	27.5

* 20 mg/kg of dimercaprol subcutaneously, 5 min. before, and 60 and 180 min. after the administration of the poison.

** 20 mg/kg of dimercaprol subcutaneously, 30, 90, and 210. min. after the administration of the poison.

administered subcutaneously 3 times for 1 day it augmented toxicity, increasing the ratio of spontaneous deaths.

The toxic effect (4.6 mg per kg) of intraperitoneal mercury bichloride was fully prevented by 3 times 20 mg per kg of subcutaneous dimercaprol given as a prophylactic means; when administered therapeutically, the same dose had a 95% efficiency. These data are in agreement with the values obtained by NEUMAN and ALLEN [4]. Except for one animal which died on the 10th day of observation, the rats poisoned with mercury bichloride and treated with dimercaprol showed a satisfactory gain in weight throughout the experiment; a transitory weight loss was invariably observed on the first 2 or 3 days. In the rat which had died spontaneously the kidney displayed changes similar to those observed in the untreated group, while the animals which survived showed at most some minor changes which resembled those seen in convalescence from mercury bichloride poisoning.

Against PMA, dimercaprol was fully efficient, both in preventive and therapeutic treatment. In the case of the other mercurials, the antagonizing potency of dimercaprol decreased in the order EMC, DPM, PMC. All survivors exhibited an almost normal gain in weight and practically no symptoms of poisoning. In the animals which had died spontaneously necropsy revealed gross and microscopic renal changes somewhat similar to those observed in mercury bichloride poisoning. The survivors displayed normal kidneys and occasionally signs of reparation.

With the combination of MEMC and dimercaprol, the mortality rate increased from 67.5% to 82.5%, irrespective whether dimercaprol had been applied as a preventive or as a therapeutic measure. Soon after the first dose of dimercaprol, the animals exhibited restlessness, then 20 to 30 min. after the first or second injection tonic-clonic convulsions developed which resembled the symptoms occurring after the administration of toxic and lethal doses of dimercaprol; the fits tended to occur twice or three times and in such case the animals died in consequence of respiratory arrest during the comatose state that followed the convulsions.

MEMC + dimercaprol + phenobarbital (Table III). Sodium phenobarbital was potent in preventing lethal convulsions in 31 of the 40 rats treated with MEMC and dimercaprol; the acute mortality rate was significantly reduced as compared to the group receiving only MEMC and dimercaprol. Phenobarbital, however, failed to prevent death on the 4th to 6th days, characteristic of MEMC poisoning. The overall mortality rate, thus, was not different from that after MEMC alone. The necropsy findings were similar to those found in MEMC poisoning (BORDÁS [20]).

MEMC + protracted dimercaprol treatment. Results are shown in the second part of Table III. In contrast to the unfavourable results obtained with 3 times 20 mg per kg of dimercaprol, in 24 hours, either 10 mg per kg 6

Table III

Effect of protracted dimercaprol treatment in acute MEMC poisoning of the rat

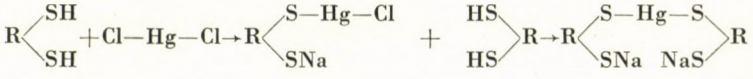
Mode of treatment	MEMC, mg per kg ip.	BAL, mg per kg sc.	Spontaneous deaths per No. of animals	Mortality rate, %
1. Dimercaprol treatment for 1 day	12.0	3×20.0*	33/40	82.5
2. Dimercaprol + phenobarbital sodium for 1 day	12.0	3×20.0**	12/20	60.0
Control of tests 1 and 2	12.0		27/40	67.5
3. Dimercaprol treatment for 2 days	12.0	6×10.0***	7/20	35.0
4. Dimercaprol treatment for 3 days	12.0	12×5.0****	6/20	30.0
Control of tests 3 and 4	12.0		18/20	90.0

* Injection of dimercaprol 0.5, 1.5 and 3.5 hours after MEMC poisoning.
** Injection of dimercaprol + phenobarbital sodium, 40 mg per kg subcutaneously, 0.5, 1.5 and 3.5 hours after MEMC poisoning.
*** Injection of dimercaprol 0.5, 1.5, 3.5, 7, 24 and 28 hours after MEMC poisoning.
**** Injection of dimercaprol 0.5, 1.5, 3.5, 7, 23, 25, 27, 29, 46, 48, 50 and 52 hours after MEMC poisoning.

times in 2 days, or 5 mg per kg 12 times in 3 days proved efficient in preventing convulsions and reducing mortality rate.

Discussion

According to present knowledge [1], the therapeutic efficiency of dimercaprol in mercury bichloride poisoning is based on the chemical binding of the poison.

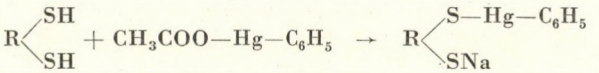


dimercaprol + $HgCl_2$ → dimercaprol—$HgCl_2$ complex I + dimercaprol → dimercaprol—$HgCl_2$ complex II

This reaction occurs when dimercaprol is present in the organism in sufficient amounts. Thus, two molecules of dimercaprol neutralize each molecule of mercury bichloride, and the product of the reaction is a water-soluble, non-dissociating complex which is rapidly eliminated through the kidneys.

According to polarographic studies of JOSEPOVITS and VÉGH [21], PMA readily forms a complex with dimercaprol in vitro, and the resulting complex I,

which is not easily transformed into dimercaprol—PMA complex II, obviously owing to the presence of the slowly dissociating phenyl radical.

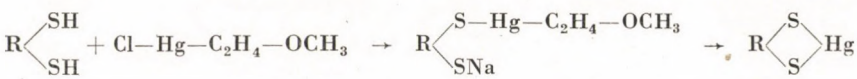


dimercaprol + PMA → dimercaprol—PMA complex I

Thus, the dimercaprol—PMA complex I seems to be a substance dissociating slowly or not at all, which is water-soluble and is rapidly eliminated from the organism. This is why dimercaprol is an efficient antagonist of PMA.

According to the observations of JOSEPOVITS and VÉGH [21], formation of the dimercaprol—DPM complex occurs slowly in vitro; this might be responsible for the weak effect of dimercaprol in DPM poisoning.

MEMC and dimercaprol tend to form a complex I, and then the dimercaprol—MEMC complex II (JOSEPOVITS and VÉGH [21]).



dimercaprol + MEMC → dimercaprol—MEMC complex I → dimercaprol—MEMC complex II

There is reason for assuming that (i) as the formation of the dimercaprol—MEMC complex I takes at least 30 to 60 min. There is time for the MEMC to be absorbed and bound at various sites in the organism; (ii) the lipoid-soluble alkyl radical of the dimercaprol—MEMC complex I may in a hitherto unknown way augment the toxicity of dimercaprol low concentrations of which cause convulsions which can be antagonized by barbiturate derivatives [19]; (iii) finally, dimercaprol—MEMC complex II is capable of dissociating mercury [1], which may aggravate the clinical picture of the poisoning. These considerations may offer an explanation why acute dimercaprol treatment (3 times 20 mg per kg within a day) failed to have any appreciable effect on the overall mortality rate. If the administration of dimercaprol is protracted, the convulsions responsible for death of the animals tend to occur less frequently, presumably as a result of the MEMC + dimercaprol synergism. Also, with protracted administration, the neutralization of MEMC, of the complexes of dimercaprol and MEMC, and their further metabolites, and the elimination of these substances are enhanced and thus the poisoning takes a favourable course.

As regards EMC, DPM, and PMC, the respective complexes of the first type are probably formed with dimercaprol. Distribution, neutralization, and elimination of these complexes are favourable enough to ensure a certain degree of control.

Acknowledgements

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REFERENCES

1. GILMAN, A., ALLEN, R. P., PHILLIPS, F. S., ST. JOHN, E.: J. clin. Invest. **25,** 549 (1946).
2. BRAUN, H. A., LUSKY, L. M., CALVERY, H. O.: J. Pharmacol. exp. Ther. **87,** Suppl. 119 (1946).
3. LONG, W. K., FARAH, A.: J. Pharmacol. exp. Ther. **88,** 388 (1946).
4. NEUMAN, W. F., ALLEN, R. P.: J. Pharmacol. exp. Ther. **96,** 95 (1949).
5. LEVY, R. P., MOIR, T. W., MILLER, M.: Proc. Soc. exp. Biol. (N. Y.) **73,** 498 (1950).
6. LONGCOPE, W. T., LUETSCHER, J. A. JR.: J. clin. Invest. **25,** 557 (1946).
7. JALILI, M. A., ABBASI, A. H.: Brit. J. industr. Med. **18,** 303 (1961).
8. JORON, G. E.: Occup. Hlth Rev., **15,** 12 (1963).
9. KLIMMER, O. R.: Pflanzenschutz- und Schädlingsbekämpfungsmittel. Abriss einer Toxikologie und Therapie von Vergiftungen. Hundt-Verlag, Hattingen 1964.
10. AHLBORG, G., AHLMARK, A.: Nord. Med. **41,** 503 (1949).
11. HÖÖK, O., LUNDGREN, K.-D., SWENSSON, A.: Acta med. scand. **150,** 131 (1954).
12. LUNDGREN, K.-D., SWENSSON, A.: J. Amer. industr. Hyg. Ass. **2,** 308 (1960).
13. GLÖMME, J., GUSTAVSON, K. H.: Acta med. scand. **164,** 175 (1959).
14. HAYES, W. J. JR.: Clinical Handbook on Economic Poisons. Emergency Information for Treating Posisoning. U. S. Government Printing Office, Washington 1963.
15. SWENSSON, A.: Acta med. scand. **143,** 365 (1952).
16. SWENSSON, A., ULFVARSON, U.: Occup. Hlth Rev. **15,** 5 (1963).
17. DEICHMANN, W. B., LEBLANC, T. J.: J. industr. Hyg. **25,** 415 (1943).
18. MILLER, LL. C., TAINTER, M. L.: Proc. Soc. exp. Biol. (N. Y.) **57,** 261 (1944).
19. DURLACHER, S. H., BUNTING, H., HARRISON, H. H., ORDWAY, N. K., ALBRINK, W. S.: J. Pharmacol. exp. Ther. **87,** Suppl. 28 (1946).
20. BORDÁS, S.: A hazai növényvédőszerek foglalkozási méregtani vizsgálata. FM Növényvédelmi Szolgálata, Budapest 1962.
21. JOSEPOVITS, GY., VÉGH, A.: Personal communication.

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HISTOCHEMISCHE VERLAUFSBEOBACHTUNGEN DER β-HYDROXYBUTTERSÄUREDEHYDROGENASE UND UNSPEZIFISCHEN ESTERASE GANZKÖRPER-BESTRAHLTER RATTEN

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Die Aktivität der unspezifischen Esterase (Indoxylacetat) und HBDH wurde nach Ganzkörperbestrahlung mit 650 und 1950 R in 10 Rattenorganen im Verlaufe von 72 Std. histochemisch untersucht. In Leber, Niere, Ovar, Pankreas, Nebenniere, Darm wurde nach anfänglicher Aktivierung eine Abnahme der Enzymaktivität — in einzelnen Organen bis zum völligen Schwund — festgestellt. Im Herzen dagegen konnte nach 1950 R eine Aktivierung der HBDH beobachtet werden. Mitursächlich für diese Enzymveränderung ist eine Störung des Fettabbaues. Die Hauptursache ist jedoch in der allgemeinen Aktivierung des Stoffwechsels zu Beginn der Strahlenkrankheit zu erblicken. Dieser Aktivierung folgt nach 1950 R am 3. Tag ein allgemeiner Zusammenbruch des Stoffwechsels. Bei diesem Verhalten der HBDH im Hinblick des Fettsäurezyklus ist an eine zusätzliche Bereitstellung von Radikalen für den Krebszyklus zu denken. Zu erwähnen ist, daß sich Gehirn und Milz hinsichtlich der untersuchten Fermente als relativ strahlenunempfindlich erwiesen.

Durch die zunehmende Verwendung ionisierender Strahlen in Therapie und Diagnostik wird die Wirkung dieser energiereichen Strahlung auf die einzelne Zelle und den Organismus in den Mittelpunkt des Interesses gerückt. Die radiobiologische Grundlagenforschung der letzten Jahre hat sich daher zunehmend mit strahlenbedingten Schäden des lebenden Organismus, insbesondere solcher des Enzymmusters der Zelle, befaßt. Den Enzymen kommt als Katalysatoren biochemischer Reaktionsabläufe eine wesentliche Bedeutung zu.

Auf lebende Substanz auftreffende Energiequanten bewirken reversible und irreversible Veränderungen an der Zellmembran, dem Zellkern und den Zellorganellen. Man unterscheidet zwischen einer direkten und indirekten Strahlenwirkung. Als Folge der direkten Strahlenwirkung treten Radikale verschiedener chemischer Reaktionsgruppen auf. Es erfolgt eine Änderung der Ionenkonzentration sowie der spezifischen Aktivatoren und Inhibitoren. Weiterhin kann der Coenzymgehalt und auch die chemische Konstitution des Fermentes selbst geändert werden (BERGEDER, BREUER und PARCHWITZ). Aus dem Zusammenwirken dieser Faktoren resultieren Aktivierungen oder Hemmungen der Enzymaktivitäten, die den Ablauf des intrazellulären und

auch intermediären Stoffwechsels beeinträchtigen. Hierdurch bedingte Stoffwechselentgleisungen sind als indirekte Strahlenwirkung auf den Gesamtorganismus aufzufassen. Dem Strahlentherapeuten sind solche Reaktionen seit langem bekannt. Sie werden unter dem klinischen Begriff der Strahlenkrankheit zusammengefaßt.

In der vorliegenden Arbeit soll über das Verhalten zweier im Fettsäurestoffwechsel beteiligter Enzyme — der β-Hydroxybuttersäuredehydrogenase und der unspezifischen Esterase — unter Zugrundelegung histochemischer Untersuchungsmethoden in den Zellen lebenswichtiger Organe von Ratten nach Ganzkörperbestrahlung berichtet werden. Die Untersuchungsergebnisse werden im Zusammenhang mit dem Ablauf des Fettsäurestoffwechsels diskutiert.

Der erste Schritt des Abbaues der Fette ist die enzymatische Spaltung der Esterbindung zwischen Glyzerin und Carboxysäure:

$$RCOOR' + H_2O \rightleftharpoons RCOOH + R'OH$$

Man spricht von einer Lipase, wenn das Substrat ein Ester einer höheren Fettsäure mit Glyzerol oder anderen Alkoholen ist. Die übrigen esterspaltenden Fermente werden als Esterasen bezeichnet. Je nach dem angebotenen Substrat spricht man von einer spezifischen oder nichtspezifischen Esterase. Im vorliegenden Falle handelt es sich bei Verwendung von Indoxylacetat um den Nachweis einer Aliesterase.

Neben der Darstellung der unspezifischen Esterase (UE) wurde, um besseren Einblick in den Fettsäureabbau zu erhalten, die β-Hydroxybuttersäuredehydrogenase (HBDH) histochemisch nachgewiesen. Dieses Enzym ist NAD-abhängig und katalysiert die Umwandlung von D-β-Hydroxybuttersäure in Azetoessigsäure:

$$\text{D-}\beta\text{-Hydroxybutyrat} + DPN^+ \rightleftharpoons \text{Acetoacetat} + DPNH + H^+$$

Die wichtigere Funktion der HBDH ist jedoch, mit Coenzym A an der Oxydation der Fettsäuren – gekuppelt mit der Synthese des aktivierten Coenzyms A — teilzunehmen. Wenn die HBDH als D-Butyratcoenzym-A-Dehydrogenase wirksam ist, kann man die Reaktion wie folgt zusammenfassen:

$$\text{D-}\beta\text{-Hydroxybutyryl-CoA} + DPN^+ \rightleftharpoons \text{Acetoacetyl-CoA} + DPNH + H^+$$

Durch die Acetyl-CoA-Transacylase kann Acetoacetyl-CoA und CoA in 2 Mol Acetyl-CoA umgelagert werden. Über dieses aktivierte Coenzym A ist somit die Verbindung zum Zitronensäurezyklus gegeben.

Das Ferment spaltet β-Hydroxybuttersäure, oxydiert jedoch auch höhere β-Hydroxycarbonsäuren bis zu einer Kettenlänge von 9 C-Atomen. Es ist stereospezifisch und spaltet die linksdrehenden Formen nicht.

Die höchste Enzymaktivität von den Organen der Ratte weist die Leber auf. Es folgen danach Niere, Herzmuskel sowie Nebenniere und in größerem Abstand Pankreas, Gehirn, Skelettmuskel und Milz (LEHNINGER und Mitarb.). Die Aktivität der UE ist in der Leber aller Säugetiere relativ hoch (FODOR, FALCONER und TAYLER, OMACHI und Mitarb.). In Darm (ALDRIDGE), Pankreas (MYERS und Mitarb.), Ovar und Skelettmuskel (MATLACK und TUCKER) sind diese Aktivitäten niedriger. Sehr wenig Aliesterase findet sich im Hirngewebe (MYERS).

Material und Methode

Zu den Untersuchungen wurden 34 weibliche geschlechtsreife Ratten eines Wistarstammes verwendet. Die Tiere erhielten im Überschuß Standardkost der Intermast GmbH sowie Wasser ad libitum. Zur Zeit des Todes hatten sie ein Körpergewicht von 170—180 g. Die Ganzkörperbestrahlung erfolgte in einem Plexiglasbehälter entsprechend der Feldgröße von 10×15 cm mit dem Röntgengerät »Stabilipan« der Firma Siemens—Reiniger bei 250 KV Röhrenspannung, 15 mA Stromstärke und 1 mm Kupferfilterung. Die Halbwertschicht betrug 2;08 mm Cu, der Focus-Tierabstand 40 cm, die Dosisleistung 110 R/min. Zwei Gruppen von je 12 Tieren erhielten eine einmalige Ganzkörperbestrahlung von 650 R und 1950 R. 24, 48 und 72 Stunden nach der Bestrahlung wurden je 4 Tiere zur gleichen Tageszeit durch Dekapitation getötet, seziert und sofort histochemisch aufgearbeitet, so daß die zeitliche Differenz zwischen Tötung und Inkubation der letzten Gewebsschnitte nicht mehr als 3 Stunden betrug. Als Vergleichsgruppe dienten 10 Tiere, die unter gleichen Bedingungen gehalten und getötet wurden.

Zur Untersuchung gelangten frische 10 μ starke Kryostatschnitte von Leber, Niere, Herz, Großhirn, Muskel, Pankreas, Nebenniere, Ovar, Milz und Dünndarm.

Die Darstellung der HBDH erfolgte nach der von PEARSE angegebenen Kobalt-Formazan-Technik, die von HESS und Mitarb. zusammengefaßt beschrieben wurde. Wir benutzten MTT [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl-tetrazoliumbromid] als Wasserstoffakzeptor. Dem histochemischen Nachweis der Aktivität der UE wurde die von BARRNETT und SELIGMAN und HOLT angegebene Methode zugrunde gelegt. Als Substrat verwendeten wir Indoxylacetat. Die Reaktion verläuft wie in Abb. 1 dargestellt.

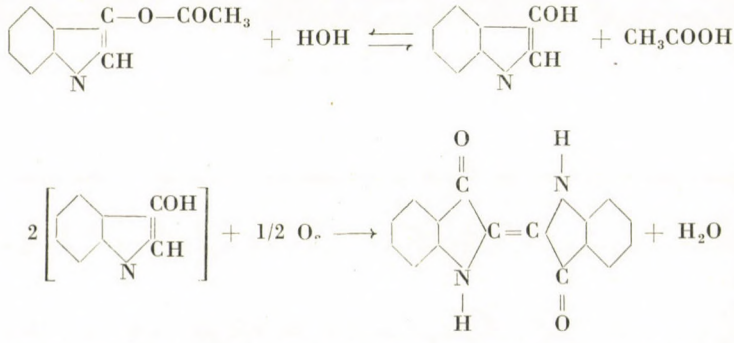

Abb. 1. Histochemische Esterasereaktion mit Indoxylacetat. Bei der hydrolytischen Spaltung wird Indoxyl frei, welches durch den im Gewebe und in der Inkubationslösung vorhandenen Sauerstoff sofort zu Indigo oxydiert wird. Indigo ist lichtecht, beständig und in den bei histochemischen Reaktionen vorhandenen Medien nicht löslich

Ergebnisse

Enzymhistochemische Nachweise sind in erster Linie qualitative Methoden, die Informationen über die Topik der Enzyme vermitteln. Zur Beurteilung der Stoffwechselvorgänge ist aber auch die Höhe der Enzymaktivität von Bedeutung. Es ist jedoch anhand der Größe und Dichte der Formazangranula auf die Intensität der Fermentreaktion zu schließen. Die Höhe dieser Fermentaktivität wurde in Beziehung zum Enzymverteilungsmuster der Kontrolltiere gesetzt.

β-Hydroxybuttersäuredehydrogenase (Übersicht — Abb. 2)

Leber:

Nach Bestrahlung mit 650 R zeigte sich nach 24 Std. eine starke Zunahme der Formazangranula, die auch nach 48 Std. erhalten blieb. Am 3. Tag bestand immernoch eine deutliche Erhöhung. Nach 1950 R war die Aktivierung nach dem ersten Tag nicht ganz so stark, aber 48 Std. p. r. und 72 Std. p. r. gleichartig ausgebildet. Die auch bei den Kontrolltieren vorhandene leichte Betonung der läppchenzentralen Gebiete wurde bei der bestrahlten Gruppe deutlicher. Daneben waren besonders bei den mit 1950 R bestrahlten Tieren am 2. und 3. Tag eine deutlich fleckförmige Anordnung ohne Beziehung zum Läppchenbau des Organes zu beobachten. Außerdem fiel eine Vergröberung und Verklumpung der Granula auf.

		24 Std.	48 Std.	72 Std.
Leber	650 R	+++	+++	++
	1950 R	++	+++	++
Niere	650 R	0	—	—
	1950 R	+++	+	0
Darm	650 R	++	+	0
	1950 R	+++	—	—
Muskel	650 R	— —	— —	— —
	1950 R	—	— —	———
Herz	650 R	(—)	(—)	0
	1950 R	0	++	+++
Nebenniere	650 R	++	0	(—)
	1950 R	0	+	0
Pankreas	650 R	—	0	0
	1950 R	0	(—)	—
Ovar	650 R	++	(+)	0
	1950 R	+++	+++	+++

Abb. 2. β-Hydroxybuttersäuredehydrogenase (HBDH). Die Aktivität der Normalgruppe wurde gleich Null gesetzt und eine Zu- oder Abnahme in den Organen der bestrahlten Tiere jeweils mit + oder — angegeben. (+) ganz geringer Aktivitätsanstieg; + geringer Aktivitätsanstieg; ++ deutlicher Aktivitätsanstieg; +++ starker Aktivitätsanstieg; ——— starker Aktivitätsabfall; —— deutlicher Aktivitätsabfall; — geringer Aktivitätsabfall; (—) ganz geringer Aktivitätsabfall

Niere:

Die mit 650 R bestrahlten Tiere zeigten am 2. und 3. Tag eine geringe Minderung der Formazangranula. Nach 1950 R war 24 Std. p. r. eine starke Aktivierung, die auch nach 48 Std. noch gering bestand, zu beobachten. 72 Std. p. r. zeigten sich keine Veränderungen im Vergleich zur unbestrahlten Kontrollgruppe. Einzelne Tiere dieser Gruppe ließen am 3. Tag besonders in der Rinde grobfleckige Aussparungen in den Tubuli contorti erkennen.

Darm:

Nach Bestrahlung mit 650 R zeigte sich nach 24 Std. eine deutliche Ablagerung von Formazangranula in den sonst relativ aktivitätsarmen Zottenepithelien. Auch 48 Std. p. r. war diese noch gering vorhanden, während sich am 3. Tag das Bild der Vergleichsgruppe bot. Nach einer Dosis von 1950 R bestand 24 Std. p. r. eine starke Aktivierung, nach 48 und 72 Std. war jedoch eine Aktivität nicht mehr nachweisbar.

Milz:

Nur einige Tiere der Versuchsgruppe zeigten an einzelnen Tagen unregelmäßig eine ganz gering stärkere Ausfällung von Formazangranula bei insgesamt äußerst geringer Aktivität.

Nebenniere:

Bei den mit 650 R bestrahlten Tieren konnte nach 24 Std. ein deutlicher Anstieg der Fermentaktivität innerhalb der Rindenschicht beobachtet werden. 48 Std. p.r. war wieder das normale Verteilungsmuster zu erkennen, und nach 72 Std. fiel eine ganz geringe Minderung der Formazangranula auf. Nach 1950 R zeigte sich 24 Std. p.r. eine geringe Vermehrung der Formazangranula und 72 Std. p.r. bestand das Bild der unbestrahlten Kontrollgruppe. Zwei Tiere ließen jedoch eine ganz geringe Abnahme erkennen.

Pankreas:

Nach einer Dosis von 650 R fand sich nur am 1. Tag eine geringe Minderung der Formazangranula. Während nach 1950 R am 1. Tag keine Veränderung beobachtet werden konnte, zeigte sich bis zum 3. Tag eine leichte Abnahme der Aktivität.

Ovar:

Die mit 650 R bestrahlten Tiere wiesen eine geringe Zunahme der Formazangranula auf, die sich bis zum 3. Tag jedoch zurückbildete. Nach 1950 R dagegen war die Zunahme stark und es bestand am 3. Tag immer noch eine deutliche Vermehrung der Granula.

Skelettmuskel:

Nach Bestrahlung mit 650 R war nach 24 Std. eine deutliche Minderung der Formazangranula zu erkennen, die bis zum 3. Tag anhielt. Bei der Dosis von 1950 R bestand 24 Std. p.r. eine äußerst geringe Zunahme der Aktivität, jedoch nach 48 Std. eine deutliche Minderung, und am 3. Tag war praktisch keine Fermentaktivität mehr nachweisbar.

Herzmuskel:

Die mit 650 R bestrahlten Tiere reagierten 24 Std. p.r. nur mit einer äußerst geringen Abnahme der Formazangranula, die nach 48 Std. etwas deutlicher ausgebildet war. 72 Std. p.r. bot sich wieder das normale Bild. Nach 1950 R 48 Std. p.r. konnte eine deutliche Vermehrung der Fermentaktivität beobachtet werden, die nach 72 Std. stärker ausgebildet war. Am 2. und 3. Tag fiel bei einigen Tieren eine leicht fleckförmige Anordnung auf.

Gehirn:

Im Gehirn zeigten sich bei beiden Bestrahlungsgruppen keine Veränderungen im Vergleich zur Kontrollgruppe.

Unspezifische Esterase (Übersicht — Abb. 3)

Leber:

In der Leber war 24 und 48 Std. p.r. mit 650 R ein geringer Aktivitätsanstieg nachweisbar, der 72 Std. p.r. wieder abnahhm, jedoch über der Aktivität der Kontrolltiere lag. Von der Zunahme der Fermentgranula war das gesamte Läppchen betroffen.

Niere:

Ein geringer Aktivitätsabfall fand sich in der Niere nach 48 und 72 Std. sowohl bei den mit 650 als auch bei den mit 1950 R bestrahlten Tieren in den tubulären Anteilen der Rinde.

		24 Std.	48 Std.	72 Std.
Leber	650 R	+	+	(+)
	1950 R	0	(+)	+
Niere	650 R	0	(−)	(−)
	1950 R	0	−	− (−)
Darm	650 R	0	(−)	−
	1950 R	−	− −	− − −
Muskel	650 R	1	0	0
	1950 R	(+)	0	0
Herz	650 R	0	0	0
	1950 R	+	0	0
Gehirn	650 R	0	0	0
	1950 R	(+)	(+)	(+)
Nebenniere	650 R	++	0	− −
	1950 R	+++	+	− −
Pankreas	650 R	+	++	++
	1950 R	+ (+)	++	++(+)
Ovar	650 R	+	(+)	(+)
	1950 R	++	++	++
Milz	650 R	0	(+)	0
	1950 R	0	0	0

Abb. 3. Unspezifische Esterase (UE). Erklärung der Zeichen siehe Abb. 2

Darm:

Nach 650 und 1950 R konnte im Darm ein kontinuierlicher Aktivitätsabfall beobachtet werden; dieser war nach 1950 R besonders deutlich. Von der Abnahme waren vornehmlich die Epithelien betroffen.

Milz:

In der Milz fand sich nur am 2. Tag nach der Bestrahlung mit 650 R ein geringfügiger Aktivitätsanstieg.

Nebenniere:

In der Nebennierenrinde zeigte sich 24 Std. nach Bestrahlung mit 650 R ein deutlicher Aktivitätsanstieg mit Vergröberung und leichter Verklumpung der Granula und 72 Std. p.r. ein auffallend fleckförmiges Bild mit insgesamt erheblicher Fermentaktivitätsminderung. Ähnlich war das Verhalten nach Bestrahlung mit 1950 R.

Pankreas:

Im Pankreas war eine geringe bis deutliche Zunahme der Fermentgranula nach 650 und 1950 R nachzuweisen.

Ovar:

Im Ovar zeigten die Esterasereaktionen ein ähnliches Verhalten wie im Pankreas. Von der Aktivitätsänderung waren die Graafschen- und Primärfollikel und weniger das Zwischengewebe betroffen (Abb. 4).

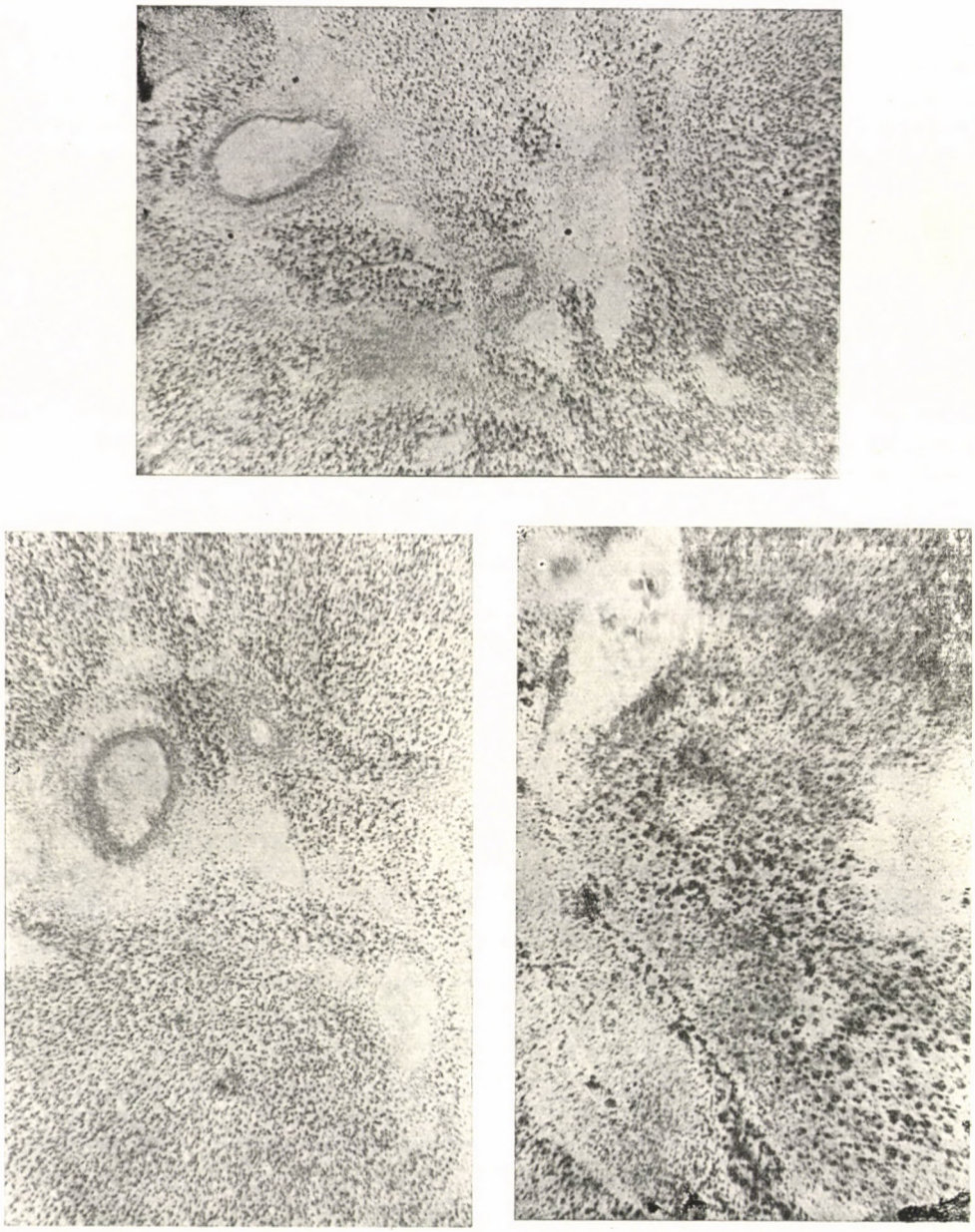

Abb. 4. Veränderung der Esterasereaktion im Ovar. Das obere Bild zeigt die Fermentverteilung im Ovar der unbestrahlten Ratte. Keine Gegenfärbung. Das linke Bild läßt eine ganz geringe Zunahme am dritten Tag nach 650 R erkennen, während nach 1950 R (rechtes Bild) diese Zunahme deutlicher ist. Hier ist eine Vergröberung und Verklumpung der Granula auffällig

Skelettmuskel:

Im Skelettmuskel zeigten die mit 650 R bestrahlten Tiere nach 24, 48 und 72 Std. keine Aktivitätsänderung. Die mit 1950 R bestrahlten Tiere ließen nur nach 24 Std. eine ganz geringe Vermehrung der Granula erkennen.

Herzmuskel:

In der Herzmuskulatur waren praktisch die gleichen Veränderungen wie im Skelettmuskel zu beobachten.

Gehirn:

Im Gehirn war nur nach Bestrahlung mit 1950 R ein ganz geringer Aktivitätsanstieg erkennbar.

Besprechung

Die Ergebnisse lassen erkennen, daß sich nach einer Ganzkörperbestrahlung die Esteraseaktivität ändert. In den Zellen fast aller untersuchter Organe ist festzustellen, daß die esterspaltende Aktivität nach teilweiser Aktivierung erheblich abnimmt. Eine Ausnahme bilden Pankreas und Leber. In diesen Organen nimmt bei höherer Strahlendosis die Aktivität gering zu. Soweit in Einzelversuchen die Esteraseaktivität bereits von anderen Autoren untersucht wurde und die Ergebnisse vergleichbar sind, konnten die Untersuchungsbefunde im wesentlichen bestätigt werden. MAISIN fand bei biochemischen Untersuchungen am Dünndarm eine deutliche Abnahme, MASUROWSKY und NOBAK am Gehirn eine geringe Phasenänderung, PASQUALINO und BOURNE bei histochemischer Untersuchung in der Leber eine geringe Abnahme der Enzymaktivität und JONEK und Mitarb. eine Abnahme in der Nebenniere und im Herzmuskel.

Die Abnahme der Esteraseaktivität wird von JONEK und Mitarb. mit einer Störung des Fettstoffwechsels in Zusammenhang gebracht. Dieser Umstand spielt sicher insbesondere dann eine Rolle, wenn man in der Nebenniere das etwa gleichsinnige Verhalten der Lipoide und der Esteraseaktivität betrachtet. Der Lipoidgehalt nimmt im Verlaufe der Strahlenkrankheit nach einer in der ersten Stunde zu beobachtenden Erhöhung ab (KOVACS, JONEK und Mitarb.). Auch die Esteraseaktivität zeigt nach einer anfänglichen Aktivierung eine Abnahme.

Durch die angewandte Methode werden weniger die echten Lipasen, die ohne Zweifel am Beginn der Fettspaltung wirksam sind, als eher Esterasen mit einer Substratspezifität für kurzkettige Carboxysäureester — jedoch nicht mit Glycerol — dargestellt. Die in fast allen Organen beobachtete Depression der Fermentaktivität dürfte somit vielmehr im Rahmen der allgemeinen strahlenbedingten Stoffwechselentgleisung zu betrachten sein. Die Tatsache, daß bei einigen Organen mit zentraler Stellung im intermediären Stoffwechsel zu Beginn der Strahlenkrankheit eine ähnliche Aktivierung anderer Fermentsysteme (Krebszyklus, Glykolyse) zu beobachten ist, legt es nahe, die Phasenänderung der Esteraseaktivität gleichartig zu interpretieren.

Unspezifische Esterasen vermögen auch Aminosäureester zu hydrolysieren (MYERS). Hieraus ergibt sich eine Verbindung zum Eiweißstoffwechsel. Die erhöhte Esteraseaktivität würde somit auf einen erhöhten Umsatz von Aminosäuren, d. h. letztlich auf eine Aktivierung der Proteolyse schließen lassen. HAGEN hat ausgehend von völlig anderen Versuchsbedingungen die Aktivierung der Proteolyse im Rahmen der Strahlenkrankheit hervorgehoben.

Bemerkenswert ist das phasengleiche Verhalten der esterspaltenden Aktivität und der HBDH in den untersuchten Organen — ausschließlich Pankreas. Ohne Zweifel kann man diese Beobachtung — wie auch einen gewissen Teil der Esteraseaktivität — im Zusammenhang mit einer Änderung des Fettstoffwechsels betrachten.

Hydroxyacyl-S-CoA-Verbindungen (β-Hydroxybutyryl-S-CoA) sind ein wichtiges Substrat der HBDH. Sie werden durch das Enzym zu entsprechenden Ketoacyl-S-CoA-Verbindungen oxydiert. Die HBDH bildet somit einen Reaktionsablauf innerhalb des Fettsäurezyklus. Die Aktivierung der HBDH in den meisten Organen zu Beginn der Strahlenkrankheit (Niere, Ovar, Nebenniere, Pankreas, Darm) oder vor dem endgültigen Zusammenbruch des Stoffwechsels (Leber, Herz) weist auf eine Intensivierung des Fettsäurezyklus hin. Offenbar werden über dieses System zu Beginn der Strahlenkrankheit dem intermediären Stoffwechsel auf Grund eines erhöhten Bedarfes vermehrt C_2-Radikale zugeführt. Es können je nach Anzahl der Durchläufe im Fettsäurezyklus verschieden lange geradzahlige Radikale entstehen. Das Acetyl-S-CoA — das letzte Radikal beim Durchlaufen des Fettsäurezyklus — ist geeignet, mit Oxalessigsäure über das kondensierende Enzym in den Krebszyklus eingegliedert zu werden. Biochemische Untersuchungen einiger Fermente des Krebszyklus zeigten, daß in der ersten Phase der Strahlenkrankheit eine Aktivierung dieses Stoffwechselsystems zu beobachten ist (ROGOV und ROZEN, RICH und Mitarb.). Eine Funktion des nach der Bestrahlung aktivierten Fettsäurezyklus ist anscheinend diesen vermehrten Bedarf zu decken.

Nach 650 R reicht offensichtlich der Beobachtungszeitraum von 3 Tagen für diese ausgleichende Funktion aus, während nach 1950 R die Aktivierung gleichbleibt (Ovar, Leber) oder sogar zunimmt (Herz). In den meisten Organen jedoch kann man eine Depression dieser Enzymleistung beobachten. BERNDT und Mitarb. fanden bei biochemischen Untersuchungen nach 650 R in der Leber schon am 2. Tag eine Aktivitätsminderung an der Fettsäuresynthese beteiligter Fermente.

Im Rahmen dieser Betrachtung soll noch einmal auf das Verhalten der Esteraseaktivität der Leber als dem Organ mit zentraler Stellung im intermediären Stoffwechsel hingewiesen werden. Dieses Organ zeigte im Verlaufe der Strahlenkrankheit eine Aktivierung der HBDH, die ohne Zweifel auf die oben beschriebene Intensivierung zurückzuführen ist.

Von vielen Autoren wird die relative Strahlenunempfindlichkeit einzelner Organe hervorgehoben. Anhand unserer bisherigen Untersuchungen mußte jedoch immer wieder auf gegenteilige Versuchsergebnisse hingewiesen werden. Der vorliegende Beitrag über die UE- und HBDH-Aktivität ließ jedoch erkennen, daß Gehirn und Milz keine oder nur eine ganz geringe Phasenänderung zeigten.

LITERATUR

ALDRIDGE, W. N.: Some esterases of rat. Biochem. J. **57,** 692 (1954).

BARRNETT, R. J.: The distribution of esterolytic activity in the tissues of the albino rat as demonstrated with indoxylacetate. Anat. Rec. **114,** 577 (1952).

BARRNETT, R. J. und SELIGMAN, A. M.: Histochemical demonstration of esterases by production of indigo. Science **114,** 579 (1951).

BERGEDER, H.: Beeinflussung durch strahlenbedingte Elektrolytverschiebungen auf die Aktivität der Pyruvatkinase. Strahlentherapie **119,** 513 (1962).

BERNDT, J., GAUMERT, R. und ULBRICH, O.: Zur Störung der Fettsäurebiosynthese nach Strahleneinwirkung in vivo. Biochim. biophys. Acta (Amst.) **137,** 43 (1967).

BREUER, R. und PARCHWITZ, H. K.: Analyse der strahleninduzierten Elektrolytverschiebungen in Nierenschnitten. Z. Naturforsch. **15b,** 671 (1960).

FALCONER, J. S., TAYLER, D. B.: The initial stages in the purification of pig liver esterases. Biochem. J. **40,** 831 (1946).

FALCONER, J. S. und TAYLER, D. B.: A specific property solubility test for protein purity and its application to the preparation of pure liver esterase. Biochem. J. **40,** 835 (1946).

FODOR, P. J.: Co-existence of two different esterases in liver juice. Nature (Lond.) **159,** 375 (1947).

HAGEN, U.: Untersuchungen über die Entstehung der Thymusatrophie nach Röntgenbestrahlung. IV. Über die Strahlenaktivierung von Fermenten. Strahlentherapie **117,** 201 (1962).

HAGEN, U.: Über die unterschiedliche Strahlenempfindlichkeit von Enzymen an verschiedenen Orten der Zelle. Naturwissenschaften **44,** 402 (1957).

HAGEN, U.: Aktivität zellulärer Endopeptidasen (Kathepsine) im strahlengeschädigten Organismus. Z. Naturforsch. **126,** 546 (1957).

HESS, R., SCARPELLI, D. G. und PEARSE, A. G. E.: Cytochemical localization of pyridine nucleotide linked dehydrogenases. Nature (Lond.) **181,** 1531 (1958).

HOLT, S. J.: A new principle for the histochemical localization of hydrolytic enzymes. Nature (Lond.) **169,** 271 (1952).

JONEK, J. und KOSMIDER, S.: Histochemische Untersuchungen über das Verhalten einiger Enzyme im Herzmuskel nach Ganzkörper-Röntgenbestrahlung sowie nach Lokalbestrahlung der Herzgegend. Strahlentherapie **122,** 198 (1963).

JONEK, J., STOKLOSA, E. und KONECKI, J.: Das Verhalten der sauren Phosphatase, der unspezifischen Esterasen, der E-600-resistenten Esterase und der Lipoide in den Nebennieren von Meerschweinchen nach Ganzkörper- sowie lokaler Röntgenbestrahlung. Strahlentherapie **123,** 226 (1964).

KOVACS, L.: Über die von ionisierender Strahlung in supraletaler Dosierung verursachten morphologischen und funktionellen Veränderungen an der Nebennierenrinde. Strahlentherapie **129,** 238 (1966).

LEHNINGER, A. L., SUDDUTH, H. C. und WISE, J. B.: D-β-hydroxybutyric dehydrogenase of mitochondria. J. biol. Chem **235,** 2450 (1960).

MAISIN, J. R. und POPP, R. A.: Effect of AET on sodium, potassium and esterases of the alimentary tract of irradiated mice. Amer. J. Physiol. **199,** 251 (1960).

MASUROWSKY, E. D. und NOBAK, O. K.: Changes in the esterases of rat brain and blood serum following acute cranial exposure to X-rays. Nature (Lond.) **200,** 847 (1963).

MATLACK, M. B. und TUCKER, J. W.: An esterase from muscular tissue. J. biol. Chem. **132,** 663 (1940).

MYERS, D. K.: in Boyer—Lardy—Myrbäck: The enzymes. 2nd Ed., Vol. 4. Academic Press, New York 1960.

MYERS, D. K., SCHOTTE, A., BOER, H. und BORSJE-BAKKER, H.: Studies on ali-esterases and other lipid-hydrolyzing enzymes. Inhibition of esterases of pancreas. Biochem. J. **61,** 521 (1955).

OMACHI, A., BARNUM, C. P. und GLICK, D.: Quantitative distribution of an esterase among cytoplasmic components of mouse liver cells. Proc. Soc. exp. Biol. (N. Y.) **67**, 133 (1948).
PASQUALINO, A. und BOURNE, G. H.: Histochemical effects of radiation. Acta anat. **42**, 1 (1960).
PEARSE, A. G. E.: Intracellular localization of dehydrogenase systems using monotetrazolium salts and metal chelation of their formazans. J. Histochem. Cytochem. **5**, 515 (1957).
RICH, J. G., GLAGOV, S., LARSEN, K. und SPARGO, B.: Histochemical studies of rat kidney after abdominal X-irradiation. Arch. Path. **72**, 388 (1961).
ROGOV, A. A. und ROZEN, V. B.: The effect of penetrating radiation on the succinic dehydrogenase activity of the parenchymatous organs of white rats. Bull. exp. Biol. Med. **47**, 207 (1959).

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HABITUATION AND REGENERATION AS REFLECTED BY EXAMINATIONS PERFORMED BY USING THE CRITICAL FLICKER FREQUENCY SERIES EFFECT METHOD

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There are essential similarities between habituation and the Critical Flicker Frequency Series Effect (CFFSE). On this basis, it is assumed that at least part of the phenomena within the idea of habituation are analogous with the latter. This, in turn, means that habituation is — at least partly — a stress phenomenon: the repetition of identical stimuli exerts a load on the nervous system. Thus, the connection assumed between habituation and fatigue seems justified.

On grounds of the good agreement of habituation with CFFSE, the latter method may seem adaptable for a more thorough understanding and more precise analysis of habituation.

By comparing the "recovery" associated with habituation to the effect brought about by the so-called regenerative (F_2) factor of the CFFSE, it may be assumed that the process of "recovery" could also be measured and clarified. By using the example of the F_2 factor, it is illustrated how the regenerative effect in the CFFSE methodology can be characterized by numerical data in a most exact manner (which might be adaptable for the purposes of clinical differential diagnostics as well).

Its analogy with habituation reveals that the CFFSE is subject to a very extensive nervous system influence.

Introduction

Fatigue is one of the definitions constantly used in every-day life. At the same time, science is generally very suspicious as far as this definition is concerned. MUSCIO suggested as early as 1921 that "the term fatigue be absolutely banished from precise scientific discussions" (BARTENWERFER, 1961). Instead of fatigue, therefore, the introduction of definitions depending on various much more readily understandable criteria is usually recommended. One of these ideas is the definition of habituation.

Although habituation is a much clearer definition than fatigue, it cannot be considered unequivocally defined. The different views are in best agreement as to the two features referred to in the first place of the 9 parametric characteristics listed by THOMPSON and SPENCER (1966). While stressing that habituation does not cover entirely uniform phenomena, in our opinion the process characterized by the following two criteria might be essentially accepted as habituation.

1) Given that a particular stimulus elicits a response, repeated applications of the stimulus result in a decreased response. The decrease is usually a negative exponential function of the number of stimulus presentations.

2) If the stimulus is withheld, the response tends to recover with time (spontaneous recovery).

As mentioned above, habituation is generally considered a phenomenon pertaining to the scope of fatigue although literature is not quite uniform on this aspect. Thus, for example, SCHMIDTKE (1965) does not discuss the problem in his monograph on fatigue, while THOMPSON and SPENCER exclude from the definition of habituation the effector fatigue phenomenon.

In the following we shall discuss the problem whether it is justified to connect habituation to fatigue. Furthermore, the presentation of a possibility for the deeper analysis of habituation will be attempted.

Method

The investigations have been performed with the Critical Flicker Frequency Series Effect method (ÁKOS and ÁKOS, 1966).

As it is well known, critical flicker frequency (CFF) represents the interruptions per second of the light of a source as expressed in cycles per second (cps), in the very moment when the observer would see the hitherto uniform light as flickering, due to the reduction of the frequency. Thus CFF is actually a psychophysical threshold. In our investigations, the CFF values were always determined for the same individual, under identical conditions in 50 consecutive instances, and always in a descendant manner, that is, by frequency reduction. The light source was an E.M.4 cathode-ray tube controlled by a Solartron generator (OS 103.2A) accurately producing even the tenths of cps values. Instead of averaging the 50 CFF data, the fluctuation value was studied in chronological succession, whereby a CFF series effect could be found, the existence of which was then readily traced back to five different factors. The analysis of the CFFSE by factors represents the CFFSE method.

Results

The analogy of the determination of identical threshold values recurring 50 times with the same individual, to the recurring stimuli bringing about habituation is quite conspicuous. Here the question may arise whether this is only a superficial similarity, or could the CFFSE be actually compared to habituation? To decide the problem, let us start from the most typical characteristic of habituation, referred to by THOMPSON and SPENCER in the first place: the reduction of the effect of recurring stimuli according to a negative exponential curve.

The frequency values of the 50 CFF data in the CFFSE decrease similarly, in 94.5% of the cases, with the continuous data collection progressing. (Hence the insignificant number of ascending sequences will not be taken into consideration for the development of averages.)

If this decrease actually follows a negative exponential curve, the reduction within the first 30 data of the series must relatively exceed that of the entire sequence. Were the value decrease uniform, the difference between the maximum and the minimum of the first 30 data (variation range = VR) would exhibit a ratio of 30 : 50 to that of the entire series. Or, expressed in percentages, with the VR of the 50 data taken as 100, the VR calculated from the first 30 CFF data would amount to 60%.

In practice, however, calculating the average of this ratio for a group of 25 patients differing in age and sex, and suffering from different diseases and, again, calculating this average ratio from 234 continuous sequences, it would amount to 76.59%. This means that the series values decrease, on the average, to a much higher degree initially than later on. Initially, the slope of the curve is steep but will flatten subsequently. This, in turn, corresponds to a negative exponential curve, that is, it corresponds to habituation.

The ratio thus obtained may be checked upon with another group of homogeneous diseases: psoriasis. This group involves other subjects than the first one did. Now the average of 243 series rendered by 26 patients of similarly different age and sex data is studied. The VR calculated from the first 30 CFF data as expressed in the percentage of the VR exhibited by the entire series of 50 amounts, on the average, to 77.57%.

Thus the ratios of the two groups are in good agreement and it may be concluded that from the aspect of effect reduction following a negative exponential curve, CFFSE conforms to habituation.

Now let us study the problem of spontaneous recovery.

With respect to CFFSE, the gradual decrease of the frequency level of CFF data may be considered analogous to the stimulus reduction in habituation. According to a further analogy, the insertion of interruptions is expected to make the CFF values return to the initial level or, at least, to make their reduction to decrease as compared to the data measured continuously. If there actually exists such an interruption effect, it may be verified as follows. Let us compare the VR of a continuous series consisting of 50 continuously recorded data to that of such a series of 50 where an interruption of 30 sec was inserted after the determination of every 10 data. An interruption of 5 min was provided for between each continuous and intermittent series. If there is an intermission effect, then the VR data of the continuous and intermittent series pairs should exhibit a difference, since the "recovery" effect of interruptions would decrease the series reduction.

The VR average of 234 intermittent series of 50 was, in the percentage of 234 continuous ones, 77.74% in the group consisting of patients suffering from different diseases. Thus, the insertion of intermissions has actually reduced the habituation decrease of value reductions. The flattening in conformity with a negative exponential curve was supplemented by the interruption effect

decreasing the reduction or, in other words, attempting to restore the original value level. If in the two sequences the ratio of the VR figures revealed by the 30 and 50 data sequences are compared to the average of the 234 series pairs, the flattening of intermittent sequences will be found increasing. While the VR average of the first 30 data in continuous sequences amounts to 76.59% of the full series, the same figure in the intermittent sequences will be as high as 84.0%. In the other group of homogeneous disease, the ratio is similar: the intermittent VR of 50 data amounts to 86.05% of that in continuous sequences, while the VR of 30 is 77.57% in continuous, and 82.42% in intermittent series.

Thus, this again shows that the CFFSE corresponds to habituation: interruptions exert a recovery effect in both cases.

Of the characteristics referred to by THOMPSON and SPENCER, another one was tested similarly. Accordingly, if repeated series of habituation training and spontaneous recovery are given, habituation becomes successively more rapid (this might be called potentiation of habituation). It should be expected, therefore, that on the successive daily averages of three series pairs per day, the percentage of the VR displayed by the first 30 data as related to that of the full series would increase continuously, that is, the flattening of the curves would appear as increasingly definite. This, however, did not take place:

the average VR per cent of the first 30 data

in 248 continuous series was, on the first day,	78.19
the same for 244 continuous series, on the second day,	76.37
the same for 238 continuous series, on the third day,	77.08

Although from this aspect the description of habituation differs somewhat from the CFFSE behaviour, the first two characteristics justify the assumption of a partial, if not full, analogy between CFFSE and habituation.

Discussion

According to the general opinion, habituation is an extremely widespread phenomenon covering everything from the behaviour of the entire organism to the function of certain parts of the nervous system. This widespread character itself may awake sufficient suspicion as to the possibility of different processes being encountered. This impression is confirmed by the number of contradictions concerning the idea of habituation. THOMPSON and SPENCER, for example, excluded from the idea of habituation the phenomenon of receptor adaptation which ASHBY (1959) considered identical with habituation proper. WALTER (1959), on the other hand, separated the two and identified habituation with learning.

If it is accepted that CFFSE and habituation are, at least to a certain extent, analogous, this will lead to an easier interpretation of habituation on the basis of CFFSE analysis.

Previously, CFFSE was demonstrated to represent the result of a load or stress. The relevant evidence will be repeated here in brief.

If the series actually represents a stress which is caused by the serial CFF data registration proper, the result of which can be measured by means of the VR, then the first 30 data of the series must represent a much lesser stress than what the full series of 50 does. Thus, comparing the VR magnitude calculated from the first 30 data to that of the entire series must give, both in continuous and intermittent sequences, a significantly lower VR average as that calculated from the first 30 data.

In the above group consisting of different patients, this ratio was as follows.

Continuous series	(234) VR of 30:	2.31 cps, VR of 50:	3.08 cps
Intermittent	(234)	2.01 cps,	2.40 cps

If, furthermore, starting from the assumption that the insertion of intermissions would reduce the stress effect, the respective intermittent series averages should always be lower than those of the continuous sequences. The above data actually reflect this statement.

The existence of stress can be verified, in addition, as follows. The stress must appear more intensive for old subjects than for young ones, since younger individuals are capable to withstand higher stresses. In our group consisting of different subjects, the situation was as follows.

Age group		Continuous		Intermittent	
		VR of 30	VR of 50	VR of 30	VR of 50
17—35	12	1.97	2.64	1.73	2.10
35—50	8	2.57	3.32	2.04	2.47
over 50	5	3.00	3.80	2.67	3.03

The consistent age gradient indeed shows that, in accordance with the biological reduction of load bearing capacity, an identical stress would be lower for young subjects than for old ones.

Thus, CFFSE is the result of a load or a stress. If CFFSE is analogous to habituation, the flattening of the sequences should reveal the negative exponential feature characteristic of habituation and, according to age and the relative increase of the stress, this flattening should increase with age. What is then the actual situation?

In the group consisting of different patients, and in a resolution by age groups, the VR average of the first 30 data in the percentage of the VR exhibited by the complete series was

Age group	Continuous	Intermittent
17—35	74.54	82.57
35—50	77.43	82.72
over 50	78.96	88.26

This is what had been expected: in addition to representing a stress, CFFSE illustrates also in its habituation character the gradient corresponding to the load bearing capacity of the different age groups decreasing with age. Thus CFFSE and habituation may, at least to a certain extent, be considered identical. Since, however, CFFSE is the result of a stress, habituation may also be assumed to represent a stress phenomenon. The repetition of stimuli causing habituation would exert a load on the nervous system. With respect to this conclusion, it is remarkable that recently LURIA, SOKOLOV, and KLIMKOVSKY (1967) have pointed out the significant connections between habituation and inhibition. Thus the outlines of a correlation of habituation to inhibition to stress are taking shape.

The connection between habituation and stress, in turn, supports the opinion of those claiming an essential connection between habituation and fatigue.

If, furthermore, the analogy between CFFSE and habituation is accepted, new possibilities will open up for habituation research.

In the case of CFFSE, the findings such as the subsidence of the series following a negative exponential curve have not been considered sufficient. The value fluctuations of the series have been traced back to the effects of factors of variable intensity but specific direction. In the series, value reduction is caused by the effects of an F_1 factor of decreasing character, whereas the attenuation and, perhaps, the elimination of this reduction or even an increase of ascending character in course of the further continued measurements (that is, during a stress increase) might be attributed to the effect of an F_3 factor. And, finally, "recovery" in the case of habituation seems equivalent to the manifestation of the F_2 factor action in intermittent CFFSE sequences.

Recovery or regeneration

In intermittent sequences, CFFSE will develop not only according to the contradictory effects of factor F_1 decreasing the data standard, and of

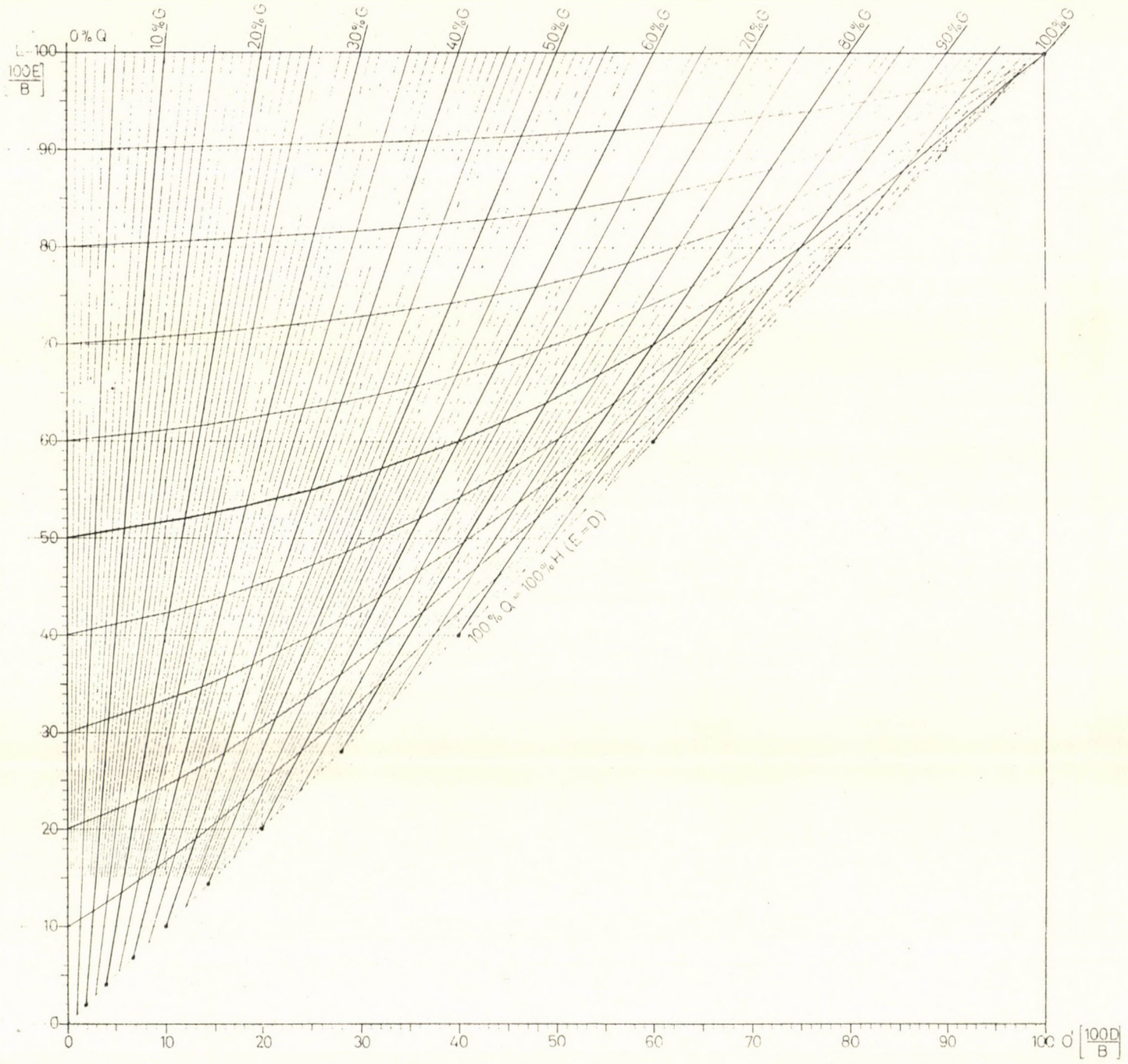

Fig. 1. Diagram of the uniform F_2 effect and F_2 factor intensity. Axis L defines E in the percentage of B. Here B represents the difference between the maximum and minimum of the 50 CFF data recorded by using the continuous method and expressed in cps. E, in turn, is the difference between the maximum and minimum of the intermittent series (that consisting of 50 CFF data recorded with four 30 sec interruptions).

Axis O' determines D in the percentage of B, where D is the difference between the maximum and the minimum of the first 30 data in the intermittent series of 50, expressed in cps.

The line ascending from point O in an angle of 45°, that is, the H = 100 per cent line, corresponds to the points to which E = D applies, or, in other words, where the difference between the maximum and minimum of the first 30 data of the intermittent sequences is identical to that between the maximum and minimum of the complete series of 50.

Lines G partly specify the difference between the maximum and minimum values of the first 30 data in the continuous sequences, expressed in the percentage of the difference between the maximum and minimum of the entire series of 50. These possibilities increase from 0 to 100%. On the other hand, they determine the location of those points which, in case of a given percentage, and under uniform F_2 effect conditions, may occur for the intermittent series (L, O') registered as the counterpart of the continuous sequence; thus, in the case of a uniform interruption effect, the L and O' values may only be located along the respective G line.

The intensity (Q) of factor F_2 amounts to 100% on the H = 100% line, in conformity with the given G percentage, thus this line represents — at the same time — the Q = 100% line as well. The Q = 0% line is at the height of L = 100. The other ratios are indicated on the individual G lines by those curves which ascend in the Figure in an exponential ratio. On axis L, the values of 10, 20, 30, etc., at the Q line starting points correspond, respectively, to the 90, 80, 70, etc., per cent values, and accordingly intersect the rest of the G lines in the diagram.

(Due to technical reasons, the G lines have not been plotted down to the corner of the co-ordinate system.)

factor F_3 increasing it, but will be affected — as against both F_1 and F_3 — by factor F_2 representing the regenerative influence of intermissions.

By using the CFFSE method, the relatively abstract description of habituation may be made much more exact. As an example, it will be illustrated how two simple figures can characterize, by series pairs, the regenerative F_2 factor.

Let us indicate the magnitude of the VR calculated from the first 30 data of the continuous series by A, the VR of the full series by B, the VR of the first 30 data in the respective intermittent series by D, and that of the first 50 data by E. Thus A, B, D, and E will be expressed in cps.

Let us indicate the following ratio by G.:

$$G = \frac{100\,A}{B}$$

Since the first 30 data of the sequences are included in the first 50 data, A will never exceed B and, consequently, the maximum of G will be 100 per cent. Theoretically, its minimum will be at the point where each datum of the first 30 values is equal, as in that case G is zero.

If, in the intermittent series assumed as the counterpart of the continuous one, the intermissions do not exert any influence whatsoever, that is, if the effect of factor F_2 cannot be demonstrated, the VR figures of the continuous and intermittent series will coincide or, in other words, $D = A$ and $E = B$.

The D and E values of the intermittent sequences may be made comparable by expressing them in the percentage of B, and by illustrating these values along the two axes of a co-ordinate system. The horizontal axis represents $L = 100E/B$, the vertical one $Ó = 100D/B$, and the two meet in the zero point whence their values will linearly increase up to 100.

One point in the L, Ó co-ordinate system is represented by the case when the complete continuous series coincides with the complete intermittent sequence and, at the same time, both sequences arrive at their lowest points as soon as within the first 30 values. Here G is 100 per cent, L is 100 per cent, and Ó is similarly 100 per cent. Along the perpendicular drawn from this point to axis L according to the possibilities offered by the 30 to 50 VR ratio of the continuous series, that is, in correspondence to all the G values, those points may be found, where no influence by factor F_2 can be observed along the intermittent series recorded as a counterpart of the continuous sequence. Thus, in these points any 30 to 50 VR ratio of the two series would coincide: $A = D$, and $B = E$.

In our co-ordinate system, points L and Ó produce a G line corresponding to different G values in the case of a uniform F_2 effect. These lines start at the height of $L = 100$, where the F_2 effect is zero, and terminate in the point where the VR calculated from the first 30 data of the intermittent series is identical

to that of the complete series, that is, where D = E. A D (VR of the first 30 data of the intermittent series) higher than that cannot occur, as the first 30 values are included in the first 50 values, that is, D = E, and the uniform F_2 effect is at its maximum. In our co-ordinate system, this ratio is expressed by L = Ó and the L = Ó points are found, from zero to L = Ó = 100, on a line of 45° inclination. (In this latter point G = 100, as mentioned above.) The 45° line represents the lower limit of the G lines which cannot go beyond it. This is the H = 100 per cent line (H = 100 D/E).

In our co-ordinate sytem, the G lines run in an oblique way. Why is that so?

The G lines represent the intersections of the L and Ó values, possible at any G per cent, in the case of a uniform F_2 effect.

In this case, however, L and Ó have a predetermined ratio. The first 50 data of the intermittent sequences involve 4 interruption effects, the first 30 have 2. It follows that each of the first 30 CFF data is under the influence of a 2/30 interruption effect, whereas the same for each of the whole 50 is 4/50. In case of a uniform F_2 effect, this means that 83.33 per cent of the total interruption effect of 100 per cent is manifested within the VR of the first 30 data, since 2/30 : 4/50 = 83.33 : 100.

It follows, in turn, that if the VR of the first 30 and 50 data, respectively, of the intermittent series form a ratio corresponding to the uniform effect of F_2, and if this effect is related in percentage to the reduction of the VR values exhibited by the respective continuous series, then

$$\frac{100(A - D)}{A} = \frac{83.33}{100} \cdot \frac{100(B - E)}{B} \tag{1}$$

D or E can be calculated from this equation, if the other is known, and the effect of F_2 is uniform. In the complete series, the effect of F_2 is indicated by the VR difference between the continuous and intermittent pairs, that is, by (B—E). Expressed in the percentage of B, this will be

$$\frac{100(B - E)}{B}$$

and its 83.33 per cent corresponds to the VR difference as expressed in the percentage of A, thus:

$$A - D = \frac{A}{100} \cdot \frac{83.33}{100} \cdot \frac{100(B - E)}{B}$$

Consequently,

$$D = A - \frac{83.33\,A(B - E)}{100B} = \frac{16.67\,AB + 83.33\,AE}{100B} \tag{2}$$

and

$$E = \frac{B(100\,D - 16.67A)}{83.33A} \tag{3}$$

On the basis of these equations D or E can be calculated for any G value, in the form of L and Ó, if the other is known. From Equations (1), (2), and (3):

$$\frac{B - E}{A - D} = \frac{B - \dfrac{B(100\,D - 16.67\,A)}{83.33A}}{A - D} = \frac{83.33\,AB - 100\,BD + 16.67\,AB}{83.33\,A(A - D)} =$$

$$= \frac{100\,AB - 100\,BD}{83.33\,A(A - D)} = \frac{100\,(A - D)\,B}{83.33\,A(A - D)} = \frac{100 \cdot 100B}{100 \cdot 83.33\,A} = \frac{10000}{83.33\,G}$$

Thus in any per cent of G:

$$E = \frac{83.33\,BG - 10000\,(A - D)}{83.33\,G} = B - \frac{120\,(A - D)}{G}$$

Calculating the G lines expressing the uniform F_2 effect, and plotting them in the L, Ó co-ordinate system yields a diagram with two data for any F_2 effect therein.

The first information is on the intensity of the F_2 effect. Conforming to H = 100 per cent, each G line has its lowest point where L = Ó, that is, where the F_2 effect arrives at its possible maximum, if the F_2 effect is uniform. Along the given G line, the F_2 effect will decrease from that maximum up to the L = 100 height where it will be zero. The possibilities characteristic of F_2 may be expressed by the size of the G_x section. This G_x begins on the H = 100% line at its intersection with G, and will ascend, parallel to L, up to the height of L = 100.

G_x indicates to what a maximum extent can a uniform F_2 effect reduce B in the case of the given G per cent. In other words, it indicates the possible extent of the fluctuation of a uniform interruption effect in the case of a given 30 to 50 continuous VR ratio.

$$G_x = \frac{100\,(B - E)}{B}, \text{ if } E = D \quad (L = Ó)$$

If the effect of F_2 is uniform, and E = D then, on the basis of (3):

$$E = \frac{100\,BE - 16.67\,AB}{83.33\,A} = \frac{16.67\,AB}{100\,B - 83.33\,A}$$

$$G_x = \frac{100\,B - 100\,E}{B} = 100 - \frac{1667\,A}{100\,B - 83.33A}$$

and from the formula of G,

$$A = \frac{BG}{100}$$

$$G_x = 100 - \frac{1667\,BG}{10000\,B - 83.33\,BG} = 100 - \frac{20}{\frac{120}{G} - 1} \qquad (4)$$

The intensity of the actually manifest F_2 effect can be compared to the intensity of the one calculated with its uniformity assumed. The difference of the actual E as expressed by L from 100, i.e. 100 — L, gives, in the percentage of G_x, the relation of the actual magnitude of the F_2 effect to the one possible with its uniformity assumed. This relation (Q) is characteristic of the intensity of the F_2 effect:

$$Q = \frac{100(100 - L)}{G_x}$$

It can be calculated similarly, how and to what extent the actual F_2 effect would differ from the uniform one. In the case of a uniform F_2 effect, the calculated L or Ó values would be located on the G line corresponding to the continuous curve. If, on the other hand, the F_2 effect is not uniform, the actual Ó and L values would not coincide with the given G line but be located at a numerically definable distance therefrom and, with Ó taken as a basis, either to the left (—) or to the right (+).

Under uniform F_2 effect conditions, D of Equation (2) must be substituted into the formula

$$Ó = \frac{100\,D}{B}$$

whereby

$$Ó = \frac{16.67\,AB + 83.33\,AE}{B^2}$$

The difference of the Ó value thus calculated, and characteristic of the uniform F_2 effect, from the actual Ó value represents the measure of F_2 effect uniformity:

$$F_2 = \frac{100\,D}{B} - \frac{100\,AB + 500\,AE}{6B^2} \qquad (5)$$

In order to explain how exact comparisons are feasible by using the CFFSE method, a comparative Table based upon the F_2 data is presented below. This Table presents the F_2 data as calculated according to (5), for two patient groups and categorized by two syndromes, by making use of the second (10 o'clock) series pairs of the total of 3 such series pairs recorded daily for 3 consecutive days. (Naturally, 18 and 21 patients per group, respectively, are sufficient only for a superficial information.)

It is easy to realize the different distribution of $\pm F_2$ values in the two groups.

If it is accepted that, at least to a certain extent, the phenomenon of CFFSE and that of habituation are analogous, this analogy might be extended to the phenomena of recovery and regeneration. Thus, investigations into the

Clinical epilepsy (18 patients)

	Serial No.	Name	Sex	Age	+	—	Note
1	36	I. M.	f	31	10.64		
2	113	Gy. N.	f	24		7.88	+ neurosis, hysteria?
3	123	I. V.	m	20		4.15	
4	132	I. K.	f	31	5.27		
5	136	K. K.	m	27		2.41	
6	138	J. S.	f	18		1.62	
7	139	S. T.	m	27		0.06	
8	141	G. Sz.	m	15		13.86	
9	143	F. P.	f	27		6.63	
10	144	E. M.	f	18		13.05	
11	145	J. M.	m	30		8.50	
12	147	F. P.	m	23		0.44	
13	149	M. K.	f	35		1.90	
14	153	J. R.	m	38		0.08	
15	154	B. B.	m	35		8.22	
16	156	G. M.	m	20		23.73	
17	161	B. S.	f	39		3.96	
18	162	I. T.	f	51	11.79		

Clinical neurosis (21 patients)

	Serial No.	Name	Sex	Age	+	—	Note
1	40	D. F.	m	33	14.83		
2	107	S. N.	f	45	12.44		+ hypotension, climax
3	109	P. V.	f	53	10.84		+ hypertension, climax
4	110	A. N.	m	48	20.58		
5	114	K. N.	f	44		29.79	
6	115	S. L.	f	38	14.65		
7	127	K. I.	f	32	7.64		
8	129	I. K.	f	28	4.05		
9	134	I. J.	m	37	2.44		
10	137	I. E.	f	36		13.22	
11	140	A. D.	m	18	9.99		
12	142	J. O.	f	19	0.18		
13	146	B. G.	m	34		1.82	
14	150	E. Cs.	m	43		7.33	
15	151	F. T.	m	41	1.27		
16	152	A. M.	f	32	7.16		
17	155	Gy. F.	f	56		33.77	
18	157	J. V.	f	21		8.96	+ hypotension
19	158	M. Zs.	f	63		1.98	
20	159	K. H.	f	43	22.68		
21	160	F. T.	m	21		21.93	+ hypertension

regenerative factor of the CFFSE or, more precisely, their possibilities outlined above could allow detailed studies on the recovery associated with habituation.

Understanding the CFFSE phenomenon is similarly promoted by the analogy with habituation. The latter is a very extensive process. As for the CFF, there is further evidence available to verify its being affected by an extremely multilateral influence. Thus, for example, in encephale isolé experiments, the potentials following light impulses may be facilitated or impeded from different parts of the non-specific systems of the brain (STERIADE and DEMETRESCU, 1960). This does not contradict the well-known finding by BARTLEY (1959), according to which the critical, final point of CFF origin is the cortex. The extensive connections of the cortex, in turn, are well known (see, for example, PARIKASHVILI, 1962). CFFSE demonstrates this extensive influence, and its stress variations, just like its relation with habituation confirms the opinion on its extensively affected nature.

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REFERENCES

Ákos, K., Ákos, M.: The Critical Flicker Frequency Series Effect. Publishing House of the Hungarian Academy of Sciences, Budapest 1966.
Ashby, W. R.: Mechanisation of Thought Processes. Her Majesty's Stationery Office. London 1959.
Bartenwerfer, H.: Psychische Beanspruchung. In: Handbuch der Psychologie. Ed. Larsch—Sonder—Thomae. Verlag für Psychologie. Hogrefe. Göttingen 1961, Vol. 9.
Bartley, S. H.: Handbook of Psychology: Neurophysiology, Vol. I, 1959. American Physiological Society. Washington. Ed: Field, Magoun, Hall.
Luriya, A. R., Sokolov, E. N., Klimkovsky, M.: (Лурия, А. Р., Соколов, Е. Н., и Климковский, М. Журн. высш. нервн. деятельности III. XVII. 2. 1967.)
Ruch, T. C.: Handbook of Experimental Psychology. Wiley, New York 1951.
Schmidtke, H.: Die Ermüdung. Huber, Bern 1965.
Steriade, M., Demetrescu, M.: J. Neurophysiol. **23,** 602 (1960).
Thompson, R. F., Spencer, W. A.: Psychol. Rev. **73,** 16 (1966).
Parikashvili, S. P.: (Парикашвили, С. П., Вопросы психологии. 1962. 56—72.)
Walter, G.: Mechanisation of Thought Poıcesses. Her Majesty's Stationery Office. London. 1959.

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AN ATTEMPT TO EXPLAIN DIFFERENCES IN THE HYPOPHYSEAL–ADRENOCORTICAL RESPONSE TO REPEATED STRESSFUL STIMULI BY THEIR DEPENDENCE ON DIFFERENCES IN PATHWAYS

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Certain non-specific stimuli that raise the plasma corticosterone level when administered on a single occasion fail to raise it when administered repeatedly whereas others raise it independently of the number of administrations. It is suggested that stressor agents reach the CRF-secreting hypothalamic cells by different pathways and that the CRF-activating mechanism of some stressors does adapt itself to repeated stimuli, that of others does not.

It is only since we know that a wide variety of acute stress — physical, chemical, emotional and other stimuli — evoke hypophyseal-adrenocortical hyperfunction that a better understanding of the function and the physiological role of the system has emerged. Acute stresses increase the corticosteroid concentration in the blood, cause adrenal ascorbic acid and lipoid depletion, involution of the thymus, eosinopenia, and lymphocytopenia (SELYE 1936, 1950, 1961). According to SELYE (1936, 1950, 1961) repeated exposure results in the stage of resistance in which the adrenal is hypertrophied, adrenal ascorbic acid and lipoid concentrations are again normal, or above normal, and lipoid granules accumulate. Not so long ago it was widely believed that this indicated adrenocortical hyperfunction. Other data, however, suggested that in the stage of resistance the thymus regains its weight, the number of lymphocytes and eosinophils returns to normal, and other indirect signs of hypercorticalism disappear (SELYE 1950, 1961). These findings seem to show that repeated exposure to non-specific stimuli fails to elicit adrenocortical hyperfunction.

While it is difficult to find one's way in the maze of literary data concerning the changes in hormone secretion and morphology that follow acute stress of the hypophyseal-adrenocortical system, surprisingly little attention has been devoted to the response of the hormonal axis to repeated stressful stimuli. SELYE's investigations and his reasoning were based on morphological findings (SELYE 1936, 1950, 1961). WEISZ and his coworkers (1959) found that repeated injections of formalin caused no rise in the corticosterone level of the adrenal venous blood. SCHÖNBAUM (1960) observed no increased hormone production in vitro by the adrenals of animals exposed to cold for several weeks. We showed (STARK et al., 1965) that the last of 14 daily injections of formalin produced a

significantly lesser rise in the corticosterone level of the peripheral blood than did a single formalin injection administered to control animals pretreated for the same number of days with physiological saline.

A considerable body of experimental evidence suggests that the operative surgery and cannulation which necessarily precede the collection of adrenal venous blood intensify the corticosterone production in a degree beyond which it cannot be considerably increased even with exogenous ACTH (HOLZBAUER and VOGT, 1957; CSALAY and FRENKL, 1962; PAPP et al., 1964). We, however, have always used the pooled blood of four animals to determine plasma corticosterone levels.

The present experiments were performed for the purpose of ascertaining whether our earlier results obtained with formalin were valid for other stressor agents, and whether a method more sensitive (GUILLEMIN et al., 1959) for the determination of corticosterone would confirm them.

In selecting stressor agents for the present experiments we were guided by the consideration that since in the view of some authors (MATSUDA et al., 1963; DE WIED, 1964; MIALHE-VOLOSS, 1958; ROCHEFORT et al., 1959; MANGILI et al., 1966; SMELIK, 1959; FORTIER, 1951) non-specific stimuli activate the hypophyseal-adrenocortical system by different mechanism, it might be possible that their repeated application will lead to different degrees of adaptation.

Materials and methods

Male Wistar rats weighing 160 ± 10 g were used (exept the ether-group). While being treated, the animals were kept in a constant-temperature room (24 ± 1 °C) and fed a standard diet with water ad libitum.

Stressful stimuli

Ether. Rats were subjected to etherization stress once daily for 14 days. Each animal was placed for 1 minute in a 4.5-litre jar immediately after 10 ml of ether (Aether ad narcosim Ph. Hung. V.) had evaporated in it during 3 minutes. The control animals were each subjected to mild restraint by being placed in an empty jar for the same length of time. In both groups, 24 hours later treatment was followed by one more 1-minute application of ether stress and restraint, respectively. All animals were killed by decapitation 30 minutes after the last interference.

Endotoxin. Two groups of rats were treated daily for 14 days with an intraperitoneal injection of 0.2 ml of an endotoxin suspension (germ count 100,000/ml). Twenty-four hours later the animals in one group were given an additional endotoxin injection and those in the other an injection of physiological saline. Another two groups were treated in the same manner with 0.2 ml of physiological saline and one of them received 24 hours later an additional injection of physiological saline and the other group an injection of 0.2 ml of endotoxin. Having found in preliminary experiments that intraperitoneal endotoxin took about 3 hours to produce maximum blood corticosterone concentration, all animals were exsanguinated 3 hours after the last injection.

Electroshock. For administering the electroshock current a cage having walls of plexy and a bottom of parallel metal bars was used. Every other bar was connected with a positive and negative pole, respectively, thus ensuring a closed circuit through the limbs and the tail of the animal on the bars. The source of the shock was a condenser charged up with 300 V direct current. The average of measurements showed the animals resistance to be 15 kohm;

thus the value for the current arising on initial stimulation was about 20 mA, decreasing exponentially until complete discharge of the condenser. Shock duration was 0.3—1 seconds. Separate groups of animals were shocked on a single occasion and once a day on 5, 14 and 30 consecutive days.

All animals were exsanguinated by decapitation 25 minutes after the administration of the last electric shock because practical experience had revealed that between 20 and 30 minutes had to elapse before the shock raised the blood corticosterone level to peak value.

Vasopressin — Tonephin. One group of rats was treated subcutaneously with 1 I.U. of lysine-vasopressin (Sandoz) and another one with 5 I.U. Tonephin (Hoechst A. G.) once daily for fourteen days. Twenty-four hours later one half of the animals in each group was given an additional injection of the stressor, the other half one of physiological saline. All animals were decapitated one hour after the last injection (Table 4).

Formalin. Two groups of rats were injected subcutaneously with 1% formalin (0.25 ml/100 g b. wt.) once daily for 14 days. The animals in one group received 24 hours later an additional formalin injection, and those in the other group an intraperitoneal injection of endotoxin (0.2 ml). Two control groups were treated daily for 14 days with physiological saline (0.25 ml/100 g b. wt.) administered subcutaneously. Twenty-four hours later this treatment was followed in one group by an additional injection of physiological saline, in the other by an injection of formalin. The animals were bled to death 1 hour after formalin, and 3 hours after endotoxin, administered as the last injection.

Corticosteroid determination

The trunk blood caught in heparinized tubes was centrifuged and plasma corticosterone content was measured by the fluorometric method of GUILLEMIN et al., (1959).

The adrenals of the animals used in our experiments with endotoxin and electric shock, which had been removed, cleaned, and weighed immediately after killing, were cut into four parts and placed into flasks containing Krebs—Ringer-bicarbonate-glucose solution. A mixture of 95% O_2 and 5% CO_2 was bubbled through the medium continuously, and incubation carried out in a bath for 1 hour at 37°C, after SAFFRAN and SCHALLY (1955). At the end of this incubation period the medium was discarded and the adrenals were reincubated with fresh medium for 1 hour in the presence of 0.1 I.U. of crystalline ACTH per 100 mg of adrenal (Corticotrophinum, Organon, Oss). From the two incubation media the corticosteroids were extracted with chloroform, isolated by paper chromatography, developed in a solution of tetrazolium blue, and eluated; corticosterone and aldosterone were then measured spectrophotometrically, using a Unicam SP 500 apparatus (FACHET et al., 1962).

Results

Ether. Administered 24 hours after 14 daily exposures to mild restraint, a single exposure to etherization stress produced a statistically significant rise of the plasma corticosterone level. Administered after 14 daily etherization stresses, it also raised the corticosterone concentration but to a significantly lower level which, however, was higher than the control level (Table 1).

Endotoxin. Administered 24 hours after 14 daily injections of physiological saline, a single exposure to endotoxin caused a significant rise in the plasma corticosterone level, but when it followed 14 daily injections of endotoxin it had no appreciable effect on corticosterone concentration. Treatment with endotoxin for 14 days caused adrenal hypertrophy. In vitro, the adrenals produced about as much corticosterone and aldosterone during the first hour of incubation as did the controls, but in the presence of ACTH (reincubation) the corticosterone production increased (Table 2).

Table 1

Effects of single and repeated ether stress

14-day treatment	Followed 24 hours later by	Body weight g	Weight of adrenals mg	Corticosterone μg/100 ml plasma
daily 1-min. restraint	1-min. restraint	386±8	43.8±1.1* (25)	16.3±3.2 (13) (a)
daily 1-min. restraint	1-min. etherization			37.4±3.0 (12) (b)
daily 1-min. etherization	1-min. etherization	392±8	48.0±1.6 (23)	29.6±3.3 (11) (c)
daily 1-min. etherization	1-min. restraint			22.1±3.8 (12) (d)

mean ± S. E.
number of determinations

a—b $p < 0.05$
a—d $p < 0.05$
c—d $p < 0.05$
b—c $p < 0.05$

Electric shock. Whether given on a single occasion or once a day on 5, 14 and 30 consecutive days, electric shock resulted in largely the same statistically significant rises of the blood corticosterone level. Irrespectively of the number of applications, electroshock had no effect on adrenal weight. In relation to the controls, corticosterone production in vitro increased significantly in response to electric shock in all four experimental groups, but the increase in aldosterone production was insignificant. The amount of corticosterone produced in the presence of ACTH (during reincubation) significantly exceeded the control value in all groups while that of aldosterone fell below it (Table 3).

Vasopressin and Tonephin. A single exposure to either of these two stressor agents raised the plasma corticosterone level significantly. Twenty-four hours after 14 daily injections of vasopressin or Tonephin the corticosterone concentrations were on the whole equal to those in the controls. Preceded by 14 daily injections of vasopressin, an additional vasopressin elicited nearly the same significant rise as did a single exposure. Preceded by 14 daily injections of Tonephin, an additional Tonephin also produced a significant rise, but this was significantly lower than that produced by a single exposure (Table 4).

Formalin. A single exposure to 1% formalin raised the plasma corticosterone level significantly, but one administered 24 hours after 14 daily formalin injections, had a lesser effect. Given 24 hours after a 14-day treatment with formalin, a single endotoxin injection increased the corticosterone concentration; this increase was significantly higher than that produced by a single formalin following 14 daily formalin injections (Table 5).

Table 2

Effects of single and repeated endotoxin

14-day treatment	Followed 24 hours later by	Weight of adrenals mg/100 g B. W.	Corticosterone μg/100 ml plasma	Corticoid production in vitro			
				without ACTH		with ACTH	
				Corticosterone μg/h/100 mg	Aldosterone μg/h/100 mg	Corticosterone μg/h/100 mg	Aldosterone μg/h/100 mg
0.9% NaCl	0.9%NaCl	14.4 ± 0.6 (12)	23.3 ± 2.5 (a) (12)	3.1 ± 0.5 (6)	1.9 ± 0.2 (6)	9.3 ± 1.6 (e) (6)	5.6 ± 0.9 (g) (6)
0.9% NaCl	endotoxin	20.9 ± 1.0 (15)	52.1 ± 7.3 (b) (17)	—	—	—	—
endotoxin	endotoxin	24.6 ± 0.7 (22)	26.8 ± 7.4 (c) (27)	—	—	—	—
endotoxin	0.9% NaCl	23.7 ± 1.0 (24)	24.9 ± 2.3 (d) (24)	3.4 ± 0.6 (6)	2.1 ± 0.1 (6)	12.2 ± 0.1 (f) (6)	4.1 ± 0.5 (h) (6)
			a—b $p < 0.001$ a—c $p > 0.40$ a—d $p > 0.60$ b—c $p < 0.001$			e—f $p > 0.10$ g—h $p > 0.05$	

Table 3

Effects of single and repeated electric shocks

Daily shocks applied for	Weight of adrenals mg/100 g B. W.	Corticosterone μg/100 ml plasma	Corticoid production in vitro			
			without ACTH		with ACTH	
			Corticosterone μg/h/100 mg	Aldosterone μg/h/100 mg	Corticosterone μg/h/100 mg	Aldosterone μg/h/100 mg
Control	16.8 ± 0.8 (18)	19.5 ± 1.7 (20)	2.04 ± 0.37 (5)	1.90 ± 0.33 (5)	8.30 ± 1.18 (5)	3.22 ± 0.52 (5)
1 day	16.1 ± 0.4 (10)	49.1 ± 2.0* (11)	6.40 ± 0.94* (5)	2.60 ± 1.06 (5)	13.40 ± 0.55* (5)	2.46 ± 0.24 (5)
5 days	16.6 ± 0.4 (9)	41.4 ± 2.8* (9)	9.46 ± 0.71* (9)	1.96 ± 0.32 (8)	13.52 ± 1.24* (9)	2.06 ± 0.50 (9)
14 days	16.3 ± 0.8 (9)	46.4 ± 4.3* (9)	10.96 ± 1.02* (9)	1.83 ± 0.24 (9)	15.78 ± 1.68* (9)	2.96 ± 0.34 (9)
30 days	15.5 ± 0.8 (10)	51.6 ± 1.7* (12)	6.76 ± 2.19** (4)	2.40 ± 0.79 (4)	11.92 ± 1.27*** (5)	1.86 ± 0.48 (5)

* $p < 0.01$ vs control ** $p > 0.05$ vs control *** $p < 0.05$ vs. control..

Table 4

Effects of single and repeated Vasopressin and Tonephin, respectively

14-day treatment	Followed 24 hours later by	Body weight	Weight of adrenals mg	Corticosterone μg/100 ml plasma
0.9% NaCl	0.9% NaCl	213.0 ± 3.2	43.9 ± 1.5 (a)	13.0 ± 0.4 (10)
0.9% NaCl	Vasopressin	(20)	(20)	63.4 ± 2.7 (10)*
Vasopressin	0.9% NaCl	209.8 ± 2.8	49.9 ± 1.8 (b)	12.2 ± 1.7 (10)
Vasopressin	Vasopressin	(20)	(20)	61.8 ± 3.1 (9)*
0.9% NaCl	0.9% NaCl	200.4 ± 3.5	46.7 ± 1.6 (c)	15.8 ± 2.2 (6)
0.9% NaCl	Tonephin	(12)	(12)	73.2 ± 3.0 (6)*
Tonephin	0.9% NaCl	205.0 ± 3.7	50.5 ± 2.2 (d)	11.6 ± 2.0 (6)
Tonephin	Tonephin	(13)	(13)	58.1 ± 3.2 (7)*

a—b $p < 0.05$ * $p < 0.001$ vs. controls
c—d $p > 0.10$

Table 5

Effects of formalin and endotoxin

14-day treatment	Followed 24 hours later by	Corticosterone μg/100 ml plasma	Significance
0.9% NaCl	0.9% NaCl	12.1 ± 1.7 (11) (a)	a—b $p < 0.01$
0.9% NaCl	1% formalin	67.5 ± 4.3 (6) (b)	b—c $p < 0.05$
1% formalin	1% formalin	43.3 ± 3.6 (12) (c)	a—c $p < 0.05$
1% formalin	endotoxin	57.2 ± 3.5 (6) (d)	d—c $p < 0.05$

Discussion

In the present work, prolonged exposure to endotoxin, ether, or formalin produced no rise of the corticosterone level or, at most, one that was significantly lower than the rise elicited by a single administration. Although they measured adrenal activity by methods different from the one we used, earlier investigators made similar observations with repeated formalin (VECSEI, 1962), with insulin (KRAICER and LOGOTHETOPOULOS, 1963), with cold stress (SCHÖN-

BAUM, 1960) and with traumatization in the Noble-Collip drum (MIKULAJ and KVETNANSKY, 1966). The present experiments showed, however, that electric shock, vasopressin, and Tonephin elicited hypophyseal-adrenocortical hyperfunction whether administered repeatedly or on a single occasion.

How is one to explain why certain of the stressors that raise the plasma corticosterone level when administered on a single occasion fail to raise it when administered repeatedly, whereas others raise it independently of the number of administrations? To explain the absence of adrenocortical hyperfunction during repeated exposure three possibilities offer themselves for consideration:

(i) Repeated exposure is not accompanied by repeated hypersecretion of ACTH.

(ii) Repeated exposure is accompanied by repeated ACTH hypersecretion but the adrenal fails to respond with a corresponding increase in corticosteroid secretion.

(iii) There may be increased need for corticoid hormones in the tissues, i.e. accelerated hormone metabolism.

The absence of a rise in the plasma corticosterone level after repeated exposure to endotoxin and a presence of a lesser rise to formalin, cannot be ascribed to reduced adrenal responsiveness to ACTH (Table 2). Nor does increased corticosteroid elimination explain it since, at least in formalin-treated animals, there is no increased elimination (STARK et al., 1965). This observation agrees well with the finding of KRAICER and LOGOTHETOPOULOS (1963) that repeatedly administered insulin no longer increases the plasma corticosterone level and leaves corticosteroid elimination unchanged.

Thus it would appear that prolonged exposure to certain stressor agents fails to raise the plasma corticosterone level because it is not accompanied by repeated hypersecretion of ACTH.

In an earlier work (STARK et al., 1965) we showed that operative trauma caused ascorbic acid depletion in the adrenal of animals treated with formalin. The present experiments produced evidence that endotoxin raised the plasma corticosterone level in the formalin-treated animals. These facts indicate that the hypophysis of animals treated with formalin is capable of secreting ACTH 24 hours after prolonged treatment.

The conclusion is that neither decreased adrenal or hypophyseal responsiveness (Stark et al., 1963, 1968) nor increased plasma corticosteroid elimination (Stark et al., 1965) can be evoked to explain why repeated exposure to certain stressors (ether, formalin, and endotoxin) is not followed by a rise in the plasma corticosterone level, or only by one significantly lower than that seen from a single administration.

The mechanisms of stressors activating ACTH secretion have been studied by various approaches (FORTIER, 1951; SMELIK, 1959; DEWIED, 1964; MIALHE-VOLOSS, 1958; ROCHEFORT et al., 1959; ARIMURA et al., 1965), and several

attempts have been made to subdivide the stimuli according to the ways ("neurogenic", "systemic", "somatic") by which they give rise to ACTH secretion under acute conditions.

The final common pathway controling ACTH secretion is the corticotropin releasing factor (CRF) produced, according to HALÁSZ et al. (1962, 1967), by the cells in the hypophyseotrophic area.

The finding that repeated exposure to certain stressor agents is not, whereas that of others is, accompanied by repeated ACTH hypersecretion may be interpreted along the line that stressor agents reach the CRF-secreting hypothalamic cells by different mechanisms (pathways); and that the CRF-activating mechanism (neural or humoral) of some stressors does adapt itself to repeated stimuli, that of others does not.

If this is a valid interpretation it may lead to an answer to the question why hypophyseal-adrenal response is always the same to acute stresses but widely varies after repeated exposures.

*

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REFERENCES

ARIMURA, A., YAMAGUCHI, T., YOSHIMURA, K., IMAZEKI, T. and ITOH, S.: Jap. J. Physiol **15,** 278 (1965).
CSALAY, L. and FRENKL, R.: Acta Phys. Acad. Sci. Hung. **22,** 135 (1962).
DE WIED, D.: J. Endocrin. **29,** 29 (1964).
FACHET, J., STARK, E., VALLENT, K. and PALKOVITS, M.: Acta Med. Acad. Sci. Hung. **18,** 461 (1962).
FORTIER, C.: Endocrinology **49,** 782 (1951).
GUILLEMIN, G. R., CLAYTON, W. G. LIPSCOMB, H. S.: and SMITH, J. D. J. Lab. Clin. Med. **53,** 830 (1959).
HALÁSZ, B., PUPP, L. and UHLARIK, S.: J. Endocrin. **25,** 147 (1962).
HALÁSZ, B., SLUSHER, M. A. and GORSKI, R. A.: Neuroendocrinology **2,** 43 (1967).
HEDGE, G. A., YATES, M. B., MARCUS, R. and YATES, F. E.: Endocrinology **79,** 328 (1966).
HOLZBAUER, M. G. and VOGT, M.: J. Physiol. **138,** 449 (1957).
KRAICER, J. and LOGOTHETOPOULOS, J.: Acta Endocrin. (Kbh) **44,** 259 (1963).
LEEMAN, S. E., GLENISTER, D. W. and YATES, F. E.: Endocrinology **70,** 249 (1962).
MANGILI, G., MOTTA, M. and MARTINI, L.: Control of Adrenocorticotropic Hormone Secretion, in: Neuroendocrinology Vol. I. Chapter 9, pp. 298—370 (1966) Ed. by MARTINI, L. and GANONG, W. F. (AP.)
MATSUDA, K., KENDALL, J. W., DUYCK, C. and GREER, M. A.: Endocrinology **72,** 845 (1963).
MIALHE-VOLOSS, C.: Acta Endocrin. **28,** Suppl. 35. 1 (1958).
MIKULÁJ, L. and KVETNANSKY, R.: Physiol. Bohemoslov. **15,** 439 (1966).
PAPP, M., STARK, E., ÁCS, ZS. und VARGA, B.: Endokrinologie **46,** 280 (1964).
ROCHEFORT, G. J., ROSENBERGER, J. and SAFFRAN, M.: J. Physiol. **146,** 105 (1959).
SAFFRAN, M. and SCHALLY, A. V.: Endocrinology **56,**, 523 (1955).
SCHÖNBAUM, E.: Fed. Proc. **19,** 85 (1960).
SELYE, H.: Brit. J. exptl. Pathol. **17,** 234 (1936).
SELYE, H.: Stress. Acta Inc. Montreal (1950).
SELYE, H.: Nonspecific Resistance, in: Ergebnisse der Pathologie. Bd. **41,** 209 (1961). Springer-Verlag.

SMELIK, P. G.: Autonomic Nervous Involvement in Stress-Induced ACTH Secretion (N. V. Drukkerij V.) H. H. Born, Assen, Netherlands, 80 pp. (1959).
STARK, E., FACHET, J. and MIHÁLY. K.: Can. J. Biochem. Physiol. **41**, 1772 (1963).
STARK, E., FACHET, J. und MIHÁLY, K.: Endokrinologie **49**, 27 (1965).
STARK, E., ÁCS, Zs., MAKARA, G. B. and MIHÁLY, K. Can. J. Physiol. Pharm. **46**, 567 (1968).
VECSEI, P.: Endokrinologie **42**, 154 (1962).
WEISZ, P., KÁDAS, T., KÖVES, P. und RITTER, L.: Endokrinologie **38**, 80 (1959).

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RECENSIONES

COLLETTE, I. M., JANTET, G., SCHOFFENIELS, E. *New trends in basic lymphology.* Proceedings of a Symposium held at Charleroi on 11 to 13 July, 1966. Birkhäuser Verlag, Basel and Stuttgart 1967.

This volume of 235 pages, the 14th Supplement of Experientia, fully documents the Symposium held under the auspices of the Council for International Organizations of Medical Sciences with Professor M. FÖLDI presiding. The guests were clinicians and theoretical scientists engaged in research on the production of lymph and on the dynamism of lymphatic circulation. The subjects included the ultrastructure of the lymph channels in health and disease, the passage of various substances into the lymph, the relationships between structure and function in the lymphatic network of the mesentery, contactility of human lymph vessels, problems of the lympho-venous junctions and their response to various factors. Papers were read on the histochemical and histoenzymological aspects of the lymphatic pathways, others dealt with the functional and structural signs of lymph stasis. Radiographic exploration of the lymphatic structures and the results of intralymphatic application of cytostatic drugs were documented with impressive photographic material. The text of the individual papers had been handed out to the members for previous study. This accounts for the relevant and interesting discussions following the reports.

The transendothelial transport of fluids and solids was suggested to be due to the interplay of dynamic factors. Fluid movement across the interendothelial junctions of the lymph capillaries was discussed in detail. It would have been welcomed if the exchange of these materials within the cell through the intermediary of the intracellular vesicular elements resulting from an active function of the endothel cell would have received more attention. On the evidence of electron microscopic studies, parallel with muscle relaxation, the wall of the lymph capillary opens like a valve, giving thus access to the inflow of interstitial fluid and solids. On the other hand, on muscular contraction, the materials are returned to the interstitial compartment. The lymph is forwarded toward the heart, in addition to muscle contraction, by an active contraction of the functional unit of the lymphatic system, the "lymphangion", in response to an elevation of the intralymphatic pressure mediated via sympathetic pathways. Ample evidence has been furnished that dye uptake, lymphographic filling and patency of lymphatic anastomoses are responsive to various drugs. Histochemical and histoenzymological studies failed to produce evidence of any distinctive character between blood and lymph capillaries. On the other hand, certain metabolic abnormalities arising in organs after experimental obstruction of their lymph flow were identified by histochemical methods. The preliminary results of intralymphatic application of cytotoxic drugs seem to be promising. The animated debates and interesting comments show that the individual contributions had been thought-provoking and that the athmosphere was one of fertile criticism. The production of the volume is exemplary, only the legends on pages 14 and 193 are incorrect. The student of the lymphatic system will find here a rich source of information and of stimulating ideas.

M. PAPP

WANDREY, D. and LEUTNER, F. *Neuro-Psychopharmaca in Klinik und Praxis.* F. K. Schattauer Verlag, Stuttgart 1967.

From year to year it becomes more and more difficult to survey the existing psychotropic drugs. The authors of the present volume have made the best of this task by bringing the vast material up to date until 1967. The book is intended for clinicians and other practising

physicians. Although the authors were primarily concerned with practical issues and clear presentation of the information was one of their main objectives as seen from the well-arranged tables, they give nevertheless due consideration to the theoretical background as well. It is to be welcomed that the references are given separately instead of being inserted in the text which would have made it too bulky. In this manner the reader has less difficulty in finding the information he may require.

The authors have not attempted to give any new classification of the psychotropic drugs, still less to present them in a new light. What they did intend in fact, and we believe, with success, was to give a complete list of these agents with a short and accurate but none the less full account of their pharmacological and clinical properties. It must be emphasized that the book is free from any advertising character. Special sections deal with the psychotropic aspects of clinical medicine, dermatology and surgery. The authors have filled a gap by having compiled evidence on these little studied relationships.

A critical presentation of the material would have added much to the value of this informative volume, for instance by marking out certain drugs as efficient, certain others as being devoid of any value. In the absence of any indication reflecting the authors' view, even the most exhaustive source of information remains incomplete.

E. VARGA

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РЕЗЮМЕ

ПОБОЧНОЕ ДЕЙСТВИЕ ПРЕПАРАТА ДОКАКВОЗУМ (ГЛЮКОЗИД ДЕЗОКСИКОРТИКОСТЕРОНА) НА ФУНКЦИЮ ЩИТОВИДНОЙ ЖЕЛЕЗЫ

К. БАРТА-ГОЛИАН

Проводились эксперименты по выяснению побочного действия препарата Докаквозум на поглощение йода и на синтез гормонов щитовидной железой. Действие препарата на обмен йода исследовалось на основе накопления йода щитовидной железой, а также процентного состава его йодгормонов и гормонов плазмы. Для исследования использовалась методика радиоактивных индикаторов. Количественная и качественная оценка кривых аккумуляции йода показала значительное торможение поглощения йода: при введении 10 мг на кг веса в день оно понизилось с 33% до 20%. В щитовидной железе фаза неорганического йодида повысилась почти до двухкратной исходной величины, в ущерб фазы дийодтирозина. В плазме состав гормонов указал на нарушение мобилизации. При введении 10 мг йода на кг веса тела фазы ди- и трийодтирозина повысились до пятикратной исходной величины, и содержание тироксина понизилось до величины, ниже половины исходной.

БАКТЕРИАЛЬНАЯ ФЛОРА В ВЫДЕЛЕНИИ ИЗ БРОНХОВ

Э. СЕКЕЙ, и Э. ФАРКАШ

Авторы разработали данные 3867 бактериальных культур и 1407 исследований резистентности, проводившихся на 3682 пробах выделения из бронхов, полученных в связи с 3602 бронхоскопическими исследованиями 2432 детей в возрасте от 0—18 лет. Больных они распределили на девять групп: интактную, активный туберкулез, неактивный туберкулез, посттуберкулезное расширение бронхов, прорыв процесса в бронх, бронхиальная астма, хронический бронхит, хроническая пневмония, бронхоэктазия.

Установлено, что

1. У здорового человека выделение из бронхов не обязательно стерильное.
2. Наличие нейссерий и стрептококков в бронхиальном секрете обуславливается не загрязнением бронхоскопом, а это патологическое явление.
3. Между бактериальной флорой бронхиального секрета и степенью выраженности бронхиального процесса наблюдается определенная связь, независимо от того, является ли основное заболевание специфическим или неспецифическим.
4. Ответственным за заселение патогенных микробов следует считать анатомические и тканевые изменения, а также биохимические изменения совместно.
5. Чувствительность к антибиотикам микробов, выращенных из выделений больных туберкулезом и из нетуберкулезного материала различная.
6. Для «слепого» лечения острых вспышек хронических туберкулетиков лучше всего оправдывается хлорамфеникол и эритромицин.
7. Для длительного профилактического лечения больных с хроническим легочным процессом, особенно в раннем детском возрасте, лучше всего оправдался эритромицин.

ИССЛЕДОВАНИЕ СВЯЗИ МЕЖДУ СОДЕРЖАНИЕМ МУКОПОЛИСАХАРИДОВ В ЖЕЛУДОЧНОМ СОКЕ И ВЕЛИЧИНАМИ ЛИПОИДОВ В КРОВИ

Ю. СЕКЕЙ, М. БИХАРИ-ВАРГА и Ш. ГЕРЁ

Авторы проводили исследования на 150 контрольных лиц и 75 больных с различными расстройствами липоидного обмена.

1. В контрольной группе и в группе больных с гиперхолестеринемией связь между содержанием гексозамина, сиалиновой кислоты и мукоида Винцлера и уровнем сывороточного холестерина показывает подобный характер.

2. При заболеваниях, сопровождающихся холестеринемией средние величины содержания гексозамина, связанной белками гексозы, мукоида Винцлера и сиалиновой кислоты в желудочном соке достоверно более низкие, чем в контрольной группе.

3. В желудочном соке исследовавшиеся компоненты МПС оказались самыми низкими в группе больных с артериосклерозом.

4. При исследовании связи между концентрацией одинаковых компонентов МПС, обнаруживаемых в желудочном соке и в сыворотке тех же лиц, содержание гексозамина показало как в группе больных с гиперхолестеринемией, так и в контрольной группе линейную связь, в количестве мукоидов Винцлера в желудочном соке в контрольной группе получалась параболоидная кривая в функции содержания серомукоида.

5. Между кислотностью желудочного сока и его содержанием МПС не удалось выявить связи.

6. В группе артериосклеротиков распределение содержания МПС в стенке желудка не отличается от величин контрольной группы.

ДЕЙСТВИЕ ЛИГИРОВАНИЯ ПЕЧЕНОЧНОЙ АРТЕРИИ НА СОСТАВ ПЕЧЕНОЧНОЙ ЛИМФЫ

Дь. САБО и Ж. МАДЬЯР

После лигирования печеночной артерии содержание белков в печеночной лимфе остается низким и относительная концентрация альбуминов лимфы выше, чем в плазме крови. Следовательно, предположение, что различный состав белков обуславливается тем, что к жидкости, истекающей из синусоидов, состав которой идентичен с составом плазмы крови, примешивается фильтрат, образовавшийся в капиллярной системе с низкой проницаемостью (околожелчные капилляры), не подтверждается.

ИССЛЕДОВАНИЕ СВЕРТЫВАЕМОСТИ КРОВИ У БОЛЬНЫХ ИСТИННОЙ ПОЛИЦИТЕМИЕЙ

Дь. НАДЬ, Л. АСОДИ, И. ХАТВАНИ и Л. АНТАЛ

Авторы анализировали коагулограммы больных истинной полицитемией в фазе обострения болезни, а также при ремиссии, появляющейся под влиянием лечения радиофосфором. В фазе обострения болезни удалось выявить комплексные отклонения, наиболее выраженные в протромбиновом показателе, потреблении протромбина, числе тромбоцитов и инактивации тромбина. В период ремиссии эти изменения по большей части вернулись к норме.

ПЕРЕХОДНЫЕ ЭЛЕКТРОЭНЦЕФАЛОГРАФИЧЕСКИЕ ЯВЛЕНИЯ МЕЖДУ РИСУНКОМ ГЕНЕРАЛИЗИРОВАННЫХ СПАЙК-ВОЛН И ТАК НАЗ. ПРИПАДОЧНЫМИ ГЕНЕРАЛИЗИРОВАННЫМИ ПОВТОРЯЮЩИМИСЯ СУДОРОЖНЫМИ РАЗРЯДАМИ

П. ХАЛАС, Дь. ВЕЛОК, Й. ХИДАШИ и Г. БОЦАН

В целях выяснения патофизиологических причин различий, наблюдаемых между механизмом малого припадка и механизмом большого припадка, сообщаются данные четырех больных, у которых механизмы генерализированных спайк-волн или видоизменения спайк-волн переплетались с генерализированными повторяющимися судорожными разрядами. На основании этих данных можно сделать следующие установления:

1. Во время припадка между электроэнцефалографическими явлениями малого припадка, сопровождающегося со спайк-волнами и большого припадка с повторяющимися быстрыми разрядами можно выявить переходные электроэнцефалографические явления.

2. Подкрепляются наблюдения Гастаута, согласно которым при отдельных формах эпилептической энцефалопатии, показывающих видоизменения спайк-волн, во время сна часто наблюдаются тонические припадки, сопровождающиеся с повторяющимися быстрыми разрядами, со слабыми двигательными и значительными вегетативными явлениями.

3. Сон — как естественный сон, так и эвипановый наркоз —, и по всей вероятности и препараты бензодиазепина, способствуют появлению переходных явлений.

На основе электрографических данных, выясненных до сих пор фактов механизма спайк-волн и неврального механизма повторных быстрых разрядов типа большого припадка (Поллен 1964, Матсумото и Аймоне Марсан 1964), а также интерневронального торможения коллатералей возвратного нерва, играющего решающую роль в ритмических электроэнцефалических явлениях (Экклес 1965), авторы делают попытку создать единую теорию о механизмах большого и малого проиступа и объяснить электрографические и клинические различия, выявляемые в этих формах эпилепсии.

ДИНАМИКА ИЗМЕНЕНИЙ ЭЭГ, ЭДГ И ШИРИНЫ ЗРАЧКОВ У ЧЕЛОВЕКА ПОД ВЛИЯНИЕМ ГЕКСОБАРБИТАЛА

Ф. КАЙТОР и П. ХАЛАС

Авторами исследованы у 17 лиц реакции показателей активности ЭЭГ, ЭДГ и изменений ширины зрачков, вызванные периферическими раздражениями, а также их связь с различными уровнями гексобарбитального наркоза. Постепенное углубление действия барбитурата вызвало в ЭЭГ всех обследовавшихся лиц 3 одинаковых доминантных модификации. Каждая из этих модификаций стала доминантным ответным явлением в различных стадиях наркотического действия. Фаза дремоты является оптимальной для ответа пробуждения (arousal). При поверхностном сне накапливаются двуфазные ответы (синхронизация, присоединяющаяся к десинхронизации), при наркозе средней глубины — модификации типа синхронизации. Для глубокого наркоза характерно отсутствие ответной реакции.

В ходе уменьшения наркоза, обеспеченного раздражением, скопление вышеуказанных ответов ЭЭГ происходит в обратном порядке. Между ЭДГ и активностью пупилломоторной реакцией и изменениями модификаций ЭЭГ наблюдается выраженная связь.

Формальные различия электроэнцефалографических ответов, вызыванные при различных глубинах гексобарбитального наркоза, связь модификаций формы элементов ЭЭГ при тождественной глубине наркоза рассматривается как возможное доказательство дифференцирующей чувствительности к барбитуратам, иерархической организованности (понтобульбарных) систем пробуждения ствола мозга.

ДЕЙСТВИЕ ДИМЕРКАПТО-ПРОПАНОЛА (БАЛ) ПРИ ОСТРОМ ОТРАВЛЕНИИ КРЫС ОРГАНИЧЕСКИМИ СОЕДИНЕНИЯМИ РТУТИ

Ш. БОРДАШ и К. ЛЕХОЦКИ

У крыс при остром отравлении органическими соединениями ртути алкилового, алкокси-алкилового и арилового типа профилактическая и терапевтическая эффективность димеркапто-пропанола (БАЛ) в дозах 3 раза по 20 мг на кг веса тела на протяжении суток, по сравнению с сулемой в качестве контроля, показывает следующий порядок. Эффективность БАЛ и в отношение сулемы ($HgCl_2$) > фенилуксуснокислой ртути (PMA) > этилмеркурхлорида (EMC) > дифенилртути (DPM) > хлорфенилртути (PMC) > метоксихлорид этилртути (MEMC). Если применить димеркапто-пропанол в дозировке 6 раз 10 мг на кг веса тела в течение двух дней, или 12 раз 5 мг на кг веса тела в течение трех дней, то его действие на отравление MEMC более благоприятное и, по вышеприведенному порядку, оно находится между действиями на отравление DPM и PMC.

Исходя из этих результатов, полученных на крысах, авторы придерживаются того мнения, что при отравлениях людей, вызванных вышеперечисленными органическими соединениями ртути, можно рекомендовать осторожное *л* протяжное применение димеркапто-пропанола, это, предположительно даст сравнительно, благоприятный результат.

ГИСТОХИМИЧЕСКОЕ ИССЛЕДОВАНИЕ ИЗМЕНЕНИЙ ДЕГИДРОГЕНАЗЫ ГИДРОКСИМАСЛЯНОЙ КИСЛОТЫ И НЕСПЕЦИФИЧЕСКОЙ ЭСТЕРАЗЫ, НАБЛЮДАЕМЫХ НА КРЫСАХ ПОСЛЕ ОБЛУЧЕНИЯ ВСЕГО ТЕЛА ЖИВОТНЫХ

Г. ВАЛЬТЕР и К. Ю. ШМИДТ

Активность индоксилацетата и дегидрогеназы гидроксимасляной кислоты определялась в 10 органах крыс после облучения всего тела дозой в 650 р и 1950 р гистохимическим методом исследования на протяжении 72 часов. В печени, почках, яичниках, поджелудочной железе, надпочечниках и кишечнике после начальной активации наблюдалось уменьшение ферментативной активности, в отдельных органах даже полное отсутствие ее. В противоположность этому в сердце после облучения дозой в 1950 р отмечалась активация дегидрогеназы гидроксимасляной кислоты. Изменению активности энзима способствует также нарушение расщепления жира. Однако основной причиной следует рассматривать общую активацию обмена веществ, наблюдаемую в начале лучевой болезни. Под влиянием дозы лучей в 1950 р после начальной активации на третий день наблюдается, как правило, полное крушение обмена веществ. При таком поведении дегидрогеназы гидроксимасляной кислоты в отношении цикла жирных кислот следует думать о том, что организм предоставляет дальнейшие радикалы для цикла Кребса. Следует отметить, что головной мозг и селезенка оказались относительно исследуемых ферментов сравнительно резистентными к облучению.

ПРИВЫКАНИЕ И РЕГЕНЕРАЦИЯ В СВЕТЕ ИССЛЕДОВАНИЙ, ПРОВЕДЕННЫХ МЕТОДОМ ЭФФЕКТА СЕРИИ КРИТИЧЕСКОЙ ФУЗИОННОЙ ЧАСТОТЫ

К. АКОШ и М. АКОШ

Авторы выявили существенное сходство между привыканием и эффектом серии критической фузионной частоты (KFFSE). На основе этого сходства полагается, что по крайней мере часть явлений, относящихся к понятию привыкания, аналогична KFFSE. Это означает, что привыкание, по крайней мере частично, явление нагрузки, повторные одинаковые раздражения означают нагрузку для нервной системы. Следовательно привыкание можно с полным правом привести в связь с утомлением.

На основе аналогий между привыканием и KFFSE, применение методики KFFSE может иметь значение при более подробном уяснении и более точном анализе привыкания.

На основе сопоставления «восстановления», связанного с привыканием и действия, оказанного так наз. регенеративным фактором (фактор F_2) KFFSE, можно полагать, что процесс «восстановления» также можно было бы измерять и более точно выяснить.

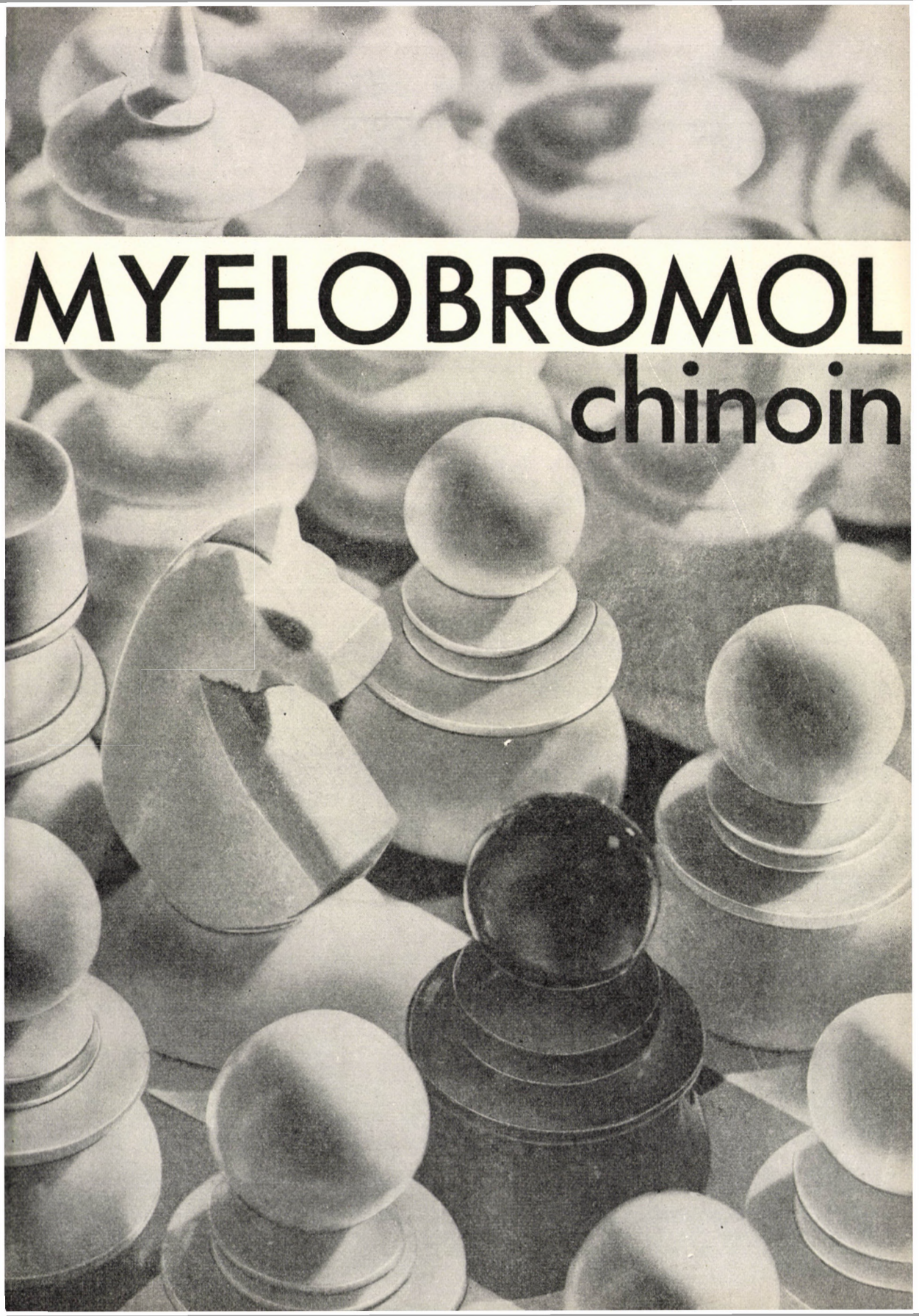
На примере фактора F_2 показывается, каким образом можно при методике KFFSE точно характеризовать цифровыми данными действие регенерации, что, предположительно, можно использовать и в клинической дифференциальной диагностике.

Аналогия между привыканием и KFFSE показывает и то, что последний подвергается значительному влиянию со стороны нервной системы.

ИССЛЕДОВАНИЕ РЕАКЦИЙ ГИПОФИЗАРНО-НАДПОЧЕЧНОЙ СИСТЕМЫ ПОСЛЕ ПОВТОРНОВО ПРИМЕНЕНИЯ РАЗЛИЧНЫХ НЕСПЕЦИФИЧЕСКИХ РАЗДРАЖЕНИЙ

Э. ШТАРК, Й. ФАХЕТ, Г. Б. МАКАРИ и К. МАХАЙИ

Отделъные неспецифические раздражения, повышающие при первом применении уровенъ кортикостерона в плазме, при повторном применении или совсем не повышают или лишъ мало повышают его, тогда как другие раздражения повышают его независимо от часла повторений. Авторы обясняют эти результаты тем, кто различные неспецифические раздражения достигают гипоталамические клетки, производящие CRF, по различным путям, и что активизирующие CRF механизмы определенных стрессоров приспособляются к новторному применению раздражений, в то время как механизмы других стрессоров не адаптируются.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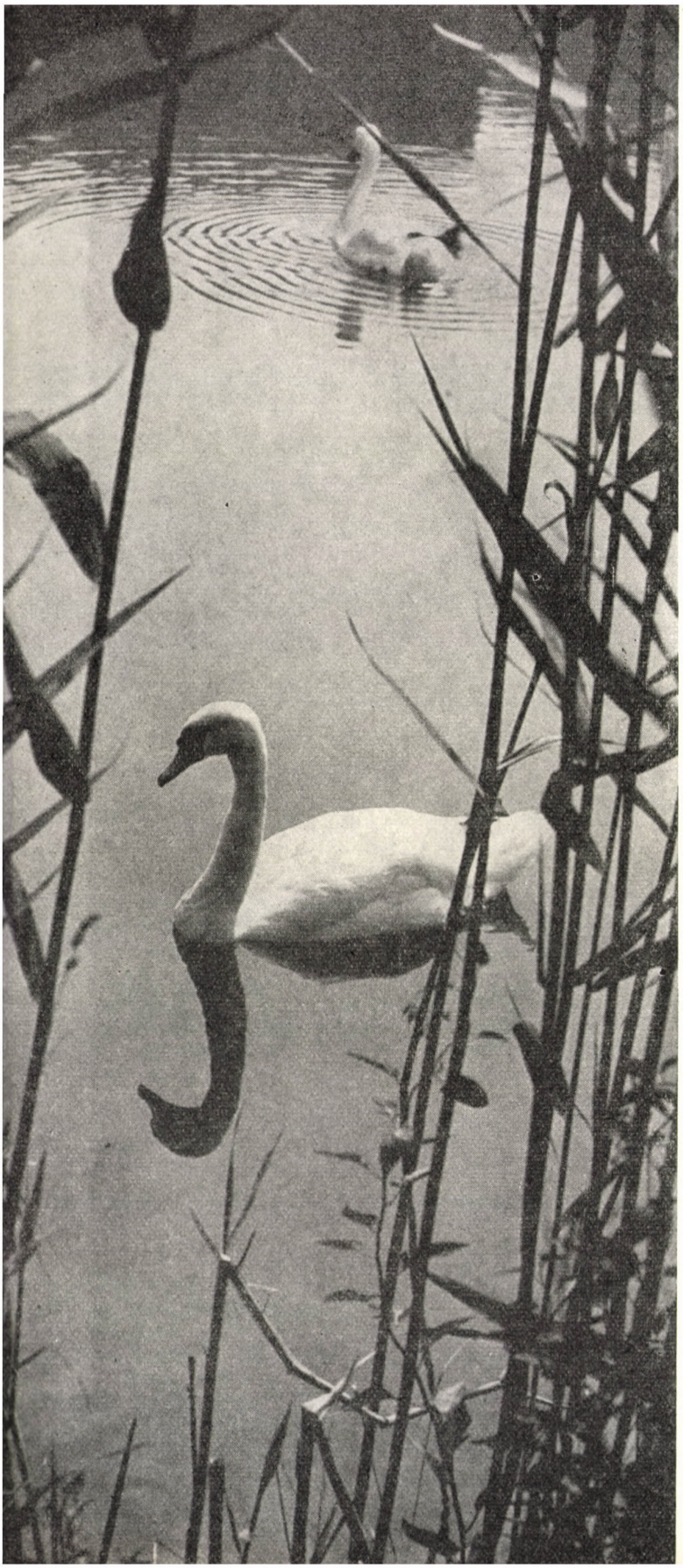

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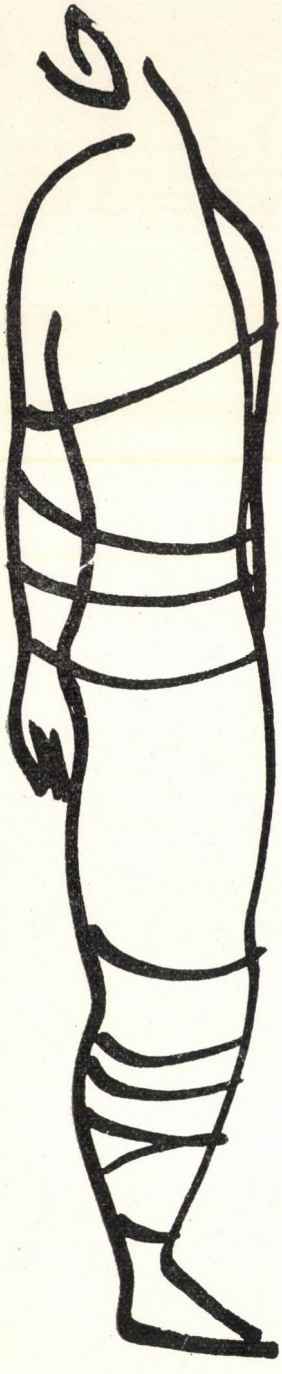

Wirksam:

a) Myotonolytisch bei hypertonischen Zuständen der quergestreiften Muskulatur;

b) Durchblutungsfördernd bei obliterativen Gefässerkrankungen

Hauptindikationen:

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Bürgersche Krankheit, Raynaudsche Krankheit u. a.

II. Durch Spastizität oder Kontrakturen bedingte Zustände
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Information on the Congress can be obtained at the Congress Office, Ist Clinic for Internal Diseases, Mickiewiczova 13, Bratislava, Czechoslovakia

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Von Prof. Dr. O. KLEINSASSER,
Oberarzt der Hals-Nasen-Ohrenklinik der Universität, Köln

Geleitwort von Prof. Dr. L. SEIFERTH, Köln

1968. XI, 128 Seiten, 133 Abbildungen, davon 100 mehrfarbig,
Format 16×24,5 cm, Balacuir DM 65,—

Die Einführung des Operationsmikroskopes in die Laryngologie eröffnet eine Reihe bisher ungenützter diagnostischer und therapeutischer Möglichkeiten. Mit Hilfe des Operationsmikroskopes kann das Kehlkopfinnere nun erstmals binokular mit fast beliebiger Vergrößerung untersucht werden.
Bei der Mikrolaryngoskopie werden eine Reihe von Veränderungen sichtbar, die bisher mit Hilfe des Kehlkopfspiegels oder durch ein Laryngoskop nicht erkennbar waren, so daß nun eine wesentlich genauere Differentialdiagnose der Erkrankung der Kehlkopfschleimhaut möglich wird.
Die Mikrolaryngoskopie dient vor allem auch zur Frühdiagnose des Stimmlippenkarzinoms und zur Feststellung der Ausdehnung von Kehlkopfkrebs. Durch die Anwendung der intratrachealen Narkose mit dünnen Kathetern, Wechseldruckbeatmung, Operationslaryngoskopen und verfeinerten Instrumenten ist es nun möglich geworden, ebenso wie bisher im Ohr oder im Auge, exakte, die Stimmfunktion erhaltende und verbessernde Operationen im Kehlkopf auszuführen. Nach einer genauen Schilde ung der Untersuchungs- und Operationstechnik werden an Hand eines Atlas von 100 mikrolaryngoskopischen Farbbildern die wichtigsten chirurgischen Kehlkopferkrankungen geschildert und deren operative Behandlung dargestellt.

ATLAS DER LAPAROSKOPIE

Von Doz. Dr. K. BECK, Freiburg · Dr. W. DISCHLER, Freiburg · Dr. M. HELMS, Freiburg · Dr. B. KIANI, Freiburg · Priv.-Doz. Dr. K. SICKINGER, Göttingen · Dr. R. TENNER, Freiburg

Herausgegeben von Doz. Dr. K. BECK, Leiter der Gastroenterologischen Abteilung der Medizinischen Universitäts-Klinik, Freiburg

Geleitwort von Prof. Dr. Dr. h. c. L. HEILMEYER, Ulm

1968. ca. 224 Seiten, 385 Abbildungen, davon 369 mehrfarbig,
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Infolge zunehmender technischer Vervollkommnung hat die Laparoskopie inzwischen weite Verbreitung erfahren. Neue Indikationsgebiete wurden erschlossen. Es bestand deshalb ein dringendes Bedürfnis nach einem Atlas, der die Vielzahl der charakteristischen Befunde in naturgetreuer Reproduktion wiedergibt.
Aus einem Archiv von über 10 000 Bildern hat das Team von 6 Autoren 385 Abbildungen (davon 369 mehrfarbig) für den Atlas ausgewählt. Die Bilder sind teils nach Organen, teils nach pathogenetischen Prinzipien folgerichtig geordnet. Die Legenden sind jeweils auf der gegenüberliegenden Seite zu finden, so daß Bild und Text leicht miteinander verglichen werden können. Das Wesentliche eines Bildes wird durch eine kurze Überschrift gekennzeichnet. Die knapp gehaltenen Erläuterungen geben neben der reinen Bildbeschreibung Hinweise auf die praktische Bedeutung der Befunde, diskutieren ihre Differentialdiagnose und enthalten Anmerkungen zur laparoskopischen Technik. Jede Seite steht unter einem Leitwort. Den einzelnen Abschnitten des Buches ist eine kurze Einleitung vorangestellt. Mit Hilfe eines ausführlichen Sachregisters lassen sich die typischen Befunde gut auffinden.



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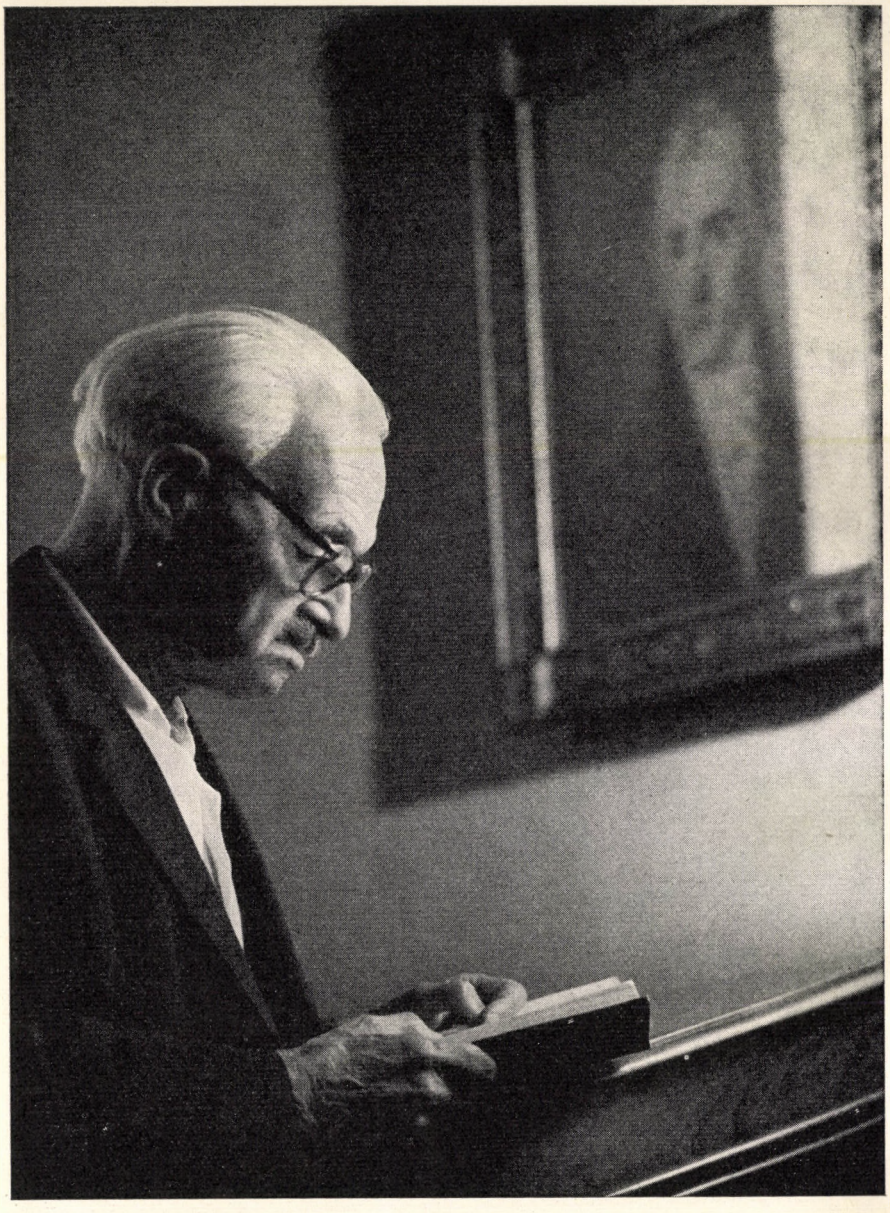

Professor Dr. István Rusznyák

Professor István Rusznyák completes his 80th year of life on January 22nd, 1969. His 70th birthday was celebrated by the 13th volume of *Acta Medica* recording the major turns of Rusznyák's life and outstanding achievements as a great teacher and research scientist. On that occasion, his pupils and coworkers and the entire Hungarian medical profession wished him "*good health and undiminished energy to lead them for many years to come and to impart to them steadfastness and courage in the pursuit of the final aim, human health and happiness*".

It gives us great pleasure that these wishes have come true. The past decade has seen István Rusznyák as keenly active as before both as the President of the Hungarian Academy of Sciences and as Director of the Institute of Experimental Medicine, as well as in the other high offices he holds.

On the solemn occasion we sincerely wish him to retain his comprehensive mind and keen interest which go to produce the true scientist and renders him ageless.

When in 1936 Sándor Korányi had retired, the exciting task of taking over and continuing his activities fell to his pupil, István Rusznyák. Many scientific achievements of István Rusznyák have since become integral parts of medicine.

The Rusznyák school means more than the mere succession of pupils highly trained as investigators and physicians. It means groups of people who represent progressive thinking and humanism.

The part of István Rusznyák's intellectual activity which has been published, will remain linked up with his name. What is not demonstrable with data is the effect he has been, and still is, exerting, both as a scientist and as a science administrator, on the whole of the medical sciences in Hungary, directly as well as through his pupils.

István Rusznyák's name has become inseparable from the Hungarian Academy of Sciences. Twenty years ago he was an outstanding advocator and reorganizer of the country's highest scientific authority in harmony with the changes in the social structure of Hungary. Since then, during his uninterrupted presidency, the sciences in our country have rapidly been moving forward on a broad front. As the Academy's president, István Rusznyák

emphasizes the importance not only of international relations in general but takes every occasion to point at the advantages of joint efforts between nations in seeking the solution of single problems.

István Rusznyák is not only a great figure of the medical sciences in Hungary; the impact of his work and his personal authority are felt far beyond the boundaries of this country. He is corresponding member of the Bulgarian Academy of Sciences, the Academy of Sciences of the Soviet Union, the Czechoslovak Academy of Sciences, the Polish Academy of Sciences, the German Academy of Sciences in Berlin, the Swiss Academy of Sciences, and the Yugoslav Academy of Sciences and Arts.

In recognition of his scientific merits he has been decorated with many honours both at home and abroad.

István Rusznyák has been Editor-in-Chief of Acta Medica since 1959.

The Board of Editors warmly congratulate him on his eightiest birthday wishing him further good health and much success. As a mark of their esteem, they present this issue to the President of the Hungarian Academy of Sciences, the Director of the Institute for Experimental Medicine and the Editor-in-Chief of Acta Medica. It contains articles written for this particular occasion by his present and a few of his past collaborators.

The Board of Editors

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ARTERIO-VENOUS SHUNTS OF THE KIDNEY

By

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(Received April 25, 1968)

A summary is presented of the author's and his collaborators' investigations into the problem of arterio-venous shunts of the kidney. In the first step, it was possible to reproduce the Trueta-type of shunt, parallel with a demonstrable impairment of renal functions including urinary output, CFR and Tm_{PAH}. Subsequent studies based on the corrosion technique furnished morphological proof of the existence of arterio-venous communications. In the latest series of experiments it has been demonstrated that these communications have not only the appearance but also the function of actual shunts.

The problem of the arterio-venous shunts of the kidney has repeatedly been discussed in the literature, but no general agreement has been reached. The possibility of this mechanism, originally suggested by SPANNER (1937, 1937/38) and later by CLARA (1956), has been definitely rejected by KÜGELGEN et al. (1958, 1960).

Interest in the questions of arterio-venous shunts received new impetus from the classical work of TRUETA et al. (1947). Their results obtained in rabbits were confirmed by DANIEL et al. (1952) in other animal species. TRUETA and his colleagues had demonstrated the existence of juxtamedullary shunts in different types of shock, giving rise to a diversion of renal blood flow via the juxtamedullary glomeruli. As a result, cortical ischaemia and medullary hyperaemia arise. The findings of TRUETA et al. and DANIEL et al., though strongly suggestive, were not conclusive enough to be uniformly accepted. The existence of arterio-venous shunts in the kidney still represents a controversial issue.

It is intended here to give a summary of our work of the last two decades by which we have attempted to throw light on the question of renal shunts. We began these investigations in 1948 (GÖMÖRI, FÖLDI, SZABÓ, 1948, 1961) with the aim of adding functional evidence to the findings of TRUETA et al. By excitation of the hilar nerves of the kidney we were indeed able to elicit in dogs a reduction of urinary output, of GFR, of total renal blood flow (C_{PAH}) as well as of maximal tubular excretory capacity (Tm_{PAH}), parallel with a distinct blanching of the cortical area in contrast to the dark red colour of the medullary region, corresponding, on the evidence of benzidine preparations, to cortical ischaemia and medullary hyperaemia. Our findings were consistent

with the existence of shunts as described by TRUETA et al. As a matter of fact, we not only had shown that their results were reproducible in some other way as well, but also that the mechanism of juxtamedullary shunts takes effect at the expense of the renal functions. Our finding raised the possibility of an involvement of similar factors in the renal failure in shock.

Our investigations into the problems of renal arterio-venous shunts were resumed at a later period (1962) on the spur of the finding that in shock C_{PAH} is significantly lower than indicated by the direct estimation of RBF (BÁLINT et al., 1961). It was believed at first that this discrepancy between the direct method and C_{PAH} might be connected with the opening up of shunts, a possibility indicated by our earlier experiments, where the Trueta-type of shunt was clearly associated with impaired renal function including C_{PAH} and C_k. It is clear that the direct measurement of RBF which merely reflects the amount of blood leaving the renal vein regardless of the perfused tissues, defines the total blood flow of the kidney, whereas C_{PAH} is obviously a measure of the blood flow through the renal parenchyma. It was on this basis that we assumed that, as described by TRUETA, arterio-venous shunts might be opening up in the course of shock. In the light of later evidence we had, however, to reconsider this supposition. Although in these conditions C_{PAH} is indeed less than the RBF measured directly from a cannulated renal vein (BÁLINT et al., 1964, GÖMÖRI et al., 1961), the cause of this lies in a retention of PAH by the oliguric kidney. Though these facts have cast considerable doubt on our original supposition, they have given, on the other hand, impetus to further investigations into the question whether the condition of shock is actually associated with shunts as claimed by TRUETA et al.

Closer study of the question was facilitated by the corrosion technique developed by MUNKÁCSI (1957). The procedure consists in injecting an acetone-cyclohexamine solution of PVC into the renal artery of the removed kidney. After the usual corrosion procedure the specimen is examined stereomicroscopically. Our studies (GÖMÖRI et al., 1962) revealed first of all a striking ischaemia in association with traumatic, ischaemic, haemorrhagic or dehydration shock, a finding consistent with our claim ascribing an important role to ischaemia in the pathogenesis of the shock-kidney. While filling of the renal veins was never seen in normal specimens, it was an invariable, distinct finding in all types of shock. The fact that filling of the renal veins was confined to the shock-kidney was taken as an indication of shunts opening up in the course of shock. It must be added that MUNKÁCSI's procedure which involves the use of a 5% solution of PVC leaves the veins empty under normal conditions, the great viscosity of this solution being incompatible with its passage through the capillaries. Therefore, in normal specimens, apart from the major arteries only the afferent arterioles, the glomerules and a small segment of the efferent arterioles are reached, whereas the postglomerular capillaries or veins remain

empty. Thus, filling of the veins in diverse conditions of shock suggested that this must occur in a retrograde way via arterio-venous anastomoses. The foregoing findings were duly reproduced in subsequent studies (GÖMÖRI et al., 1965).

Our following task was to find morphologic evidence in support of the shunt mechanism. As a result of these investigations (MUNKÁCSI et al., 1963) we succeeded in furnishing stereomicroscopic proof of the existence of well-defined arterio-venous communications measuring up to 50μ in diameter. These are scattered over the entire kidney as corticomedullary or intracortical communications and may also be found in the cortex corticis. We were the first to draw attention to the existence of glomerules opening directly into veins (paravenous glomerules) and involving by this fact a potential shunt-mechanism. Our findings were consistent with those of CLARA, SPANNER and TRUETA et al. referred to earlier in this paper.

We had, however, still failed to furnish definite functional proof of the existence of a shunt mechanism. Morphological evidence of arterio-venous communications in the kidney still does not mean that they must be capable of assuming the function of actual shunts under given conditions. We received orientation for further studies into these aspects by the results of CARGILL (1948) who had found that administration of human serum albumin resulted in a reduction of the difference between arterio-venous PAH (PAH-extraction, E_{PAH}). This finding was interpreted by numerous workers including HOMER SMITH (1951) as being due to an opening of arterio-venous shunts, supposed to divert the blood stream from the renal parenchyma to the renal vein. On reproducing these experiments in dogs (GÖMÖRI et al., 1964) we also found a reduction in E_{PAH} and the arterio-venous O_2 difference. Similar results were obtained by the administration of dihydrolazine. Similar results were obtained by ELPERS and SELKURT (1963) in dogs after administration of canine and bovine sera. In their studies Tm_{PAH} was not affected, indicating that E_{PAH} was unrelated to any tubular damage.

In further studies (GÖMÖRI et al., 1965), shock was induced in dogs subsequent to unilateral renal denervation. On the evidence of the corrosion casts it has been found that 1. denervation prevents renal ischaemia; 2. while E_{PAH} of the denervated kidney remained unaffected, its definite reduction was demonstrable in the contralateral kidney with intact nervous supply. After serum albumin administration, denervation failed to prevent the reduction of E_{PAH}, indicating that shock and serum albumin affected E_{PAH} by different mechanisms.

This presumptive evidence awaited confirmation by some functional proof. It was to be furnished by our subsequent studies (GÖMÖRI et al., 1968) in which we made use of certain recent methodological possibilities. By the

use of the electromagnetic flow meter we were able to measure total RBF in the renal vein under more or less physiological conditions, and in SAPIRSTEIN's ^{86}Rb-extraction method modified by HÁRSING and PELLEY (1965) we had found a convenient means for assessing capillary blood flow in the kidney. It is obvious that the difference between total and capillary blood flow represents that fraction of blood flow which bypasses the renal parenchyma; we have termed this "inert" blood flow. In contrast to the control animals where the inert blood flow did not account for more than 5% of RBF, after injection of serum albumin it attained 28% RBF. This inevitably suggested shunts being opened which would account for the increase in the inert blood flow. Looking for other possible causes, we might consider an increase in the medullary fraction of blood flow as a direct effect of serum albumin, since it is known from the studies of REUBI (1948) that there is no medullary extraction of PAH. This conjecture may be, however, left out of account, since administration of serum albumin was found to cause a fall in the ratio of medullary/cortical blood flow from 40.2 to 32.1%. This leaves the opening of arterio-venous shunts as the only plausible explanation of the increase in inert blood flow. HÁRSING et al. (1964) have provided a remarkable counterpart to this evidence by having raised the inert flow from 9.1 to 34.5% by injecting acetylcholine into the renal artery. The interest of this lies in the consistency of the figures despite the use of different methods. Further interest attaches to a recent work of HÁRSING et al. (1967) where it has been demonstrated by the aid of the stop flow method that the ratio between cortical blood flow measured by the ^{86}Rb technique and RBF falls from the normal value of 0.78 to 0.48 and E_{PAH} from 0.75 to 0.52 consecutive to stop-flow. A similar discrepancy between direct RBF measurement and the diameters of the perfused portion of the dog kidney during intra-arterial cineradiography in haemorrhagic shock as revealed by the studies of JIRKA et al. (1961), is likewise consistent with the mechanism of arterio-venous shunts. SHALDON and his colleagues (1963) obtained similar results by means of ^{51}Cr labelled erythrocytes and ^{131}I serum albumin.

All these data definitely indicate that the arterio-venous communications reproducible in the kidney under conditions referred to earlier, represent actual shunts not only in morphological but also in functional respects.

The results of our corrosion studies on human kidneys obtained from subjects who had died from chronic renal disease or uraemia, lend support to similar interpretations (GÖMÖRI et al., 1968). One of the features revealed by these investigations was a recanalisation, the arisal of anastomoses between afferent and efferent arterioles, gradually replacing the degenerating glomerules. This results in the formation of aglomerular vascular structures which ensure the passage of blood to the capillaries supplying the tubules when the glomerules are being gradually put out of function. Though being no actual arterio-venous shunts in the sense of the foregoing definition, they none the

less represent aglomerular communications between the afferent and efferent arterioles. The significance of this lies in our view in the possibility of ensuring tubular blood supply even after destruction of the glomerular apparatus. This accounts for the preservation of some degree of tubular blood supply and for the absence of tubular necrosis in chronic glomerulonephritis associated with widespread glomerular destruction and hyaline degeneration. It also explains the reason why in chronic renal failure the tubular secretion of certain substances such as carbamide, creatinine, phosphorus and potassium, is still preserved, in other words, elimination of these substances exceeds the clearance of inulin.

In the face of this evidence it would be no longer tenable to deny the reality of arterio-venous shunts. We not only have morphological evidence of the existence of arterio-venous communications but also functional proof that reduction of E_{PAH} resulting from the administration of serum albumin reflects an actual bypass of the functioning renal parenchyma. Whether under given pathological conditions, particularly in shock, the morphologically demonstrable arterio-venous communications are capable of assuming the role of actual shunts, awaits further functional evidence.

REFERENCES

BÁLINT, P.: Aktuelle Probleme der Nierenphysiologie. Verlag Volk und Gesundheit, Berlin 1961.

BÁLINT, P., FEKETE, Á., FORGÁCS, I.: Clin. Sci. **26,** 345 (1964).

CARGILL, W. H.: Proc. Soc. exp. Biol. (N. Y.) **68,** 189 (1948).

CLARA, M.: Die arteriovenösen Anastomosen. 2nd ed. Springer, Wien 1956.

DANIEL, P. M., PEABODY, C. N., PRICHARD, M. L.: Quart. J. exp. Physiol. **37,** 11 (1952).

ELPERS, M. K., SELKURT, E. E.: Amer. J. Physiol. **205,** 153 (1963).

FISCHER, A., LÁNG, E., SZÉKELY, J.: Acta med. Acad. Sci. hung. **14,** 179 (1959).

GÖMÖRI, P., FÖLDI, M., SZABÓ, G.: Magy. belorv. Arch. **1,** 283 (1948).

GÖMÖRI, P., FÖLDI, M., SZABÓ, G.: Experientia (Basel) **4,** 278 (1948).

GÖMÖRI, P., FÖLDI, M., SZABÓ, G.: Acta med. Acad. Sci. hung. **17,** 99 (1961).

GÖMÖRI, P., NAGY, Z., JAKAB, I., VAJDA, V.: Acta phys. Acad. Sci. hung. **20,** 379 (1961).

GÖMÖRI, P., MUNKÁCSI, I., NAGY, Z., TAKÁCS, L., KÁLLAY, K.: Acta med. Acad. Sci. hung. **18,** 119 (1962).

GÖMÖRI, P., NAGY, Z., JAKAB, I.: Acta med. Acad. Sci. hung. **20,** 153 (1964).

GÖMÖRI, P., KÁLLAY, K., NAGY, Z., SZABÓ, Z.: Acta med. Acad. Sci. hung. **20,** 159 (1964).

GÖMÖRI, P., NAGY, Z., ZOLNAI, B., JAKAB, I., MÉSZÁROS, A.: Acta med. Acad. Sci. hung. **21,** 197 (1965).

GÖMÖRI, P., HÁRSING, L., KÁLLAY, K., BARTHA, J., DEBRECZENI, L.: Orvostudomány (In press).

GÖMÖRI, P., MUNKÁCSI, I., SZALAY, E., VARGA, I., ZOLNAI, B.: Acta morph. Acad. Sci. hung. (In press).

HÁRSING, L., KÖVÉR, GY., MÁLYUSZ, M., TÓTH, G., HARZA, T.: Pflügers Arch. ges. Physiol. **281,** 346 (1964).

HÁRSING, L., PELLEY, K.: Pflügers Arch. ges. Physiol. **285,** 302 (1965).

HÁRSING, L., SZÁNTÓ, G., BARTHA, J.: Amer. J. Physiol. **213,** 935 (1967).

JIRKA, J., GANZ, V., FENCL, V., CORT, J. H., TRAVNIČEK, R.: Lancet **2,** 692 (1961).

KÜGELGEN, A. V., OTTO, K. J., KUHLO, B., KUHLO, W.: Die Gefäßarchitektur der Niere. Zwangl. Abh. Geb. norm. path. Anat. H. 5. Thieme, Stuttgart 1958.

KÜGELGEN, A. V., PASSARGE, E.: Z. Anat. Entwickl. Gesch. **122**, 86 (1960).
MUNKÁCSI, I.: Z. wiss. Mikr. **63**, 352 (1957).
MUNKÁCSI, I., NAGY, Z., TAKÁCS, L., KÁLLAY, K., GÖMÖRI, P.: Rev. Canad. Biol. **22**, 353 (1963).
REUBI, F.: Helv. med. Acta **25**, 516 (1958).
SHALDON, S., SILVA, H., LAWSON, T. R., WALKER, J. G.: Proc. Soc. exp. Biol. (N. Y.) **112**, 359 (1963).
SMITH, HOMER, W.: The Kidney. Oxford University Press, New York 1951.
SPANNER, R.: Klin. Wschr. **16**, 1421 (1937).
SPANNER, R.: Verh. Anat. Ges. (Jena) **45**, 81 (1937—38).
TRUETA, J., BARCLAY, A. E., DANIEL, P. M., FRANKLIN, K. J., PRICHARD, M. L.: Studies of the Renal Circulation. 2nd ed. Blackwell, Oxford 1948.

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NEW ADVANCES IN THE FIELD OF ANDROGENIC STEROIDOGENESIS OF THE HUMAN SKIN

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On the basis of the working hypothesis that the cause underlying hirsutism was to be sought for in the enzyme systems of the skin and its accessory structures, the compounds of sterane-structure have been studied in the skin and hairs. It has been shown earlier that in human skin Zimmermann chromogens are present, one-fifth of which proved to be ketonic Zimmermann chromogen. In this connection a significant difference was found between hairy and hairless skin extracts, in favour of the former. In human hairs, mainly in the hairs of the armpit, a number of 17-ketosteroids and considerable amounts of dehydroepiandrosterone were found. By histochemical methods Δ^5-3β-hydroxy steroid dehydrogenase activity could be shown in human skin.

1. By various soaking methods, without digestion steroids could be extracted from the hairs.

2. In the hairs most 17-ketosteroids are present in the form of sulphate ester.

3. Human skin was found to metabolize intensively radioactive dehydroepiandrosterone.

4. Male skin synthesized two and a half times as much testosterone and twice as much androsterone as the female skin, calculated for 1 g skin. Androst-4-ene-3,17--dione is synthesized at a similar rate by male and female skin. Thus, not only the endocrine glands, but also the skin is capable of synthesizing steroids of high biological activity from biologically less active ones.

5. The skin is converting dehydroepiandrosterone to dehydroepiandrosterone sulphate.

6. Pubic skin from an agonadal man was also capable of synthesizing from dehydroepiandrosterone androsterone of high radioactivity and testosterone of low radioactivity. So far, 11 radioactive metabolites could be identified in the incubate.

7. On incubating androstenedione, androsterone and testosterone were formed. In normal skin, the two compounds are produced in roughly the same amounts.

8. In hirsute patients, the skin produces twice as much of androsterone as of testosterone, i.e. the ratio is reversed. The sum of the two compounds is higher in hirsute than in normal females.

9. Human armpit sweat was found to contain considerable amounts of 17-ketosteroid sulphate ester, mostly dehydroepiandrosterone and androsterone sulphate.

Enzymopathies of the skin are assumed to be involved in some cases of idiopathic hirsutism.

We began to study the problem of hirsutism more than a decade ago. Hirsutism, a symptom manifesting itself at the periphery, may have several causes. Beside the forms of known aetiology such as adrenocortical, hypophysial and ovariogenic hirsutism, in almost half of the cases it is unknown what induces the growth of hairs. Such cases are called idiopathic, a term which conveys nothing and in these instances our diagnostic methods are of no aid (JULESZ et al., 1963; FAREDIN et al., 1964).

According to our working hypothesis, hirsutism is due to changes in the enzyme systems of the skin or its accessory structures, and will develop in the female around whose hair follicles the androgen : oestrogen ratio shifts in the direction of the androgens. It is possible that the androgenic steroids produced in the adrenal cortex or elsewhere reach the hair follicles in increased amounts. It is possible that the androgens are bound stronger at the periphery, or the target organ, in the present case the hair follicle, which is more sensitive to androgens. It is also possible that the shift in favour of the androgens is a result of a reduced production or faster breakdown of oestrogens. We have suggested the possibility that, at least in part, androgens are produced in the skin. This seemed plausible in view of the presence in the skin of cholesterol, the principal steroid precursor.

On the basis of this working hypothesis we began to study the various sterane-structure compounds of the skin and of the various kinds of hair.

First, it was shown that in the human skin Zimmermann chromogens are present of which about 1/5 proved to be ketonic (JULESZ, 1964; JULESZ, 1965a, b; JULESZ et al., 1966). Significant differences were found between hairy and hairless skin extracts, in favour of the former. This had induced us to extend our studies to the hairs. The results showed that human hairs, especially the axillary hairs, contained several 17-ketosteroids, mainly dehydroepiandrosterone (DHA). This androgenic steroid was identified by chromatography and different colour reactions.

At the same time, we studied the presence in human skin and hairs of cholesterol, the principal precursor of steroids. Significant quantities of it were found in both the skin and hair extracts and at concentrations significantly above normal in hairy skin, in axillary hairs and in the virile-type hairs of hirsute females.

The question arose whether the 17-ketosteroids are merely stored in the skin, or at least part of them was synthesized there. Histochemical studies (JULESZ, 1965b) revealed Δ^5-3β-hydroxy steroid dehydrogenase activity in

Table I

Extraction of 17-ketosteroid content

No.	Hairs, mg	Extracting solution	Extracted 17-KS, μg in subsequent extracts					Total
			Duration of extraction, hours					
			2	4	18	24	48	
1	114	Distilled water	9.6	2.8	7.6	2.4	18.8	41.2
2	113	0.5 N NH_4OH	23.6	10.4	13.8	11.0	7.2	66.0
3	112	0.5 N NH_4OH + ether	36.8	8.6	11.4	5.6	12.4	74.8
4	115	2.0 N NH_4OH	35.6	10.4	14.6	8.0	11.4	80.0

human skin. The presence of this enzyme system was confirmed by the fact that we could demonstrate in hairs androst-4-ene-3,17-dione and androstane--3,17-dione. Thus, androgenic steroids may be synthesized in the skin and its appendages.

In further experiments (JULESZ, 1968) it was determined in what form the demonstrated DHA was present in the hairs. Since the crude chemical treatments used initially (alkaline digestion + hydrochloric acid hydrolysis) could have played a role in the formation of the demonstrated androgenic steroids, to eliminate the possibility of producing artefacts the method for recovering steroids had to be refined. By various soaking methods we have succeeded in extracting them from the hairs without destroying the latter. It was found that soaking the hairs for 10 days in 2 N ammonium hydroxide and ether, almost 100% of their 17-ketosteroid content could be recovered. As seen in Table I, soaking in 2 N NH_4OH for 4 days yielded 80 μg of 17-ketosteroids, then ether was added, which increased the yield to 94.4 μg, i.e. to 821 μg per 1 g of hairs of ketosteroid. In hairs subjected to digestion following soaking, practically no 17-ketosteroid could be demonstrated.

Thin-layer chromatography of the water phase extracts (Silicagel-G and Al_2O_3-G Sarfaty—Lipsett solvent system: methanol, ammonia and ethyl-acetate, 5 : 5 : 1) proved that most of the 17-ketosteroids are present in the hairs in the form of sulphate ester. As seen in Fig. 1, on the right the authentic steroids were run. DHA-glucuronide (DHA-G) stayed at the start point, DHA-sulphate (DHA-S) ran at medium height, while free DHA moved almost with the front. The left stripe shows the hair extract, containing almost exclusively 17-ketosteroid sulphate esters (17-KS-S).

Thin-layer chromatography following sulphuric acid solvolysis of 17-ketosteroid-sulphates proved (Fig. 2) that the hairs contained beside the large amount of DHA also a small quantity of androsterone (A-S). The left chromatogram shows the authentic A and DHA. Next to it are the two steroids of the extract of the hairs. There is more DHA and little androsterone (TÓTH et al., 1967).

from axillary hairs

	Extracted 17-KS, μg in subsequent extracts			17-KS, μg in hairs following extraction and alkaline digestion	Total 17-KS, μg in hairs,	Total 17-KS, μg/g in hairs
	Duration of extraction, hours		Total			
	48	48				
Extraction continued with 2.0 N NH_4OH + ether	32.2	12.0	44.2	4	89.4	784
	11.8	5.6	17.4	2	85.4	756
	6.8	2.2	9.0	1.5	85.3	762
	11.0	2.4	13.4	1	94.4	821

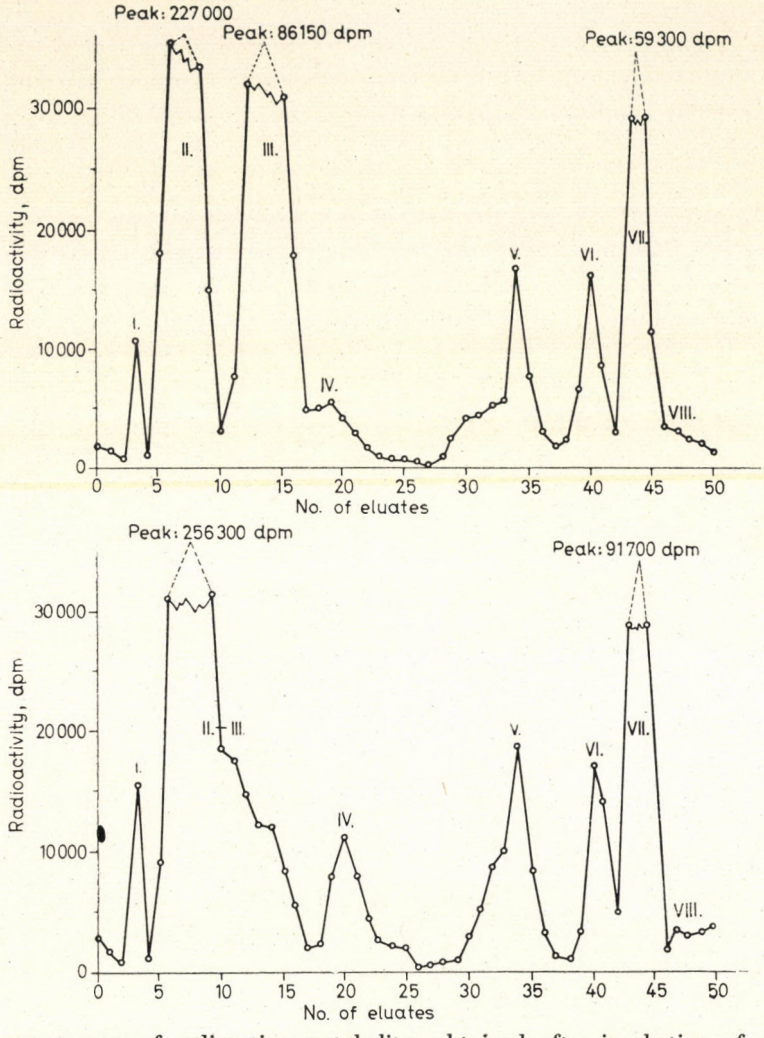


Fig. 4. Chromatogram of radioactive metabolites obtained after incubation of abdominal skin slices from a normal female (above) and male (below) in the presence of [4-^{14}C]-DHA. (Column chromatography: 3 g Al_2O_3, Brockmann III/IV activity)

The ether extract obtained by the soaking method contained the free 17-ketosteroids present in the hairs (Fig. 3).

In the left stripe the authentic steroids ran in the following downward sequence: androstane-dione, androstene-dione, A and DHA. The right stripe shows beside androstane-3,17-dione and androst-4-ene-3,17-dione, free A and DHA in the extract of hairs.

The question arose whether the androgenic steroids demonstrated for the first time by us as occurring in the skin and hairs had been transported there by the blood and then stored, or whether they were synthesized there. The problem was decided by incubation experiments.

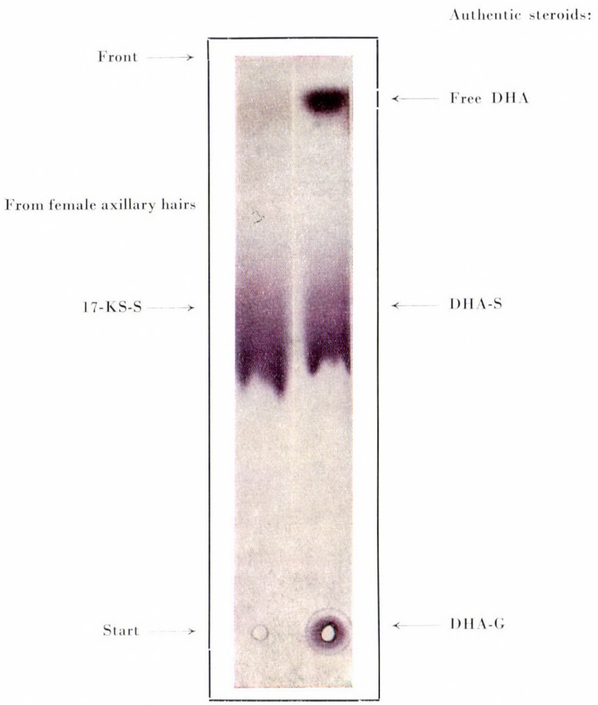


Fig. 1. 17-ketosteroid sulphates isolated from female axillary hairs [Al_2O_3-G thin-layer; Sarfaty—Lipsett solvent system (1966). Detection by Zimmermann reaction]

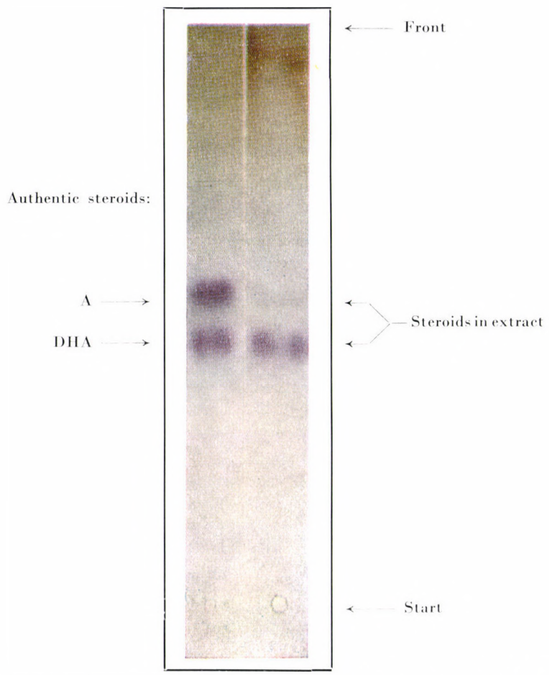


Fig. 2. Androsterone (A) and dehydroepiandrosterone (DHA) liberated from the 17-ketosteroid sulphates of female axillary hair. (Al_2O_3-G thin-layer; solvent system "C". Detection by Zimmermann reaction)

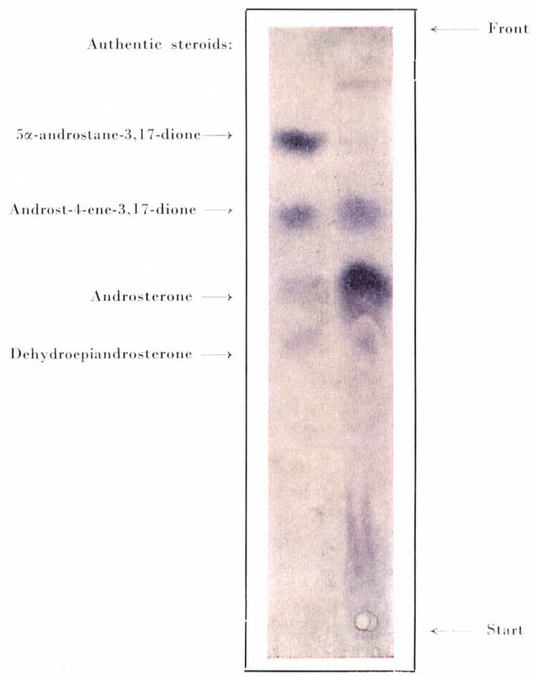


Fig. 3. Free 17-ketosteroids isolated from female axillary hair. (Al_2O_3-G thin-layer; solvent system "C"; detection by Zimmermann reaction; extraction by the soaking technique)

Removed from the living body, under suitable conditions the skin continues breathing and consuming sugar for hours. There is astonishingly scarce evidence pertinent to this. According to the investigations of JULESZ, Jr. (1965), oxygen consumption by the human skin under sterile conditions is one order of magnitude lower than it was reported some decades ago, and the skin was found to continue breathing for at least 8 hours in Krebs–Ringer-glucose or Tyrode solution at pH= 7.2–7.4.

Under such conditions, there was a possibility for incubation experiments. Slices of skin were incubated with radioactive steroids [4-^{14}C]-DHA and [4-^{14}C] -androstene-dione in Krebs–Ringer-phosphate-glucose medium at 37°C for 5 hours. To the incubating medium different co-enzymes, (DPN, TPN and ATP) were added at 10^{-3} M concentration. The radioactive steroid extract obtained after incubation was separated according to polarity into different groups on an alumina column, then the steroid groups so separated were subjected to paper or thin-layer chromatography. For identification, the isolated steroid metabolite and two of its derivatives (acetylated and oxidized, or reduced) were tested for specific activity. When the 3 specific activities thus obtained varied by less than 10%, the isolated steroid was considered identified or radiochemically pure.

This procedure served not only as a qualitative, but also as a highly sensitive quantitative method. It was suitable, of course, also for drawing conclusions from the radioactive metabolites in the medium as to what kinds of enzyme system were present in the skin.

In Fig. 4 above are the results for a normal female, below those for a normal male, showing the chromatograms of the radioactive metabolites obtained from an alumina column following incubation of slices of abdominal skin in the presence of [4-^{14}C]-DHA. The ordinate shows the activity in dpm of the single eluates, the abscissa their serial number. The numbered peaks of the chromatogram mean steroid groups of the same polarity, from which the single steroids were then identified by further paper and thin-layer chromatography (FAREDIN et al., 1968).

Peak I contains the apolar androstane-3,17-dione. Most of peak II is unchanged substrate, in our case [4-^{14}C]-DHA and a small part of it is androst--4-ene-3,7-dione formed of it. In peak III, we find epiandrosterone and a number of unknown metabolites. We attribute particular importance to the steroids in peak IV, because it contains testosterone and androsterone, i.e. the two principal virilizing steroids. Peaks V to VIII contain the highly polar radioactive metabolites (7-keto-DHA, 16 α-OH-DHA, androst-5-ene-3β, 17β--diol, 7β-OH-DHA and 7α-OH-DHA).

Fig. 4 indicates clearly that the human skin was intensively metabolizing the radioactive DHA. According to our preliminary investigations, steroids of androgenic activity may be expected to occur in peaks I to IV. We have

Table II

Steroids isolated and identified after incubation with [4-^{14}C]-dehydroepiandrosterone of skin slices from normal female and male

Steroids isolated	"Cold" carrier added, μg	Chemical reaction	Steroid derivative	Chromatographic system	Specific activity, dpm/μM	Total conversion, dpm/g	Percentage conversion
			Female skin				
Androst-4-ene-3,17-dione	253	—	Androst-4-ene-3,17-dione	Vr. 1	17 814	16 332	1.45
		$NaBH_4$ reduction	Testosterone	Vr. 2	18 472		
Testosterone	295	—	Testosterone	Vr. 2	1 730		
		Acetylation	Testosterone acetate	Vr. 6	865	885	0.07
		CrO_3 oxidation	Androst-4-ene-3,17-dione	Vr. 1	922		
Androsterone	296	—	Androsterone	Vr. 2	14 232		
		Acetylation	Androsterone acetate	Vr. 6	15 393	15 729	1.40
		CrO_3 oxidation	Androstane-3,17-dione	Vr. 1	15 103		
			Male skin				
Androst-4-ene-3,17-dione	253	—	Androst-4-ene-3,17-dione	Vr. 1	19 704		
		$NaBH_4$ reduction	Testosterone	Vr. 2	19 217	17 430	1.55
Testosterone	295	—	Testosterone	Vr. 2	3 634		
		Acetylation	Testosterone acetate	Vr. 6	2 134	2 068	0.18
		CrO_3 oxidation	Androst-4-ene-3,17-dione	Vr. 1	2 019		
Androsterone	296	—	Androsterone	Vr. 2	33 227		
		Acetylation	Androsterone acetate	Vr. 6	30 874	29 994	2.67
		CrO_3 oxidation	Androstane-3,17-dione	Vr. 1	29 442		

therefore studied the materials in these peaks with particular attention. I should like to point out again that peak IV contained the two principal androgenic steroids, testosterone and A. If we compare the female and male peaks IV, the difference in favour of the male skin is obviously significant. Quantitative assays (Table II) revealed that the male skin is capable of synthesizing 2.5 times more testosterone and twice more A than the female skin, as calculated for 1 g of skin. At the same time, it is also visible that both kinds of skin can synthesize androst-4-ene-3,17-dione with the same radioactivity one of the main precursors of these two androgenic steroids.

The steroids demonstrated in peaks I to IV are shown in Fig. 5. The substrate is DHA (on the left), of which the enzyme Δ^5-3β-hydroxy steroid dehydrogenase produces androst-4-ene-3,17-dione, and this key compound is converted by 17-carbonyl hydrogenase to testosterone, through the intermediary 5α-androstane-3,17-dione, to A, in response to 3-carbonyl hydrogenase.

These investigations proved that not only the endocrine organs, but also the periphery, so for instance the skin, is capable of synthesizing, from biologically less active ones steroids of high biological activity. The difference between the female and male human skin is not qualitative, but quantitative.

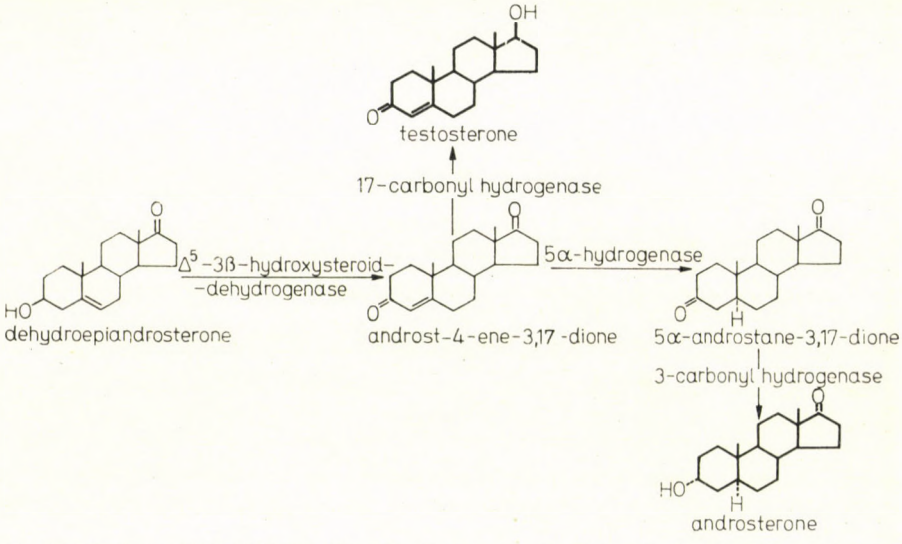


Fig. 5. Metabolism of [4-^{14}C]-dehydroepiandrosterone in abdominal skin slices of normal female and male

These investigations revealed in addition that the skin can convert DHA to DHA-S, thus it has a marked DHA-sulphokinase activity. These data have confirmed the results of the 17-ketosteroid assays of hairs (FAREDIN et al., 1968); the significance of this will be dealt with later.

Identical experiments were made with the pubic skin slices from an agonadal man. As shown in Fig. 6, this skin, too, produced A of significant radioactivity (peak IV) from DHA (FAREDIN et al., 1967).

Testosterone with low radioactivity was also present in this skin. Peak IV resembled that found in females. Fig. 7 shows that this skin contained a number of enzyme systems capable of converting DHA into other steroids.

From the incubate we could identify 11 radioactive metabolites and the hypothetical routes of the formation of these metabolites are shown, furnishing the basis for further studies.

On the grounds of our incubation studies with DHA there was reason to assume that androst-4-ene-3,17-dione had a key role in the synthesis of androgens. It was thought therefore important to carry out incubation experiments with androstene-dione. Their result is shown in Fig. 8, for the abdominal skin of a normal female above, and for that of a female with adrenocortical hirsutism below (FAREDIN et al., 1968).

Several metabolites were formed when androstene-dione was used as the substrate. Androsterone and testosterone were again found in peak IV; their radioactivity (Table III) was nearly the same in normal skin.

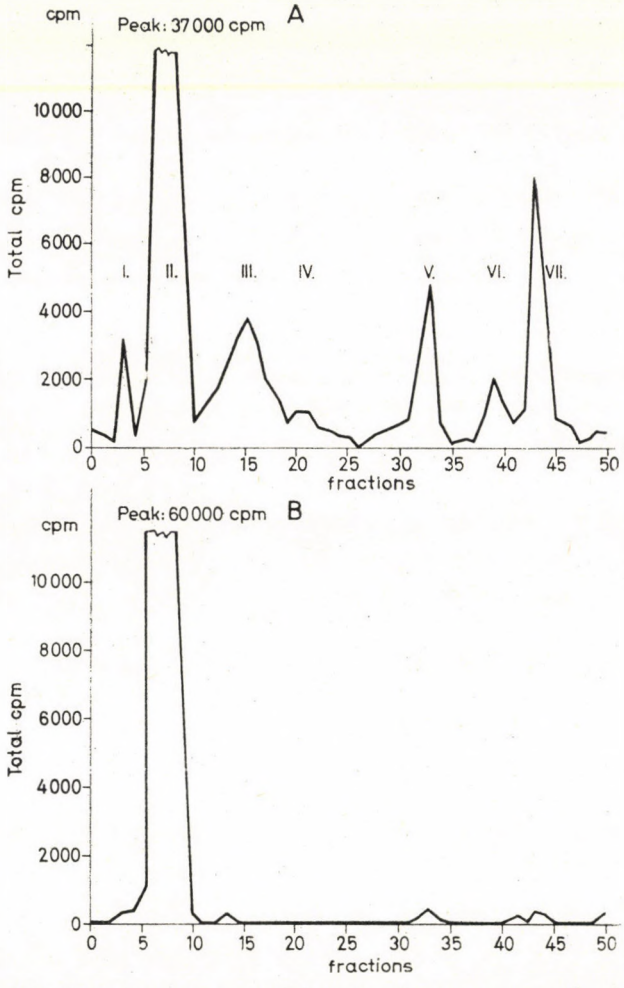


Fig. 6. Pubic skin slices from an agonadal male. Chromatogram of radioactive metabolites obtained following incubation with [4-^{14}C]-DHA. (Column chromatography: 3 g Al_2O_3, Brockmann III/IV activity) — A) Incubation of pubic skin slices from a male with agonadism with [4-^{14}C]-DHA — B) Incubation of [4-^{14}C]-DHA without skin slices

In the skin of a patient with hirsutism the radioactivity of A was double that of testosterone. The sum of the two was higher in the hirsute than in the normal female.

Thus, the human skin contains several enzyme systems capable of synthesizing and converting androgenic steroids.

The next question was, how do the androgenic steroids demonstrated in the hairs (DHA, androsterone) enter the hairs from the skin. The fact that these steroids could be eluted with aqueous solutions indicated that the water-soluble steroid sulphates could penetrate into the hairs from the surface and

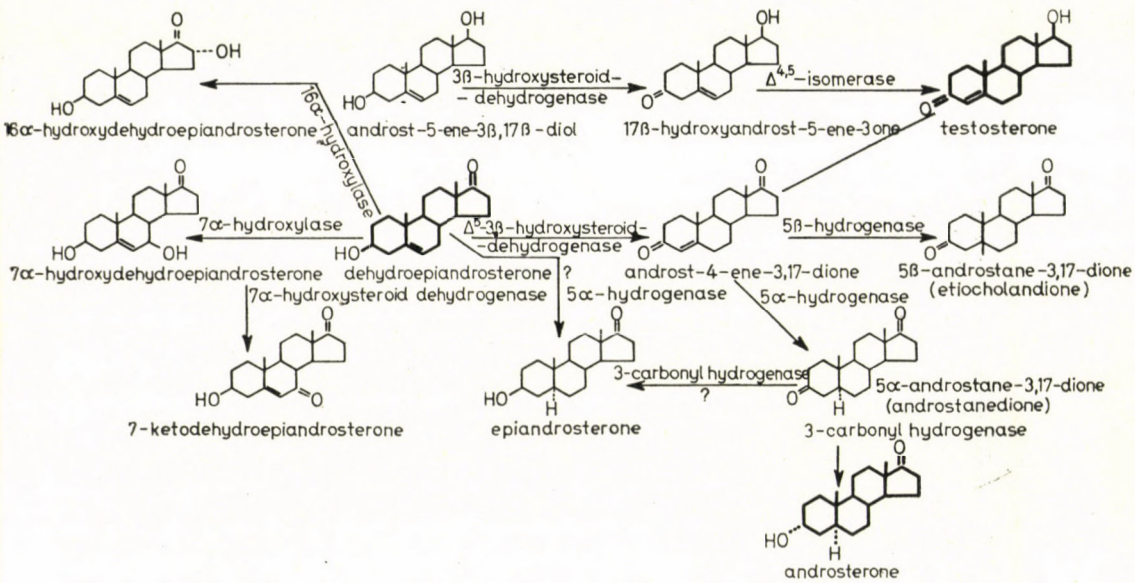


Fig. 7. Metabolism of [4-^{14}C]-dehydroepiandrosterone in pubic skin slices from an agonadal male

that the steroid transfer from skin to the hairs was effected by sweat. Therefore we have undertaken to study the androgenic steroids of sweat.

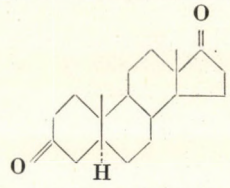
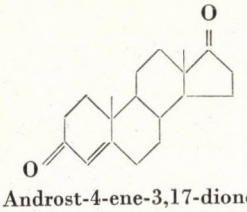
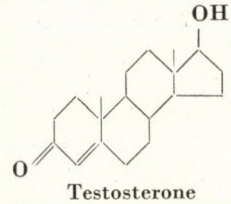
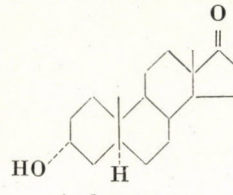
As visible in Fig. 9, the human axillary sweat contains a considerable amount of the water-soluble 17-ketosteroid sulphate ester.

These tests were made on aluminium oxide thin layer, on which the authentic DHA-glucuronide remained at the start point, DHA-S and A-S ran in the middle of the chromatogram and free DHA near the front. The spot of sweat extract developed with the Zimmermann reaction was at the level of the authentic sulphate esters.

Aluminium oxide thin-layer chromatography following solvolysis (sulphuric acid hydrolysis) of sulphates yielded much DHA and little A. These steroids were identified with radioactive DHA and A. In Fig. 10, on the left

Table III

Steroids isolated and identified from incubated
The abdominal skin slices were incubated in

Fraction No.	Steroids isolated	Chemical reactions	Steroid derivatives
I	 5α-androstane-3,17-dione	— — 1/2 part $NaHB_4$ reduction CrO_3 oxidation	Free steroid Free steroid Free steroid 5α-androstane-3,17-diol 5α-androstane-3,17-dione
II	 Androst-4-ene-3,17-dione	— — $NaHB_4$ reduction	Free steroid Free steroid Testosterone
III	 Testosterone	— — Acetylation CrO_3 oxidation	Free steroid Free steroid Testosterone acetate Androst-4-ene-3,17-dione
IV	 Androsterone	— — Acetylation CrO_3 oxidation	Free steroid Free steroid Androsterone acetate 5α-androstane-3,17- dione

* Quantity of "cold" carrier steroid (μg) in normal female skin
** Quantity of "cold" carrier steroid (μg) in hirsutism

was run the extract obtained after solvolysis, on the right the spots of authentic steroids are visible.

All these appeared to prove that sweat contains the same steroids which were found in the hairs, where they might arrive by the following route. The main precursor, cholesterol, is in the sebaceous glands, thus the androgenic steroids are synthesized partly there, and partly in the sudoriferous glands. In response to sulphokinase these become water-soluble and reach the outer

skin slices from normal and hirsute females
vitro with [4-^{14}C]-androst-4-ene-3,17-dione

"Cold" carrier steroid added, μg	Chromatographic system	Normal female Steroid identified			Hirsute female Steroid identified		
		S. A. dpm/μg	total radioactivity		S. A. dpm/μg	total radioactivity	
			dpm/g skin	per cent		dpm/g skin	per cent
243	Vr—1b	154.00			156.75		
	Vr—1a	141.00			145.15		
	Vr—"A"	138.15			152.55		
	Vr—"E"	—	214 400	9.66		280 800	12.65
	Vr—1b	149.58			157.35		
212	Vr—"C"	270.60			328.80		
	Vr—1a	275.90	1 096 000	49.37	318.10	926 000	41.74
	Vr—2	268.09			335.23		
288* 337.5**	Vr—2	51.26			31.70		
	Vr—"D"	46.33			34.04		
	Vr—1	43.60	50 640	2.28	27.96	38 864	1.75
	Vr—1	41.92			29.20		
296* 328**	Vr—2	55.30			100.90		
	Vr—"C"	52.36			92.41		
	Vr—"A"	45.63	57 120	2.57	90.20	115 600	5.20
	Vr—1	46.80			95.00		

surface of the skin and the hairs. The latter being hygroscopic, the steroids may penetrate into them.

Thus far, new facts have been presented concerning the physiology and pathophysiology of the skin. I shall not venture onto the slippery ground of speculations, but I feel justified in asking whether in addition to their theoretical significance our experiments may yield practical conclusions. The results of the incubation experiments seem to warrant an answer in the affirmative.

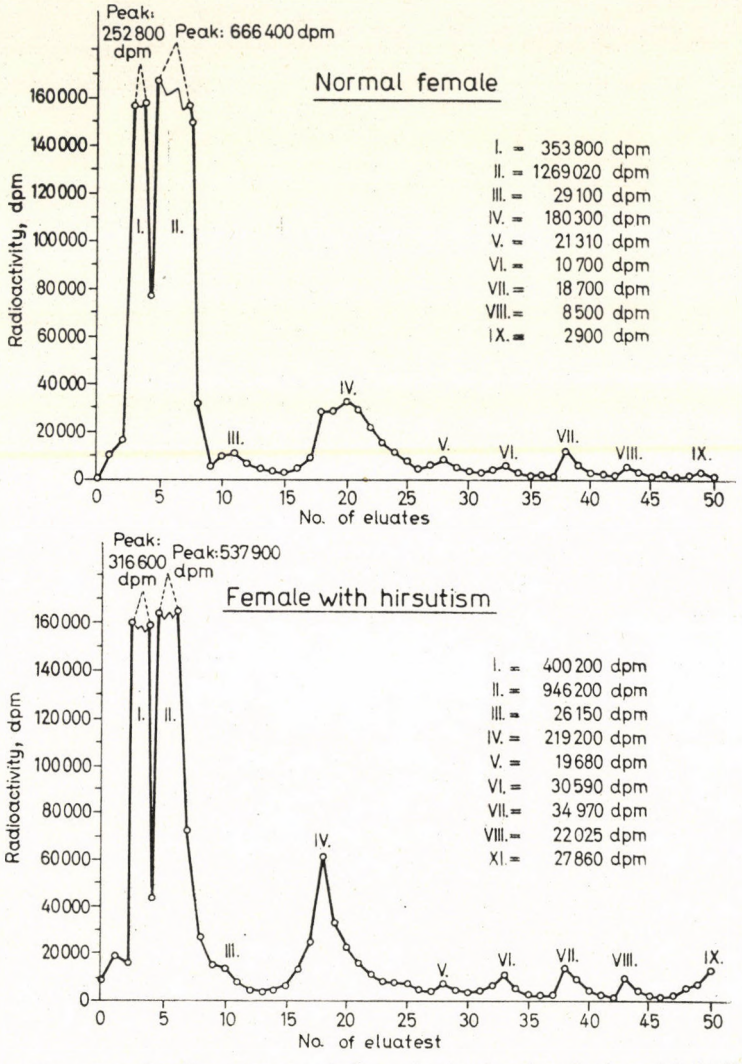


Fig. 8. Chromatograms of radioactive metabolites obtained on incubation with [4-^{14}C]-androst-4-ene-3,17-dione in abdominal skin slices from normal and hirsute females. (Column chromatography: 3 g Al_2O_3, Brockmann III/IV activity)

Our hypothesis has been confirmed by our finding in the hairs of a hirsute female spots of two such steroids which could not be demonstrated in normal hairs.

Being a clinician, my attention is focussed on therapeutic possibilities and I hope that chemists and pharmacologists will soon find the means of blocking certain dermal enzymes which are bringing about hirsutism.

In this paper I wished to emphasize the importance of the periphery in endocrinology on the basis of many decades of a collective work in which the

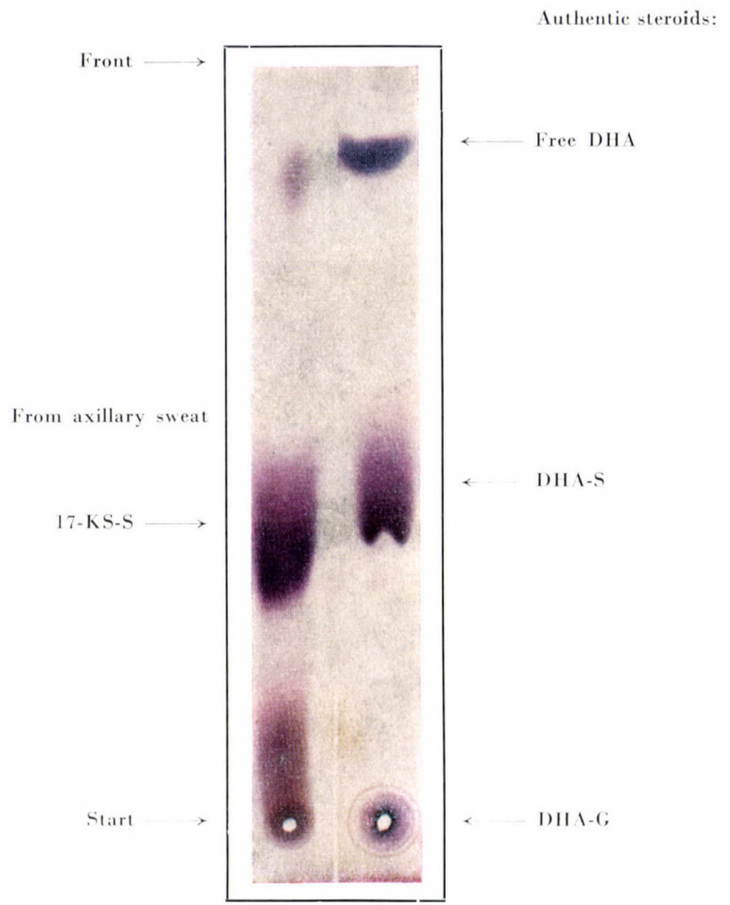


Fig. 9. 17-ketosteroid sulphates isolated from axillary sweat. [Al_2O_3-G thin layer: Sarfaty—Lipsett solvent system (1966), detection by the Zimmermann reaction]

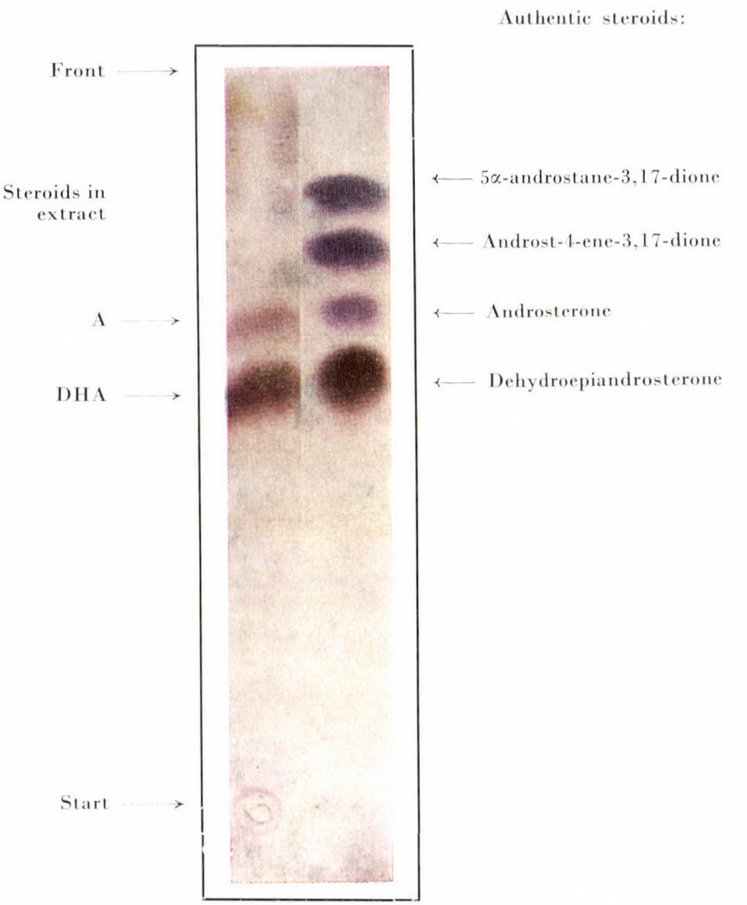


Fig. 10. Androsterone (A) and dehydroepiandrosterone (DHA) liberated from the 17-ketosteroid sulphates of axillary sweat. (Al_2O_3-G thin-layer: solvent system "C". Detection by Zimmermann reaction)

formulation of a hypothesis on grounds of theoretical considerations is not sufficient, since it calls for such chemical knowledge and methodical skill which cannot be possessed by just one individual, and also for such instruments which allow the complicated isolation and identification techniques to be carried out. In this field the clinician will be able to reach new results only when he is aided in his work by such prominent chemists as my collaborators Dr. I. FAREDIN and I. TÓTH, and a histochemist like Miss Dr. E. HORVÁTH.

Our work was supported by generous grants from the Scientific Health Council and from the Hungarian Academy of Sciences. Personally, most of my thanks and gratitude are due to Professor I. RUSZNYÁK, the outstanding research worker, who has followed my modest career from my student years, trusting, encouraging and criticising me. It is a great pleasure to dedicate him this work, a faint reflexion of my gratitude, as a gesture of admiration and respect, on his 80th anniversary.

LITERATURE

FAREDIN, I., SZARVAS, F., TÓTH, I., DÁVID, M. A., JULESZ, M.: Acta med Acad. Sci. hung. **20,** 327 (1964).

FAREDIN, I., FAZEKAS, Á. G., KÓKAI, K., TÓTH, I., JULESZ, M.: Europ. J. Steroids **2,** 223 (1967).

FAREDIN, I., KÓKAI, K., TÓTH, I., FAZEKAS, Á. G., JULESZ, M.: Europ. J. Steroids 1968, In Press.

FAREDIN, I., TÓTH, I., FAZEKAS, Á. G., KÓKAI, K., JULESZ, M.: J. Endocr. **41,** 777 (1968).

FAREDIN, I., TÓTH, I., FAZEKAS, Á. G., KÓKAI, K., JULESZ, M.: In press.

JULESZ, J.: Meddig él az emberi bőr? Az emberi bőr oxygénfogyasztása és aerob glykolysise. Tudományos diákköri pályamunka. 1965. Sub auspiciis rei publicae popularis doktorrá avatása alkalmával 1968-ban bemutatott tanulmány. In the press.

JULESZ, M.: Orv. Hetil. **3,** 118 (1964).

JULESZ, M.: A hirsutismus problémája újabb kísérleteink megvilágításában. Hungarian Acad. Sci. Meeting, 1965.

JULESZ, M.: Das Problem des Hirsutismus im Lichte unserer neueren experimentellen Untersuchungen. Dtsch. Ges. klin. Med. Sektion Urologie. 7. Wiss. Tagung, Kühlungsborn 27 (1965b).

JULESZ, M.: In press

JULESZ, M., FAREDIN, I., TÓTH, I., DÁVID, M. A., KOVÁCS, K.: Acta med. Acad. Sci. hung. **19,** 311 (1963).

JULESZ, M., FAREDIN, I., TÓTH, I.: Acta med. Acad. Sci. hung. **22,** 25 (1966).

TÓTH, I., FAREDIN, I., JULESZ, M.: Proc. 3rd Meeting of Hungarian Society of Endocrinology. Pécs 1967, p. 77.

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PATHOGENESIS OF MERCURIC CHLORIDE-INDUCED RENAL FAILURE IN THE DOG

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Parameters of renal function were investigated in dogs following the intravenous administration of 2 mg/kg mercuric chloride.

1. There is only a slight decrease in renal blood flow, which cannot account for the oligo-anuric renal failure.

2. The decisive factor in the development of renal failure seems to be the definite decrease in glomerular filtration rate. The additional role of tubular fluid leakage cannot be excluded.

3. Hyposthenuria and asthenuria are regarded as secondary consequence of the diminished filtration, thus contributing to the severity of renal failure.

4. The sharp decrease in the secretion and excretion ratio of PAH corresponds to the extensive necrosis of the proximal tubular epithelium.

Mercuric chloride (sublimate) is a well-known nephrotoxic substance: suitable doses cause death by eliciting acute anuria and uraemia. Histologic examination of the kidney reveals necrosis of the terminal two thirds of the proximal tubular epithelium; the lumina are filled by cellular debris; in case of survival calcification and regeneration of the tubular epithelium may be observed (EDWARDS [8]; OLIVER, MACDOWELL and TRACY [22]; STAEMLER [30]; GESSLER and BASS [13]; NOLTENIUS, SCHELLHAS and OEHLERT [19]; OLIVER and MACDOWELL [21]; ROTHER [25]; STEJSKAL [31]; etc.).

Generally, the oligo-anuria is thought to be connected with the necrosis of the tubular epithelium and with the obstruction of the tubular lumina by cellular debris. Rediffusion of the glomerular filtrate (primary urine) through the injured tubular wall is supposed. Inadequate circulation, caused by severe dehydration, leads to diminished renal haemodynamics and is considered an accessory factor in the development of renal failure.

Recently, however, the concept of the tubular genesis of mercuric chloride-induced oligo-anuria has been challenged by FLANIGAN and OKEN [11]. Micropuncture studies in the rat suggested that the primary decrease in glomerular filtration rate (GFR) is the cause of anuria. Similar results were obtained in rats in the early phase of glycerol-induced haemoglobinuric acute renal failure [20] and in oliguric renal failure induced by the intravenous injection of human methaemoglobin [26]. On the other hand, BANK, MUTZ and AYNEDJIAN [5] found that anuria in mercuric chloride poisoning can occur

with normal GFR and the role of "leakage" of tubular fluid in the genesis of anuria is stressed.

In other mammals (including man) the role of haemodynamic and/or tubular factors in the genesis of mercuric chloride induced renal failure was investigated prevalently by the clearance technique. It is well known, however, that in oligo-anuria clearance results are unreliable (BÁLINT [1]; etc.). In order to elucidate the question a series of experiments was performed in dogs which had been given sublethal doses of mercuric chloride and the parameters of renal function were investigated. Renal haemodynamics were assessed by the direct method (*v.* infra) which yields reliable results even in extreme oliguria or anuria. The intravenous route of mercuric chloride administration was chosen to avoid the acute gastroenteritis which dominates the picture after oral administration [15, 27]. The experiments were jointly performed with Drs A. FEKETE and M. VISY, histological evaluation was done by Dr. M. ZOMBORY in the Second Institute of Pathology, University Medical School, Budapest.

Methods

The observations were made on mongrel dogs of both sexes. Mercuric chloride dissolved in 20 ml physiological saline was administered intravenously. Preliminary experiments revealed that 4 mg/kg mercuric chloride caused death in 1 to 3 days. Therefore, in most experiments, 2 mg/kg of mercuric chloride was administered.

For some days before (control periods) and after the mercury administration the dogs were kept in metabolism cages. They were given their usual food and water *ad libitum*. Some parameters of renal function (NPN, urine volume, endogenous creatinine clearance etc.) were determined. In a number of cases spontaneous death or survival was established. As spontaneous death occurred in all cases within 10 days following the mercuric chloride administration, survival after the 10th day was considered definite.

In 37 cases an acute experiment was performed some hours or days after mercuric chloride administration. The timing of the experiment was set arbitrarily. The acute experiment was made on dogs under chloralose anaesthesia. The left kidney was approached by laparotomy and the left renal vein was connected by means of a plastic catheter to the external jugular vein. A T-extension of the tube allowed the direct measurement of renal blood flow, and the withdrawal of renal venous blood samples.

Renal blood flow (RBF) was estimated by direct measurement of renal venous outflow (ml/min/100 g kidney weight). Arterial blood pressure was estimated continuously by means of a mercury manometer. Urine was collected by means of a ureter catheter passed up into the renal pelvis. The plasma levels of PAH, inulin and creatinine required in the clearance and extraction studies were ensured by the administration of a preliminary dose and by the constant infusion of a maintenance dose. In the first part of the experiments the plasma PAH level was set to about 2 mg/100 ml (for determination of the extraction ratio), in the second part to about 20 mg/100 ml (for determination of Tm_{PAH}).

The clearances of creatinine and inulin, i.e. glomerular filtration rate (GFR) were calculated by the formulas $C_{cr} = RPF \cdot E_{cr}$, and $C_{in} = RPF \cdot E_{in}$, respectively, where RPF stands for renal plasma flow as determined from RBF measured directly and from the haematocrit value. E_{cr} and E_{in} represent the renal extraction ratios of creatinine and inulin, respectively. Determinations of the oxygen and haemoglobin content of renal venous and systemic arterial blood were made using a Doppeloxymeter (Atlas), the arterio-venous oxygen difference (AVD_{O_2} vol%) being corrected for the difference in haemoglobin content, as described by BÁLINT and FORGÁCS [2]. Oxygen consumption is the product of RBF and AVD_{O_2}.

Tubular PAH secretion (T_{PAH}) was calculated according to the formula

$$T_{PAH} = RPF(P_a - P_v) - GFR \cdot P_a \cdot 0.92$$

where P_a and P_v represent the PAH content of 1 ml arterial and renal venous plasma, respectively. T_{PAH} is considered the maximal secretory mass (Tm_{PAH}) if the tubular load of PAH ($= L_{PAH}$) is at least double of the secreted quantity. Load was calculated by the formula

$$L_{PAH} = P_a(RPF - GFR \cdot 0.92)$$

PAH was determined according to SMITH et al. [29]; inulin by the method of LITTLE [18]; and creatinine according to BÁLINT and VISY [4]. Non-protein nitrogen (NPN) was estimated by the method of CLEGHORN and JENDRASSIK [6]. Sodium was determined by flame photometry (Zeiss). Osmolality of plasma and urine was assayed by means of an osmometer (Advanced, model 63–31). Statistical evaluation was made by the usual methods, as described by FISHER [10].

Results

The total number of experiments in which dogs were administered 2 mg/kg mercuric chloride intravenously, amounted to fifty-two. In 18 cases an acute experiment was performed within 24 hours following mercury administration (Table II). Of the remaining 34 dogs 15 succumbed spontaneously and 12 were killed in the course of an acute experiment within 10 days following the mercury administration. Thus 7 dogs were alive by the 10th day and were considered definite survivors.

Concerning spontaneous mortality or survival, 22 experiments could be evaluated. Spontaneous death occurred in 15 cases and 7 dogs survived. Males seemed to be slightly more susceptible to mercury intoxication than females; the difference, however, did not prove to be significant.

Thirty-four experiments were classified according to the severity of symptoms. In 23 cases (17 males and 6 females) progressive azotaemia, oligo-anuria and clinical signs of uraemia could be observed: "severe" group. All animals which died spontaneously, belonged into this group. In 7 cases (2 males and 5 females) NPN values did not exceed 70 mg/100 ml, oliguria was absent and there were no clinical signs of uraemia: this group was called "minor" (concerning the degree of renal failure). In the remaining 4 cases (1 male and 3 females) transient azotaemia with mild uraemic symptoms was observed and the animals survived: this group was called "moderate". Significantly more males than females belonged to the severe group (evaluated by the chi^2-test; $P < 0.05$).

The results of the continuous observations are summarized in Table I. In the "severe" group NPN values increased by about 50–60 mg/100 ml each day. Within 24 hours after mercury administration polyuria was observed, with a simultaneous decrease in endogenous creatinine clearance values. The ratio of urine osmolality to plasma osmolality showed a progressive decline and from the 3rd day onward asthenuria was manifest.

In the "minor" group the mean NPN value did not increase significantly. Endogenous creatinine clearance revealed a significant decrease. Urine volume

increased on the first day and fluctuated about the control values during the entire period of observation. The ratio of urine and plasma osmolality corresponded to the controls allowed to drink water *ad libitum*. Results for the "moderate" group were in every respect between those of the "minor" and "severe" groups.

Table I

Parameters of renal function following the intravenous administration of 2 mg/kg mercuric chloride
(Chronic experiments)

$x \pm s_{\bar{x}}\,(n)$

Days after $HgCl_2$ administration		NPN mg/100 ml	P_{creat} mg/100 ml	V ml/min	$C_{end.creat.}$ ml/min	U_{osm}/P_{osm}
Controls		32± 1 (41)	1.0±0.1 (47)	0.58±0.07 (36)	48±4 (38)	2.14±0.15 (31)
1	s	60± 5 (10)	2.9±0.5 (9)	1.58±0.28 (10)	25±6 (10)	1.56±0.13 (10)
	mo	60 (4)[1]	2.3 (3)[1]	1.05 (4)	14 (4)	1.05 (4)
	mi	48± 3 (11)[1]	2.30±0.2 (7)[1]	1.17±0.22 (6)	20±3 (6)	1.42±0.32 (6)
2	s	128± 7 (8)	6.2±0.7 (6)	0.47±0.12 (8)	5±2 (6)	1.40±0.21 (7)
	mo	60 (4)[1]	2.3 (3)[1]	0.25 (4)	9 (4)	1.35 (4)
	mi	48± 3 (11)[1]	2.3±0.2 (7)[1]	0.63 (4)	18 (4)	2.45 (4)
3	s	187±30 (12)	9.1±1.0 (6)	0.20±0.07 (8)	1±0 (6)	1.07±0.15 (6)
	mo	128 (4)[2]	5.9 (4)[2]	0.27±0.05 (7)[2]	4±1 (8)[2]	1.58±0.12 (9)[2]
	mi	46± 8 (10)[2]	1.5±0.3 (8)[2]	0.80±0.17 (9)[2]	26±6 (9)[2]	1.83±0.19 (9)[2]
4	s	247±27 (11)	11.9 (4)	0.22±0.12 (5)	2 (5)	0.94±0.12 (5)
	mo	128 (4)[2]	5.9 (4)[2]	0.27±0.05 (7)[2]	4±1 (8)[2]	1.58±0.12 (9)[2]
	mi	46± 8 (10)[2]	1.5±0.3 (8)[2]	0.80±0.17 (9)[2]	26±6 (9)[2]	1.83±0.19 (9)[2]
5–8	s	276±27 (11)	18.1±2.0 (7)	0.10 (5)	3±1 (6)	0.97±0.12 (6)
	mo	109± 8 (8)	4.1±0.6 (7)	0.43±0.13 (11)	8±1 (11)	2.45±0.30 (11)
	mi	54± 7 (9)	1.2±0.2 (6)	0.52±0.08 (12)	20±4 (12)	2.36±0.19 (12)
9–14	mo	80±17 (6)	2.3±0.5 (7)	0.26±0.03 (10)	7±1 (10)	2.65±0.20 (10)
	mi	39± 6 (11)	0.9 (5)	0.45±0.11 (8)	22±5 (8)	1.96±0.27 (7)

s = severe cases; mo = moderate cases; mi = minor cases
[1] Days 1 and 2 evaluated together; [2] Days 3 and 4 evaluated together

In 20 cases an acute experiment was made within 24 hours after the mercuric chloride administration. Results are summarized in Table II. There was a wide scatter of RBF values; the means, however, did not differ significantly from those of the control series. A progressive decrease of C_{cr}, E_{PAH}

and Tm_{PAH} could be observed after the 6th hour following mercury administration. There was no correlation between RBF and the other parameters while between C_{cr} and Tm_{PAH} the correlation was highly positive (+0.85).

Table II

Parameters of renal function following the intravenous administration of 2 mg/kg mercuric chloride
(Acute experiments)

No.	Sex m: male f: female pr: pregnant	Hours after $HgCl_2$ admin.	NPN mg per 100 ml	Art. pressure mm Hg	RBF ml/min	C_{cr} ml/min	V ml/min	E_{PAH}	Tm_{PAH} mg/min
105/66	m	1	35	118	431	73	2.31	0.85	—
108/66	m	1	38	128	346	41	0.40	0.83	8.1
85/67	f	1	20	137	720	70	2.38	0.73	11.9
109/67	f	1	40	157	290	31	7.97	0.74	5.6
58/66	m	3	40	115	367	45	9.00	0.77	9.2
60/66	m	3	40	160	181	5	0.52	0.59	7.1
84/67	f, pr	3	42	120	718	73	3.05	0.67	16.7
114/67	f	3	40	107	311	24	1.96	0.70	9.0
45/66	m	6	35	133	297	21	1.85	0.65	7.0
119/67	f	6	40	100	242	12	0.94	0.59	1.8
120/67	f	6	30	163	457	42	1.53	0.64	10.3
41/66	m	12	54	112	256	3	0.04	0.05	0.1
23/65	m	12	45	133	208	15	0.60	0.57	3.2
96/67	f, pr	12	36	120	570	35	0.42	0.69	8.5
97/67	f, pr	12	26	125	430	72	0.56	0.73	15.1
95/66	m	24	70	137	326	2	0.06	0.31	4.0
10/65*	m	24	96	135	402	15	0	0.05	0.1
18/65	f	24	72	103	324	0	0	0.03	0
92/66	f	24	54	140	566	63	4.30	0.76	6.6
8/66*	f	24	98	123	492	0	0.02	0.02	0.7
Controls**		x	29	128	421	52	0.77	0.77	11.7
		s_x	1	3	14	3	0.10	0.01	0.6

* Quantity of administered $HgCl_2$: 4 mg/kg
** BÁLINT and FORGÁCS [3]

In 17 cases acute experiments were made between the 2nd and 15th days (Table III). It can be seen from Fig. 1 that NPN values were in no correlation with RBF which was reduced only moderately. On the other hand C_{cr}, E_{PAH} and Tm_{PAH} decreased in cases with azotaemia.

The relation of renal oxygen consumption to tubular Na-reabsorption is shown in Fig. 2. The regression equation is

$$Y = 3.65 + 0.76\ (\pm\ 0.07)\,x$$

i.e. the reabsorption of 1 mEq Na^+ is connected with the consumption of 0.76 ml (= 0.135 mEq) oxygen in the kidney of mercury intoxicated dogs. The difference from the controls (0.71 ml = 0.127 mEq [2]) is not significant.

Table III

Parameters of renal function following the intravenous administration of 2 mg/kg mercuric chloride

(Acute experiments)

No.	Sex m: male f: female pr: pregnant	Symptoms s: severe mo: moderate mi: minor	Days after $HgCl_2$ admin.	NPN mg per 100 ml	Art. pressure mmHg	RBF ml/min	C_{er} ml/min	V ml/min	E_{PAH}	Tm_{PAH} mg/min
28/65	m	s	2	148	164	193	4	0	0.14	0
22/66	m	s	3	152	135	469	7	0.04	0	0.6
81/67	m	mi	3	32	100	773	59	—	0.58	—
88/67	m	s	3	305	114	551	0	—	0	—
92/67	m	s	3	108	97	697	7	—	0.08	—
95/67	m	s	3	190	100	91	0	—	0	—
12/66	f	s	3	150	100	309	2	0.06	0.06	0
71/66	m	s	4	120	130	373	1	0.03	0.09	1.5
35/66	f	mi	4	22	120	363	51	0.25	0.75	10.4
49/66	f, pr	mi	5	46	145	142	18	0.11	0.72	4.0
72/66	m	s	6	164	148	326	4	0.57	0.04	3.6
55/66	f	mo	6	106	107	309	32	4.03	0.25	1.0
118/66	m	mo	10	138	150	257	10	0.17	0.52	1.7
112/66	f	mo	10	78	100	267	29	0.46	0.68	5.0
73/66	f	mi	12	26	138	223	27	0.32	0.70	6.2
116/66	f	mi	14	40	122	440	41	0.30	0.57	8.3
205/66	f	mo	15	28	130	197	16	2.35	0.42	7.9

Controls: see last row of Table III

The earliest histological alterations can be detected by light microscopy about 3 hours following the mercury administration. Glomeruli are dilated and hyperaemic. From the 6th hour onward, progressive necrosis of proximal tubuli dominates the picture (Fig. 3). From the 4th day histological signs of tubular regeneration are demonstrable (Fig. 4). The lumina are filled with

cellular debris; from the 3rd day calcium deposition and casts can be observed. Interstitial lymphocytic and plasmocytic infiltration completes the picture. In some cases a homogeneous pink staining material is to be seen in the lumina of the Bowman's capsule. According to light microscopic studies glomerular and tubular alterations appear simultaneously.

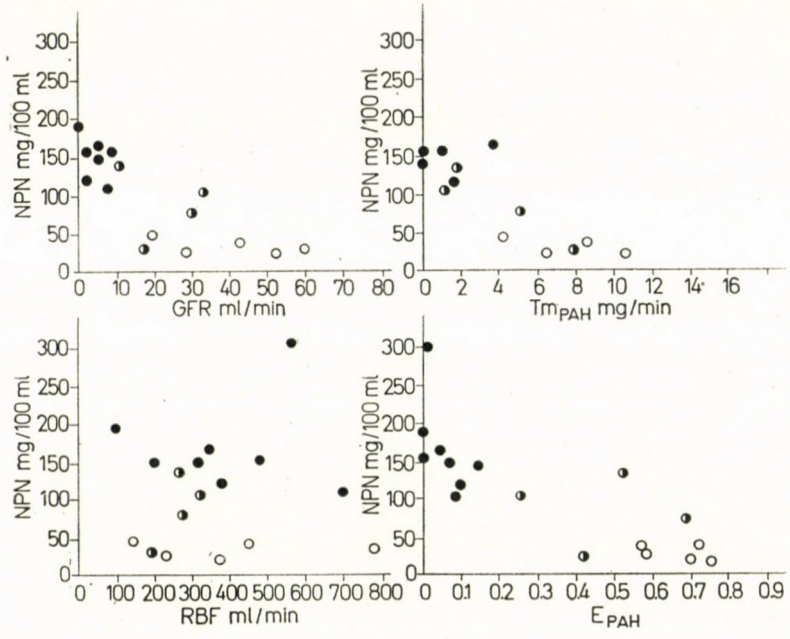

Fig. 1. Relation of NPN to GFR (= C_{cr}), RBF, Tm_{PAH} and E_{PAH}. ●: severe cases; ◑: moderate cases; ○: minor cases

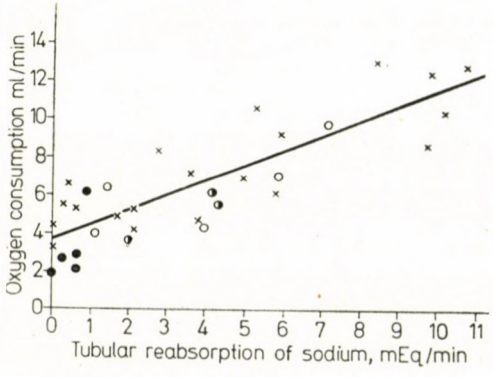

Fig. 2. Relation of oxygen consumption to tubular sodium reabsorption. ×: acute experiment made within 24 hours after mercury administration. For other signs, see Fig. 1

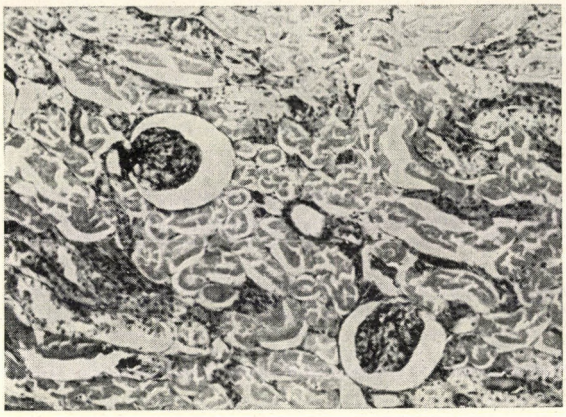

Fig. 3. Kidney of a dog 48 hours after intravenous administration of 2 mg/kg mercuric chloride. Extensive necrosis of proximal tubular epithelium. (Haematoxylin-eosin)

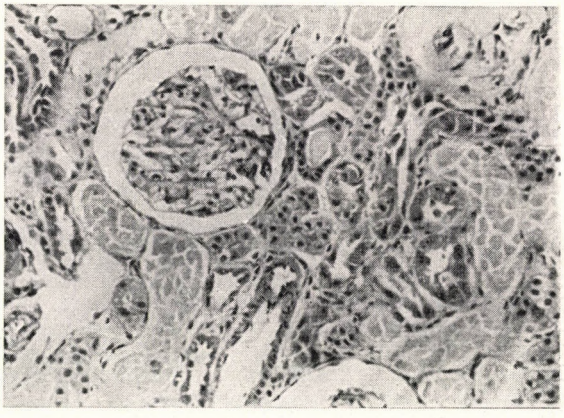

Fig. 4. Kidney of a dog 6 days following intravenous administration of 2 mg/kg mercuric chloride. Necrosis and regeneration of the epithelium. (Haematoxylin-eosin)

Discussion

Results will be discussed in the order of the various parameters of renal function.

1. The sharp decrease in the secretion and extraction of PAH corresponds to the well-known morphological feature of mercury intoxication: a massive necrosis of the proximal tubular cells. The decrease in extraction ratio is the secondary consequence of diminished secretion.

2. The direct determination of RBF revealed only slightly diminished values (Table IV). The slight renal ischaemia cannot be regarded as the cause of the fatal renal failure. Similar results were obtained by EPPINGER et al. [9]

Table IV

Mean (±S.E.) parameters of renal function in mercury induced renal failure

	Within 24 hours	Severe cases	Moderate cases	Minor cases
NPN mg/100 ml	46 ± 5 (20)	169 ±22 (8)	85 ±21 (4)	36 ± 4 (5)
RBF ml/min	398 ±35 (20)	375 ±70 (8)	263 ±32 (4)	385 ±111 (5)
C_{cr} ml/min	32 ± 6 (20)	3 ± 1 (8)	22 ± 5 (4)	40 ± 8 (5)
E_{cr}	0.13± 0.01 (20)	0.03± 0.01 (8)	0.11± 0.02 (4)	0.15± 0.02 (5)
E_{in}	0.19± 0.02 (20)	0.06± 0.02 (8)	0.12± 0.05 (4)	0.26± 0.03 (5)
V ml/min	1.90 (20)	0.14 (5)	1.75 (4)	0.25 (5)
E_{PAH}	0.55± 0.06 (20)	0.04± 0.02 (8)	0.18± 0.09 (4)	0.66± 0.05 (5)
Tm_{PAH}	6.7 ± 1.1 (19)	1.0 ± 0.6 (5)	3.5 ± 1.3 (4)	7.8 ± 1.5 (4)

In brackets: number of observations

and CONN et al. [7]. Owing to the presence of oligo-anuria, the clearance technique would have revealed complete renal ischaemia.

3. Considering the pathomechanism, evaluation of GFR is decisive. In normal dogs both exogenous and endogenous creatinine clearances are generally regarded as a reliable index of GFR. It should, however, be stressed that only those substances are suitable for the determination of GFR which are freely filterable in the glomeruli and are neither reabsorbed nor secreted by the tubular epithelium. If, as found by RICHARDS [24], part of the filtered creatinine were reabsorbed by or rediffusing through the damaged epithelium of the proximal tubuli, its clearance would be less than the filtration rate. The possibility of tubular rediffusion of filtered creatinine cannot be rejected offhand.

It can be seen in Table IV that the decrease in creatinine clearance corresponds well to the severity of clinical symptoms and to the degree of azotaemia. Does this mean that filtration rate is severely decreased or is an enhanced tubular rediffusion of creatinine with only slightly diminished filtration rate to be assumed? On the strength of the clearance results this question cannot be answered unequivocally.

Extraction ratios of creatinine and inulin were equal in control observations (0.21 ± 0.01 and 0.22 ± 0.01, respectively). In mercury-intoxicated dogs, however, the extraction ratios of inulin were significantly higher. This observation is in favour of an enhanced rediffusion of creatinine. On the other hand, extraction ratios of inulin are not reliable with varying urine volumes [3], because substantial quantities of inulin may be cleared from the kidney by the renal lymph.

An additional argument in favour of the approximate reliability of the creatinine clearance as a measure of GFR was the relation of renal oxygen consumption to tubular sodium reabsorption (Fig. 2). The regression lines did not differ from the controls. Sodium reabsorption was calculated assuming that creatinine clearance is a measure of the filtration rate.

Inferential evidence was supplied by the observation that decrease in creatinine clearance had preceded tubular necrosis. Creatinine clearances were well below control values 24 hours after mercuric chloride administration. In the "minor" series oliguria was absent, nevertheless creatinine clearance was diminished during the entire course of the observation.

Thus we are inclined to suppose that in mercury-intoxicated dogs the decisive role in the development of oligo-anuria and azotaemia is played by the decrease in glomerular filtration rate. Changes of the glomeruli can be revealed even by light microscopy; studies with the electron microscope have shown that damage of the "filtering apparatus" may take precedence of the tubular lesions [25, 28]. The dominance of glomerular factors has been stressed by REUBI [23] and GESSLER [12]; their view was based on the low values for intrarenal pressure and on the low inulin content of kidney tissue, respectively.

This, however, does not mean that the role of "leakage" of tubular fluid can be excluded. The micropuncture data [5, 11] revealed controversial results. The significant difference between inulin and creatinine extraction ratio values cannot be disregarded (Table IV). The joint role of decreased filtration and enhanced tubular rediffusion has been emphasized by LAPP and SCHAFÉ [17], and GÖMÖRI, JUHÁSZ and TAKÁCSI-NAGY [14].

4. The mercury-intoxicated dogs were not exposed to the stress of water deprivation. Nevertheless the data in the last column of Table I reveal that the concentrating capacity as compared to that in the control animals, was seriously impaired, and in the "severe" series asthenuria was manifest. This can be regarded as a secondary consequence of the diminished filtration rate [16] and contributes to the severity of the symptoms of fatal renal failure.

REFERENCES

1. BÁLINT, P.: Nierenclearance. Fischer, Jena 1965.
2. BÁLINT, P., FORGÁCS, I.: Pflügers Arch. ges. Physiol. **277,** 558 1963.
3. BÁLINT, P., FORGÁCS, I.: Acta physiol. Acad. Sci. hung. **31,** 99 (1967).
4. BÁLINT, P., VISY, M.: Acta physiol. Acad. Sci. hung. **28,** 265 (1965).
5. BANK, N., MUTZ, B. F., AYNEDJIAN, H. S.: J. clin. Invest. **46,** 695 (1967).
6. CLEGHORN, R. A., JENDRASSIK, L.: Biochem. Z. **274,** 189 (1934).
7. CONN, H. L. JR., WILDS, L., HELWIG, J.: J. clin. Invest. **33,** 732 (1954).
8. EDWARDS, J. G.: Amer. J. Path. **18,** 1011 (1942).
9. EPPINGER, H., LÁSZLÓ, D., REIN, H., SCHÜRMEYER, A.: Klin. Wschr. **9,** 633 (1930).
10. FISHER, R. A.: Statistical methods for research workers. 10th ed. Oliver and Boyd, London—Edinburgh 1946.

11. FLANIGAN, W. J., OKEN, D. E.: J. clin. Invest. **44,** 449 (1965).
12. GESSLER, U.: Experimentelle Untersuchungen zur Entstehung der akuten Anurie. In: Akutes Nierenversagen, H. SARRE and K. ROTHER (eds), Thieme, Stuttgart 1962, P. 78.
13. GESSLER, U., BASS, L.: Z. ges. exp. Med. **133,** 18 (1960).
14. GÖMÖRI, P., JUHÁSZ, I., TAKÁCSI-NAGY, L.: Acta med. Acad. Sci. hung. **21,** 169 (1965).
15. HARMON, E. L.: Amer. J. Path. **4,** 321 (1928).
16. KRAMER, K.: Das akute Nierenversagen im Schock. In: Shock, Pathogenesis and Therapy, K. D. BECK (ed.), Springer, Berlin–Göttingen–Heidelberg 1962, P. 149.
17. LAPP, H., SCHAFÉ, K.: Beitr. path. Anat. **123,** 77 (1960).
18. LITTLE, J. M.: J. biol. Chem. **180,** 747 (1949).
19. NOLTENIUS, H., SCHELLHAS, H., OEHLERT, W.: Beitr. path. Anat. **129,** 90 (1963).
20. OKEN, D. E., ARCE, M. L., WILSON, D. R.: J. clin. Invest. **45,** 724 (1966).
21. OLIVER, J., MACDOWELL, M.: Studies by microdissection and micropuncture of the abnormal kidney; the structural aspect. In: Proc. 2nd int. Congr. Nephrol., Excerpta med., Int. Congr. Ser. **78,** 13 (1964).
22. OLIVER, J., MACDOWELL, M., TRACY, A.: J. clin. Invest. **30,** 1305 (1951).
23. REUBI, F.: Schweiz. med. Wschr. **86,** 385 (1956).
24. RICHARDS, A. N.: Trans. Ass. Amer. Phycns **44,** 64 (1929).
25. ROTHER, K. O.: Experimentelle Nierenkrankheiten: Quecksilbervergiftung. In: Handbuch der experimentellen Pharmakologie, O. EICHLER, A. FARAH, H. FERKEN and A. D. WELCH (eds), Vol. XVI/4, P. 25, Springer, Berlin–Heidelberg–New York 1965.
26. RUIZ-GUINAZU, A., COELHO, J. B., PAZ, R. A.: Nephron **4,** 257 (1967).
27. SANSUM, W. D.: J. Amer. med. Ass. **70,** 824 (1918).
28. SCHÖRCHEN, CH., LÖBLICH, H. J.: Virchows Arch. path. Anat. **333,** 587 (1960).
29. SMITH, H. W., FINKELSTEIN, N., ALIMINOSA, L., CRAWFORD, B., GRABER, M.: J. clin. Invest. **24,** 388 (1945).
30. STAEMLER, M.: Virchows Arch. path. Anat. **328,** 1 (1956).
31. STEJSKAL, J.: Acta med. scand. **177,** 75 (1965).

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LYMPHOGENOUS ENCEPHALOPATHY—LYMPHOSTATIC CEREBRAL HAEMANGIOPATHY

By

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1. In contrast with the classical teaching which ascribes no significant part to the lymph circulation in cerebral fluid transport it has been shown that the mechanical insufficiency of cervical lymph flow gives rise to lymphostatic encephalopathy.

2. The mechanical insufficiency of cervical lymph flow has been further found to induce lymphostatic haemangiopathy.

3. A hypothesis has been formulated on the intramural prelymphatic fluid drainage system of the cerebral blood vessels.

4. An interpretation of the possible pathomechanism of lymphostatic encephalopathy is offered.

5. The combination of lymphostatic encephalopathy with some neurologic diseases is discussed.

When in 1954 we have shown that the experimental occlusion of the lymphatics of the heart gives rise to myocardial lymphoedema with definite functional and morphological changes [1], our findings despite their novelty found no serious opposition since it was a well-established fact known even from textbooks of anatomy that the heart possesses lymph vessels.

Our observation [2–9], namely that the experimental occlusion of the cervical lymph channels results in a functional impairment of the central nervous system with demonstrable microscopical evidence was found by many workers harder to reconcile with the view that the brain is devoid of lymphatic pathways. How should the inadequacy of lymph flow possibly affect the structure and function of an organ which possesses no lymphatic drainage system of its own?

Our finding of retinal oedema, papilloedema and exophthalmos resulting from cervical lymphatic blockade [10, 11] was likewise questioned by some critics on the ground of the alleged absence of lymphatic channels in the eye.

It must be made clear at the very start that in agreement with others we too failed to demonstrate lymphatics in the brain or in the interior of the eye. This is, however, in no way incompatible with the cerebral and ophthalmic consequences of cervical lymph blockade observed in our studies.

There is, on the other hand, undisputed evidence of the existence of endothelium-lined lymph capillaries in the mucous membrane of the nasal cavity adjacent to the fila olfactoria, furthermore in the epidural tissue sur-

rounding the spinal nerve roots and also in the orbital loose connective tissue as well as in the dura over the jugular foramen [12, 13]. These lymphatics termed perineurolymphatic pathways [14] are held to play a functionally negligible part in the drainage of CNS. Since the primary biological function of the lymphatic system involves the collection and transport of proteins released from the blood capillaries and since the CNS contains slight amounts of plasma protein, the allegedly negligible function of this perineurolymphatic system is viewed as being compatible with the transport of these proteins even though they must pass the arachnoidea and the dura to reach the lymphatics—but then this is definitely the pathway which intrathecally injected corpuscular elements have also been found to traverse.

The blood–brain barrier is regarded by the majority of neurologists an absolute obstacle to the passage of plasma proteins from the blood capillaries into the brain. Similar views are held in respect to the eye and it is asserted that the brain or the eye do not need a lymphatic system for protein drainage [15].

In opposition to traditional views I have never found it conceivable that there should exist an ideal blood–brain barrier totally impermeable to protein molecules, and I have, more than once, challenged the theory by the question, what happens if very minute amounts of protein, just a single molecule per hour, should none the less escape through the barrier into the brain?

That this is indeed the case has been demonstrated by recent isotope studies [16, 17, 18].

A further challenge to the probability of an absolute barrier is the existence of cerebral areas (area postrema, etc.) unprotected by such a blood–brain barrier. Should then these areas possess no mechanism at all for the drainage of protein molecules entering the brain tissue?

Furthermore, inflammatory processes, haemorrhages involve the presence of protein-containing exudate or of blood in the brain or in the interior of the eye. These processes are apt to heal. What can be the possible mechanism providing for the transport of these large molecules from the affected sites?

The last and also the most compelling question may be formulated as follows. The blood vessels are mesenchymal living structures admitting passage to various substances, including proteins, through their endothelial lining. For the transport of these substances the blood vessels possess lymph channels of their own [19, 20, 21, 22] between the vasa vasorum. The cerebral vessels must necessarily also have a drainage system. Which are the structures providing for its drainage?—a question which IWANOW [19] had actually raised prior to the present author without, however, following it up any further.

It was this particular question we set out to examine having recognized that the walls of the cerebral blood vessels do rely on a lymphatic drainage system. In conformity with this claim, occlusion of the cervical lymphatics

has been found to result in severe disease of the cerebral vessels, a condition which we have termed lymphostatic haemangiopathy [28].

In the following four main issues will be discussed.

After describing the features of lymphostatic haemangiopathy, a theory of the prelymphatic drainage of cerebral blood vessels and of the production of lymphogenous encephalopathy will be outlined. This will be followed by general considerations on the possible role of lymphostatic factors in certain diseases of the nervous system.

(i) Lymphostatic haemangiopathy. Studying the influence of cervical lymphatic blockade on the submicroscopic structure of cerebral vessels in the rat [23], the most typical changes were found in the blood capillaries of the area postrema. The reason why we selected this particular region is its being unprotected by a blood–brain barrier opposed to the passage of "lymph-due" substances, therefore it was expected to be the most vulnerable by lymphatic blockade.

Under normal conditions the blood capillaries of the area postrema have a double basement membrane enclosing a space of 0.1 to 0.5μ with sparse collagenous fibres. Cervical lymphatic blockade results in an accumulation of oedema fluid in this space causing a 15 to 30-fold increase in the distance between the two membranes. Parallel with the accumulation of oedema fluid the collagenous fibres increase in number and change their structure as reflected by a decrease of osmiophilia (Figs 1, 2).

The endothelial cells are also severely affected. Endothelial cells of normal cerebral blood capillaries are generally free from pinocytotic vesicles common in the endothelium of blood capillaries of other organs. Cervical lymphatic blockade is followed by the appearance of endothelial cytoplasmic vacuoles exceeding in size the pinocytotic vesicles. The cytoplasmic vacuoles obviously represent manifestations of cellular oedema, described by HILLS [24] in cerebral oedema induced by ligature of the carotid in combination with anoxia.

It must be stressed on the ground of studies of other cerebral regions that the foregoing changes are not confined to the area postrema.

Our claim that the mesenchymal elements of the cerebral capillary walls represent structures relying on lymphatic drainage has been fully borne out by the foregoing studies, mechanical insufficiency of cervical lymph flow having been found to result in lymphostatic impairment of the cerebral blood capillaries. Apart from the capillary system, the arteries and veins of the brain also exhibit signs of oedema. On the evidence of electron and light microscopic findings [25], cervical lymphatic blockade is followed by an oedematous dilatation of the Virchow–Robin-spaces.

(ii) Lymphology draws a distinct dividing line between interstitial fluid and lymph. Interstitial fluid is not regarded as lymph until it has entered the

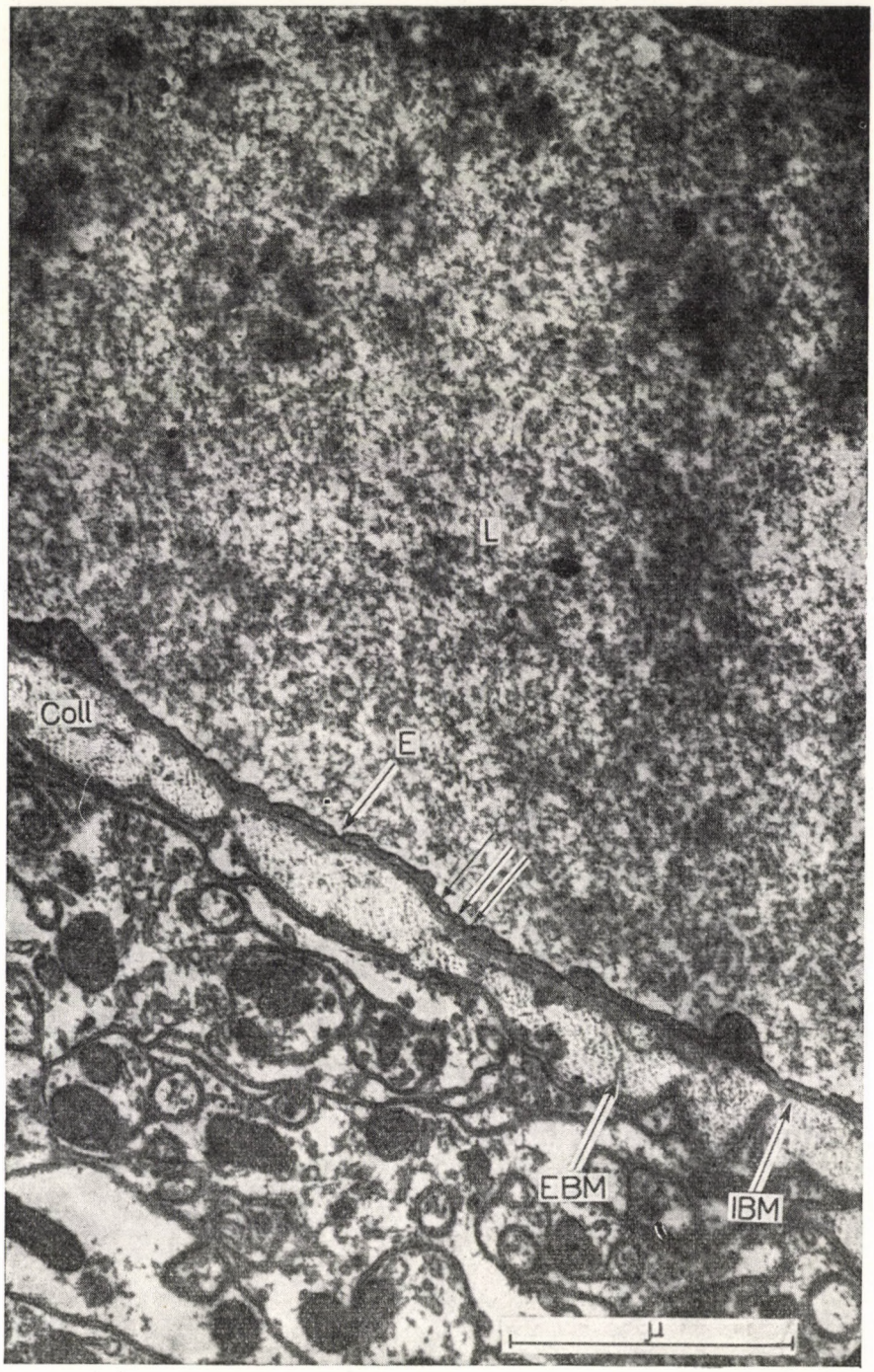


Fig. 1. Normal blood capillary in the area postrema

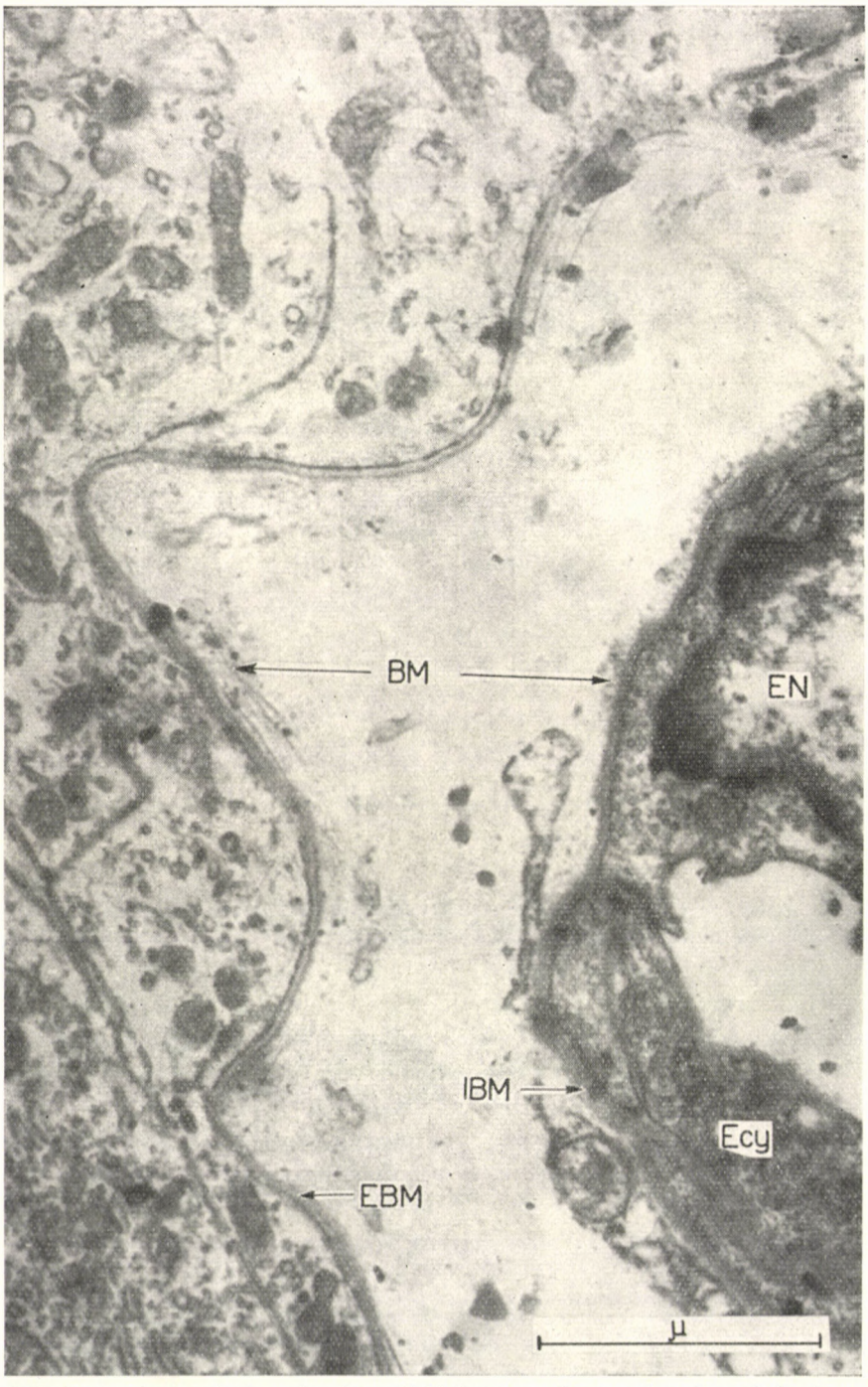


Fig. 2. Excessive accumulation of oedema fluid between the two lamellae of the basement membrane five days after cervical lymphatic blockade [23]. Coll = collagen, E = endothelium, L = lumen, EBM = External basement membrane, IBM = Internal basement membrane, BM = Basement membrane, ↗↗↗ = pores, EN = Endothelial nucleus, Ey = Endothelial cytoplasm

lymph capillaries. The pathway covered by the interstitial fluid during its passage to the lymph capillaries has been named *paralymphatic pathway* or *system* by OTTAVIANI; personally I would prefer the term *prelymphatic system*. In fact, the entire interstitium of the subcutaneous connective tissue represents a prelymphatic system since it is through its substance that the interstitial fluid leaving the blood capillaries finds access to the lymph capillaries. As regards the liver, its prelymphatic system is represented by Disse's spaces carrying the protein-rich fluid from the sinusoids to the lymph capillaries.

The finding of engorgement and dilatation of the typical intramural spaces of the cerebral blood vessels consecutive to surgical occlusion of the cervical lymphatic trunks has led us to the working hypothesis that these spaces represent elements of the prelymphatic system, carrying protein-containing interstitial fluid which must finally reach the cervical lymphatic channels under physiological conditions. Thus, in our view, the evidence of retrograde congestion, in other words, the auto-injection of these spaces by interstitial fluid consecutive to lymphatic blockade, has finally settled this much debated question.

Our findings are in full harmony with recent evidence indicating that the basement membranes may form channel systems which are often primarily involved in the transport processes in certain organs. These channels, specified as intervascular being connected with blood capillaries, are structures approximately 2000 Å in diameter with their walls formed by the basement membranes. "The network of basement membrane duplicates ensheathing the glial processes in the area postrema forms a basement membrane labyrinth . . ." [26].

"Though there is little experimental evidence on the functional significance of these channels, the very fact of their being in close communication with the perivascular spaces of blood capillaries is suggestive enough that they are in closer relations to the tissues than are the blood capillaries. This makes it possible that in those organs which are provided with a membrane system of this kind, the fluid transport takes place via these channels. Opening and dilatation of this basement membrane labyrinth has been indeed demonstrated in cerebral oedema accompanying pentetrazol seizures . . . Consequently, in addition to the blood capillaries made up of endothelial cells and of exclusively endothelial structure, there exists a communication system built up by basement membranes alone . . ." [26, 27, 28, 29].

There are further facts in support of the present theory.

In our investigations [14] into the microscopic character of lymphoedematous soft tissues of the neck following cervical lymphatic blockade in the dog, we have demonstrated in the blood capillaries the appearance of intramural spaces of exactly the same character as those known as Virchow—Robin's spaces of the cerebral vessels, with the difference that the cervical blood vessels

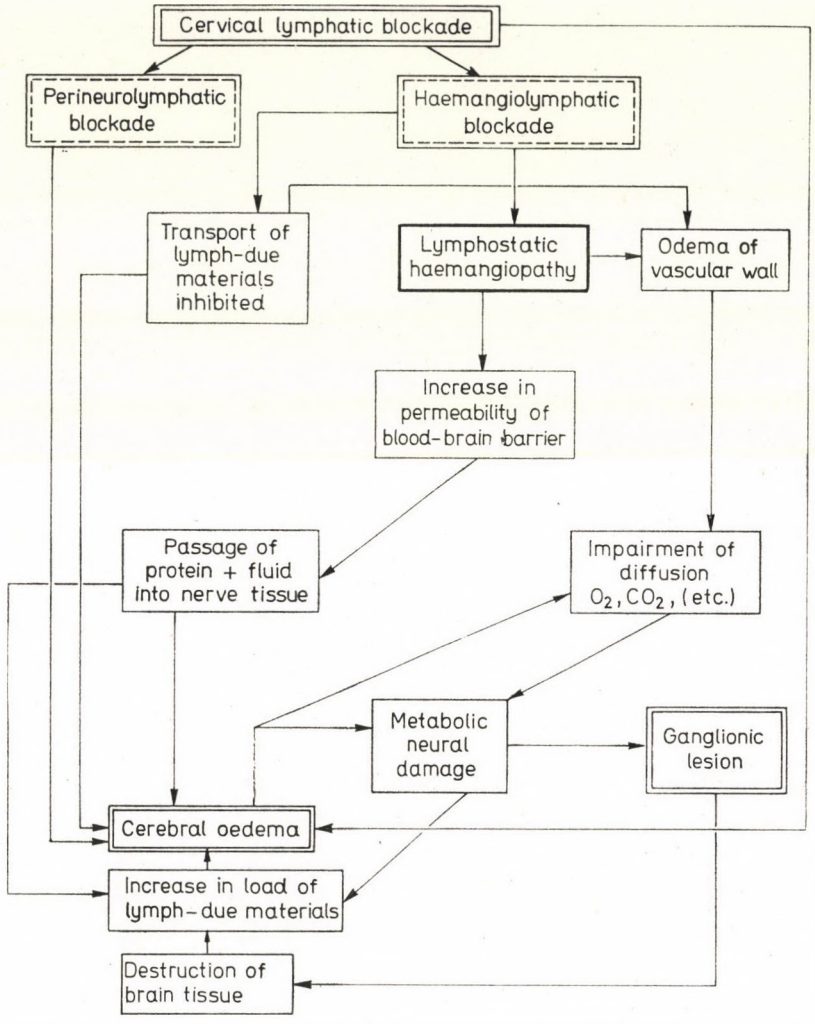
were surrounded by dilated lymphatic channels and the boundary between the "Virchow–Robin-spaces" and the perivascular lymphatic vessels was reduced to just one or two connective tissue fibres.

It is pertinent to mention here that as early as 1910 MOTT [30] suggested that the Virchow–Robin-spaces of the cerebral vessels were in fact lymphatics. So far we would not go in our claim before it has been possible to demonstrate in serial sections the existence of an unbroken endothelial lining. If future studies should prove the case of MOTT then the term prelymphatic system will have to be applied to Virchow–Robin's spaces without the prefix pre.

It is a well-established fact but, thus far, largely unaccounted for, that the fluid accumulating in the Virchow–Robin-spaces of the cerebral blood vessels may bear the marks of particular conditions. For instance in encephalitis, it has the character of an exudate, being rich in protein and cells; in cerebral tumours it contains malignant cells; in apoplexy, haemoglobin. CSANDA [31] demonstrated barium granules in the spaces after intracranial injection of a barium paste to dogs.

Our hypothesis thus sums up as follows. The cerebral arteries carry blood to, and the cerebral veins from, the tissues of the brain. In addition, the walls of cerebral arteries and veins as well as those of the blood capillaries, are sites of an intramural prelymphatic fluid passage to the lymphatic system, the direction of the fluid movement being opposite to the arterial, and identical with the venous blood flow. In my belief this is the route of transport for the lymph-due materials of the nervous tissue beside those of the vascular walls. By producing cervical lymphatic blockade we block at the same time the prelymphatic–lymphatic drainage system through which the continuous transport of lymph-due substances of nervous tissue takes place under normal conditions. This is one of the factors accounting for cerebral oedema induced by cervical lymphatic blockade.

(iii) These considerations bring us directly to the third aspect of the present discussion, the *pathomechanism of lymphostatic encephalopathy*. The manner in which this is assumed to develop has been represented in the Table. Cervical lymphatic blockade arrests the transport of lymph-due substances through the perineurolymphatic pathway. This alone might be a potent factor in the arisal of cerebral oedema. Cervical lymphatic obstruction results in a blockade of the intramural prelymphatic system of the blood vessels. Two factors prevail here. In addition to the haemangiolymphatic blockade of the transport of lymph-due substances from the brain, lymphostatic haemangiopathy associated with oedematous swelling of the blood capillaries leads to slowing of the diffusion processes vital to cerebral tissue. Increase in the permeability of the blood brain barrier, the presence of which has recently been demonstrated [32] is an important contributive factor of cerebral oedema and ganglion cell lesion by further affecting the nervous tissue.



(iv) On the basis of the foregoing facts we feel justified in drawing some *general conclusions.*

a) Diffuse cerebral haemangiopathy, whatever its cause, may lead to a blockade of the intramural prelymphatic pathways of the blood vessels where the features of lymphostatic encephalopathy join the specific manifestations of the primary disease. It is by no means accidental that cerebrovascular collagenosis shares many features of lymphostatic encephalopathy.

b) Experimental lymphostatic encephalopathy is the result of a mechanical insufficiency of cervical lymph drainage. But, in addition to the possibility of a mechanism of this kind, all organs provided with a lymphatic drainage system, so also the brain and its blood vessels, are susceptible to a dynamic

failure of lymph flow which will arise whenever the load of lymph-due materials per time unit exceeds the transport capacity of the system. Translated into clinical terms, this mechanism superimposes the features of lymphostatic encephalopathy on those of the primary disease.

c) Accordingly, the functioning of cerebro-cervical fluid drainage should be examined in every case associated with cerebral oedema. This also applies to papilloedema and retinal oedema.

Human lymphostatic encephalopathy has been discussed in an earlier paper [2]. Through a lymphologic approach to certain central-nervous and ophthalmic processes, our studies have thus led to a better understanding of their mechanism. These aspects should be duly considered by the neurologist and the ophthalmologist. In fact, neurologists have been familiar with the occurrence of an exacerbation of organic neuropathies consecutive to trivial tonsillitis. In the light of the foregoing considerations, lymphangitis, lymphadenitis or lymphangiospasm associated with tonsillitis may well activate quiescent or latent processes of the nervous system.

This view is in harmony with the result of our studies [33] in which a transitory slowing of the EEG-rhythm, confirmed by statistical analysis, has been found in 10 out of 12 otherwise healthy subjects after tonsillectomy performed because of chronic tonsillitis. Tonsillectomy may be regarded as a source of sympathetic excitation resulting in lymphangiospasm. Joined by inflammatory lymphangitis or accompanying lymphadenitis, the surgical injury may well account for the production of an acute, transitory lymphostatic encephalopathy. In our observation tonsillectomy produces far more extensive transitory EEG-alterations in subjects affected by lymphostatic encephalopathy than it does in persons with no neurologic abnormality.

REFERENCES

1. Földi, M., Romhányi, Gy., Rusznyák, I., Solti, F., Szabó, Gy.: Magy. Tud. Akad. Orv. Tud. Oszt. Közl. **5,** 63 (1954).
2. Földi, M., Obál, F., Kahán, Á., Wagner, Á., Csanda, E., Börcsök, E.: Acta paediat. Acad. Sci. hung. **8,** 171 (1967).
3. Földi, M., Csillik, B., Joó, F., Zoltán, Ö. T.: Angiologica (Basel–New York) **4,** 50 (1967).
4. Csillik, B., Földi, M.: Experientia (Basel) **23,** 835 (1967).
5. Földi, M., Csanda, E., Obál, F., Madarász, I., Szeghy, G., Zoltán, Ö. T.: Z. exp. Med. **137,** 483 (1963).
6. Obál, F., Madarász, I., Zoltán, Ö. T., Csanda, E., Földi, M.: Z. exp. Med. **138,** 26 (1964).
7. Zoltán, Ö. T., Földi, M., Obál, F., Madarász, I.: Z. exp. Med. **138,** 43 (1964).
8. Földi, M., Zoltán, Ö. T., Obál, F., Madarász, I., Lehotai, L.: Z. exp. Med. **138,** 185 (1964).
9. Csanda, E., Földi, M., Obál, F., Zoltán, Ö. T.: Angiologica (Basel–New York) **5,** 55 (1968).
10. Földi, M., Gellért, A., Kozma, M., Poberai, M., Zoltán, Ö. T.: Acta anat. (Basel–New York) **64,** 498 (1966).
11. Földi, M., Csanda, E., Zoltán, Ö. T., Dobranovics, I.: Angiologica (Basel–New York) **4,** 341 (1967).

12. FÖLDI, M., GELLÉRT, A., KOZMA, M., POBERAI, M., ZOLTÁN, Ö. T., CSANDA, E.: Acta anat. (Basel–New York) **64,** 498 (1966).
13. FÖLDI, M., KUKÁN, F., SZEGHY, G., GELLÉRT, A., KOZMA, M., POBERAI, M., ZOLTÁN, Ö. T., VARGA, L.: Acta anat. (Basel–New York) **53,** 333 (1963).
14. SWEET, W. H., LOCKSLEY, H. B.: Proc. Soc. exp. biol. (N. Y.) **84,** 397 (1953).
15. ADLER, F. H.: The Physiology of the Eye. Mosby, St. Louis 1959.
16. SKLAR, F. H., BURKE, E. F., LANGFITT, T. W.: J. appl. Physiol. **24,** 79 (1968).
17. HOCHWALD, G. M., WALLENSTEIN, M.: Amer. J. Physiol. **212,** 1199 (1967).
18. CUTLER, G. W. P., DEUEL, R. K., BARLOW, C. F.: Arch. Neurol. **17,** 261 (1967).
19. IWANOW, G.: Z. anat. Entwickl. Gesch. **99,** 669 (1933).
20. OSCHKADERCE, W. I.: Anat. Anz. **78,** 104 (1934).
21. VAN LIMBORGH, J.: Angéologie (Paris) **14,** 23 (1962).
22. JOHNSON, R. A.: Lymphatics of Blood Vessels. Second International Congress of Lymphology. Miami 1968.
23. FÖLDI, M., CSILLIK, B., VÁRKONYI, T., ZOLTÁN, Ö. T.: Vascular Surgery (New York). In press.
24. HILLS, C. P.: Amer. J. Path. **44,** 531 (1964).
25. FÖLDI, M., CSANDA, E., SIMON, M., OBÁL, F., SCHNEIDER, I., DOBRANOVICS, I., ZOLTÁN, Ö. T., KOZMA, M., POBERAI, M.: Angiologica (Basel–New York). In press.
26. VOLLRATH, L.: Dtsch. med. Wschr. **93,** 360 (1968).
27. VOLLRATH, L.: Z. Zellforsch. **73,** 107 (1966).
28. BARER, R., LEDERIS, K.: Z. Zellforsch. **75,** 201 (1966).
29. LEONHARDT, H.: Z. Zellforsch. **76,** 511 (1967).
30. MOTT, W.: Lancet **2,** 5 (1910).
31. CSANDA, E.: Thesis, Budapest 1952.
32. VÁRKONYI, T., JOÓ, F., CSILLIK, B., ZOLTÁN, Ö. T., FÖLDI, M.: Lymphology (Stuttgart). In press.
33. OBÁL, F., SZABON, J., BÖRCSÖK, E., FÖLDI, M.: This Journal. In press.

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MYOCARDIAL CONTRACTILITY AND OVERALL METABOLISM DURING TREATMENT OF CARDIAC DECOMPENSATION

By

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Changes in the myocardial contractility and metabolism in the course of digitalization have been followed in subjects with left heart failure. Contractility was determined on the basis of the first carotid derivative (dc/dt) and metabolism on that of the pressure-time index.

In those subjects who had become compensated, dc/dt was found to increase and pressure-time index to diminish in the course of treatment, thus improvement of myocardial contractility went hand in hand with a reduction in the metabolic demands. In all those cases where the metabolic requirements of the heart muscle increased together with its contractility, treatment failed to ensure compensation. In the remaining cases where treatment had no influence on contractility, the outcome was lethal.

It is inferred from the results that the digitalis glycosides primarily act on the metabolism of the heart muscle, the changes in its contractility and lasting compensation being determined by myocardial metabolism.

Haemodynamic research has thrown light on many aspects of cardiac decompensation, but the nature of the underlying myocardial changes is still unclear. Reduction in myocardial contractility has long been assumed to be the primary factor of decompensation. That myocardial contractility is definable by the velocity of the increase in the pressures arising in the individual chambers of the heart has been demonstrated in recent years. These estimations involve, however, cardiac catheterization, a procedure far from harmless, particularly to subjects with decompensation, and especially as the tests have to be repeated. In view of this we have developed a clinical method for the estimation of the rate of pressure increase [1].

The behaviour of myocardial metabolism represents another essential aspect of cardiac decompensation. According to recent data, reliable information concerning this point is supplied by the pressure-time index.

This paper will report on studies into the mechanism of cardiac decompensation performed by determining myocardial contractility and metabolism.

Methods

The pressure prevailing in the large vessels and the velocity of its rise were determined from a curve recorded from the carotid by external detection. The differentiating R–C circuit has a resistance of 50 kOhm, a capacity of 0.0002 to 0.004 farads, the time constant was 10 to 15×10^{-8} This arrangement provided for a linear amplitude differentiation up to 50 c/sec without any phase distortion [1].

The pressure-time index was calculated from the formula: mean arterial pressure × ejection time × frequency.

Ejection time was estimated on the carotid curve from the zero point to the incisure. Mean arterial pressure was measured directly by means of a cannula inserted into the femoral artery. In some instances, mean arterial pressure was calculated from the systolic and diastolic pressures on the basis of the formula

$$\text{mean arterial pressure} = \frac{S + D}{2}$$

(The relationships of the carotid derivative with myocardial contractility had been studied in animal experiments. Drugs influencing myocardial contraction were found to affect the derivative of the carotid curve both qualitatively and quantitatively in the same manner as they influenced the pressure of the left ventricle, the aorta and the carotid [1]. It was felt justified on these grounds to regard the behaviour of the first derivative of the carotid pressure curve recorded indirectly as an expression of myocardial contractility.)

(a) The patients, all with cardiac decompensation, rested in a semi-recumbent position for 30 to 60 minutes prior to recording the ECG, the carotid pressure curve and its first derivative. Then strophanthin or some other quick-acting cardiac glycoside was administered intravenously. The foregoing tracings were then repeated at 10-minute intervals during the first half hour and at 30-minute intervals during the following four hours.

(b) ECG and carotid pressure were recorded daily, at the same hour of the day and under identical conditions until full compensation. In both groups of experiments at each examination mean arterial pressure was determined and the pressure-time index was computed.

Results

A) Acute experiments

The acute effect of strophanthin was studied in 12 subjects with decompensation of different aetiology. In 5 cases the peak of the first carotid derivative became higher within the first 60 minutes, while the pressure-time index became lower. In three other cases, however, the value of the pressure-time index also increased; of these three patients one died and two needed continuous digitalization for preventing decompensation.

The peak of the first carotid derivative declined in 4 cases. Of these, in 3 the pressure-time index declined and in one it rose. Two of these four patients died (Fig. 1 and Table I).

B) Chronic experiments

The changes in the peak of the carotid curve and in the pressure-time index were regularly observed in 25 decompensated subjects. In 16 of these the peak significantly rose parallel with a diminution of the pressure-time index. Compensation was successful in all the 16 cases. In 3 cases the peak increased parallel with the increase of the pressure-time index. One of these patients soon died while in 6 the peak diminished in the course of treatment. In 3 of the latter patients the pressure-time index increased and in 3 it diminished. The first three subjects died whereas the other three regained compensation and working capacity for some length of time (Fig. 2, Table II).

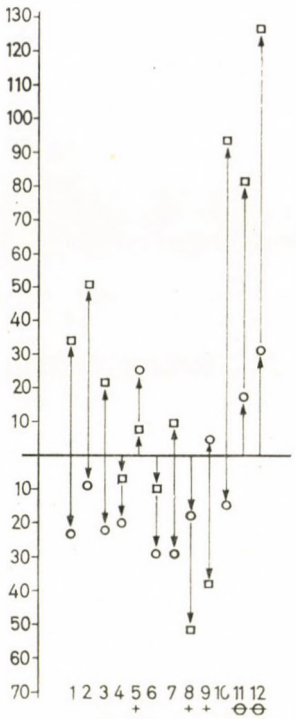

Fig. 1

Table I

Percentage changes in dc/dt at the time of restoration of compensation, respectively immediately before death (+), in cases in which compensation could not be restored (D)

No.	dc/dt, per cent	Pt, per cent	
1	+34.0	−23.0	
2	+50.0	− 8.0	
3	+22.0	−22.0	
4	− 7.0	−20.0	
5	+ 8.0	+25.0	+
6	−10.0	−29.0	
7	+10.0	−29.0	
8	−50.0	−17.0	+
9	−37.0	+ 4.0	+
10	+92.0	−12.0	
11	+80.0	+18.0	D
12	+125.0	+32.0	D

dc/dt = Peak of the first derivative of the carotid curve
Pt = Pressure-time index

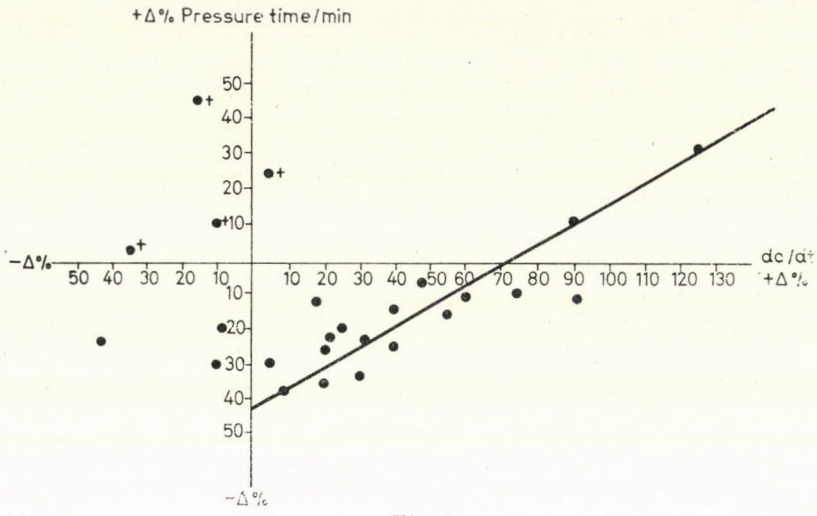

Fig. 2

Table II

The numerals in the table express the number of cases: the numerals in brackets designate the cases in which compensation was not restorable or the patients died

Pressure-time index	dc/dt		
	Increased	Reduced	Total
Increased	3 (1)	3 (3)	6 (4)
Reduced	16	3	19
Total	19	6	25

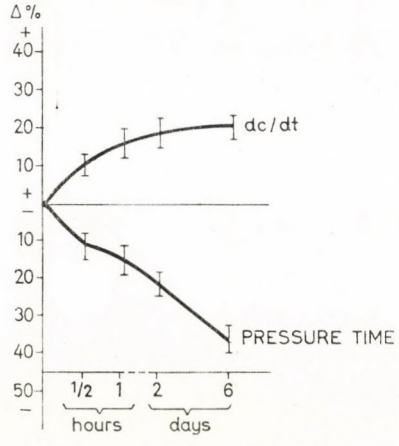

Fig. 3

Discussion

Direct measurement of myocardial contractility and of the metabolism of the heart is not possible in human subjects. It has therefore been attempted to gain information on these two essential parameters by indirect methods. From the investigations of RUSHMER [2], GLEASON and BRAUNWALD [3], and others, the rate of increase in ventricular pressure has emerged as the most reliable indicator of myocardial contractile power. The pressure changes arising in the large arteries, particularly in the aorta and in the carotid, reflect, however, myocardial ejection in the same manner as do the pressures measured directly in the individual heart chambers. Though the pressures recorded from the large vessels do not show the absolute value of myocardial contractility, the changes in the velocity of pressure elevation recorded in the same individual do correspond to changes in the contractile power of the heart muscle. This approach was the more recommendable in the present study as all subjects concerned were severely ill and it was particularly important to avoid any undue risk or discomfort. Since our purpose was to register changes rather than to compare absolute values, the peak of the first derivative of the carotid curve recorded in the course of treatment proved to offer a reliable basis for that.

For the evaluation of the changes taking place in myocardial metabolism, the pressure-time index has proved to be the most reliable indicator [4, 5, 6]. Here, too, we were concerned with changes occurring in the individual cases, therefore we expressed all the changes in per cents of the pre-treatment values.

In 5 out of 12 acute and in 16 out of 25 chronic experiments the peak of the first derivative increased and the pressure-time index diminished. In these cases compensation was more or less permanently restored.

The outcome was different in those cases where the peak of the first derivative declined, in other words where myocardial contractility diminished instead of improving in the course of digitalization. Such a negative response was registered in 6 cases, of which in three the pressure-time index increased and in three it diminished. In other words, in the first three cases the global metabolic requirements of the heart muscle increased despite its failing contractility whereas in the other three the metabolic demands of the heart muscle declined in proportion to its contractile power. The outcome was lethal in the former three cases whereas in the other three compensation could be restored though not for long.

The present observations suggest certain general conclusions. It seems that in those cases where contractility increases during treatment while the metabolic demands diminish, compensation is eventually restored. It has been furthermore found that reduction in the pressure-time index generally precedes

the increase in the peak of the first derivative, in other words, metabolism is the primary factor determining the increase in contractile power. On the other hand, in those cases where the pressure-time figures increased in opposition to a decline in the peak values of the first derivative, in other words, where despite a maximal increase in metabolic demands myocardial contractility was failing instead of gaining in power, treatment proved inefficient and the patients died, as a further proof of the metabolism of the heart muscle being the primary factor of its contractility.

By following up the lines of the present study it is hoped to gain further insight into the mechanism of cardiac decompensation. It seems justified to regard a metabolic impairment of the heart muscle as the primary factor interfering with its contractility, an assumption awaiting support by further investigations.

REFERENCES

1. GÁBOR, GY., SIMONYI, J., KISS, É., BARTHA, J., HÁRSING, L.: Orv. Hetil. 345 (1968).
2. RUSHMER, R. F.: Circulation **29,** 268 (1964).
3. GLEASON, W. L., BRAUNWALD, E.: J. clin. Invest. **41,** 80 (1962).
4. SARNOFF, S. J., CASE, R. B., WELCH, G. H. JR., BRAUNWALD, E., STAINSBY, W. N.: Amer. J. Physiol. **192,** 148 (1958).
5. ALELLA, A., WILLIAMS, F. L., BOLENE-WILLIAMS, C., KATZ, L. N.: Amer. J. Physiol. **183,** 570 (1955).
6. LAURENT, D., BOLENE-WILLIAMS, C., WILLIAMS, F. L., KATZ, L. N.: Amer. J. Physiol. **185,** 355 (1956).

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EFFECT OF PREDNISOLONE ON THE DEVELOPMENT OF CINCHOPHEN ULCER IN THE DOG

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Therapeutic doses of prednisolone administered over a period of three weeks gave rise to ulceration in 20% of the dogs so treated. The lesions were superficial and did not exceed 7 mm in diameter. These results raise the hope that human prednisolone ulcer may be detected in time, if the patient is subjected to X-ray examination every three weeks.

Prednisolone greatly promoted the progression of cinchophen ulcer. In some cases, giant ulcers developed resembling those seen in old age.

One of the possible explanations for the ulcerogenic action of prednisolone is a iatrogenic adrenal insufficiency.

The giant ulcers are not accompanied by gastritis, while in the group treated with prednisolone only haemorrhagic gastritis developed, mostly without ulcer. Consequently, peptic ulcer and gastritis result from two different mechanisms.

Among the untoward side effects of steroid treatment, gastrointestinal complications represent the gravest hazard. In the past years we, too, observed a number of cases of severe upper gastrointestinal haemorrhage and in one case there even occurred a perforation of the small intestine. We have therefore undertaken a study of the ulcerogenic effect of prednisolone under experimental conditions, to answer the questions,

(i) does prednisolone by itself induce gastroduodenal ulceration in the dog?

(ii) does prednisolone enhance the ulcerogenic effect of cinchophen?

Our results make it possible to discuss the correlations presumably existing between gastritis and peptic ulcer and to interpret the genesis of the giant ulcer of old age.

Materials and methods

Three groups of 12 dogs each were used. In each group four animals were treated with prednisolone alone, four with cinchophen alone, while four received prednisolone + cinchophen. The daily dose of cinchophen sodium was 100 mg per kg body weight, administered intravenously in the form of a 5% solution over a period of three weeks according to VAN WAGONER and CHURCHILL [8]. Prednisolone (Di-Adreson-F Aquosum, Organon) was injected intramuscularly in daily doses of 1 mg per kg. The animals were kept in isolation cages, fed a mixed diet not containing bones and were killed after 24 hours of fasting on the 22nd day of the experiment by intracardiac injection of chloroform. The animals succumbing to upper gastrointestinal haemorrhage of perforation before completion of the experiment were immediately subjected to study. The peptic ulcers were evaluated by planimetry, classifying them as small, medium and large. Medium size meant a longest diameter of 7 to 8 mm. In the presence of several ulcers the largest one was relied upon in classification. Three dogs succumbed to intercurrent disease; these were excluded from the analysis.

Finally, the normal weight of the adrenals was determined in 13 untreated control dogs.



Fig. 1. Extent of ulcers in Group 1. Shaded area: site of perforation. D: duodenal ulcer

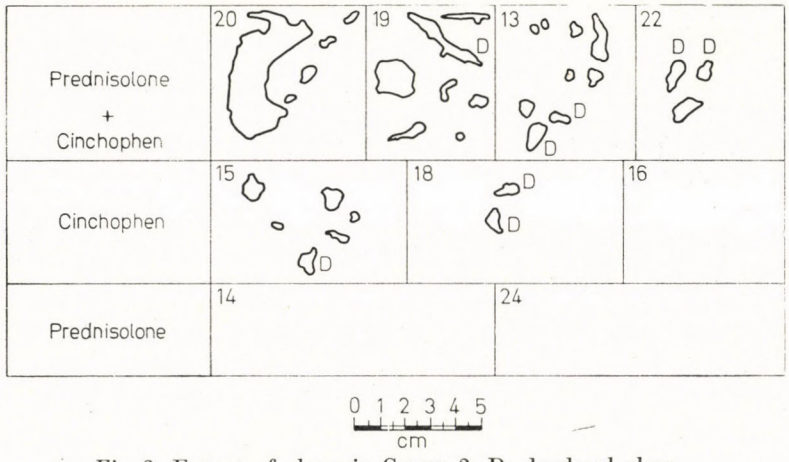

Fig. 2. Extent of ulcers in Group 2. D: duodenal ulcer

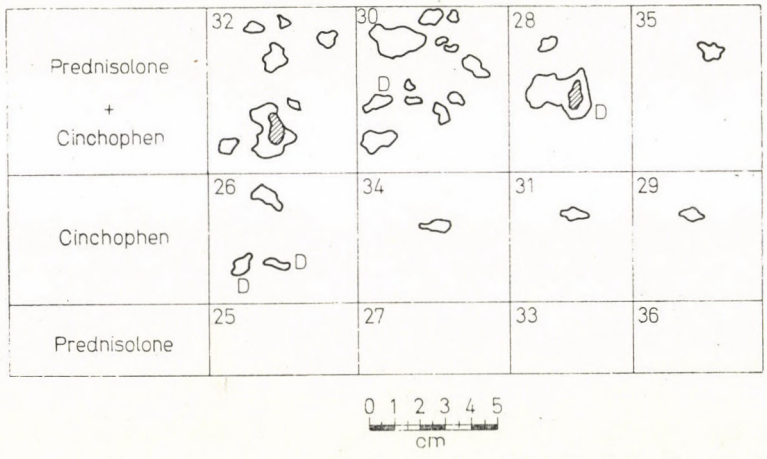

Fig. 3. Extent of ulcers in Group 3. Shaded area: site of perforation. D: duodenal ulcer

Results

Results are summarized in Table I. As visible, there was no negative finding.

Of the prednisolone-treated dogs ulceration occurred in two. The lesion was a small superficial ulcer in one case and a medium one in the other. A strik-

Table I

Results of prednisolone and cinchophen administration

Drugs used	No. of animals	Site of lesion	Inflammation	Size of ulcer			No lesions
				Small	Medium	Large	
Prednisolone + cinchophen	12	stomach	4			12	Ø
		duodenum	6		1	4	
Cinchophen	11	stomach	6		1	8	Ø
		duodenum	7		1	4	
Prednisolone	10	stomach	8	1	1		Ø
		duodenum	8				

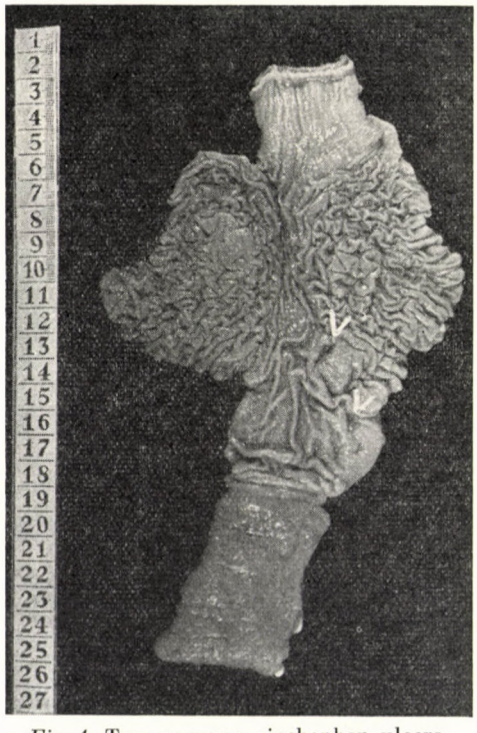

Fig. 4. Two common cinchophen ulcers

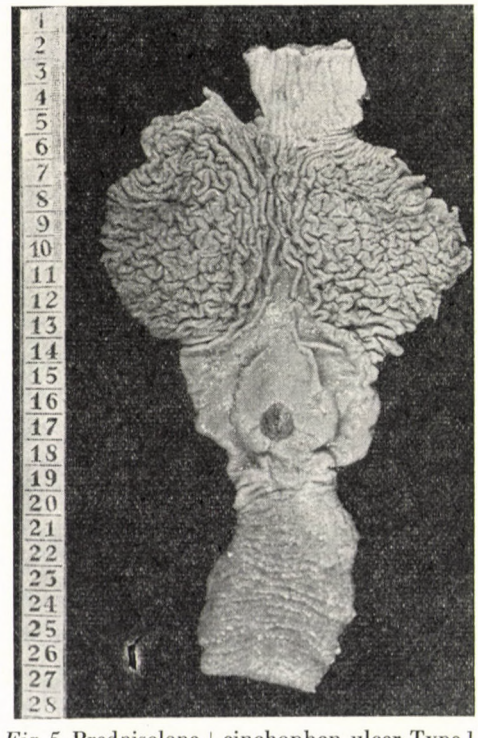

Fig. 5. Prednisolone+cinchophen ulcer. Type 1

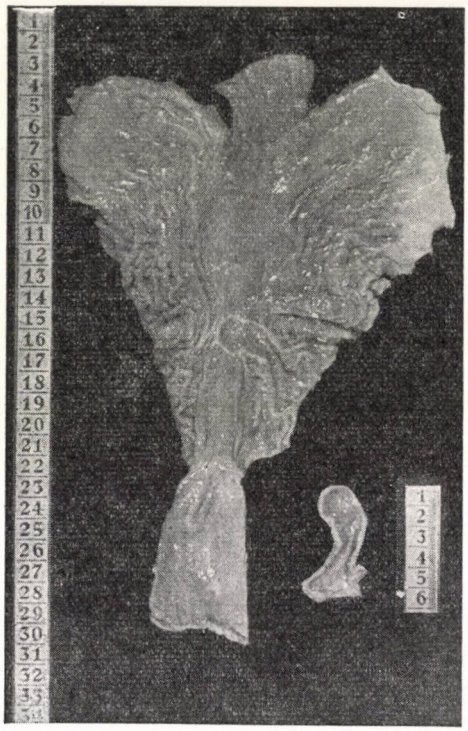

Fig. 6. Prednisolone + cinchophen ulcer. Type 2. The giant ulcer is not accompanied by gastritis

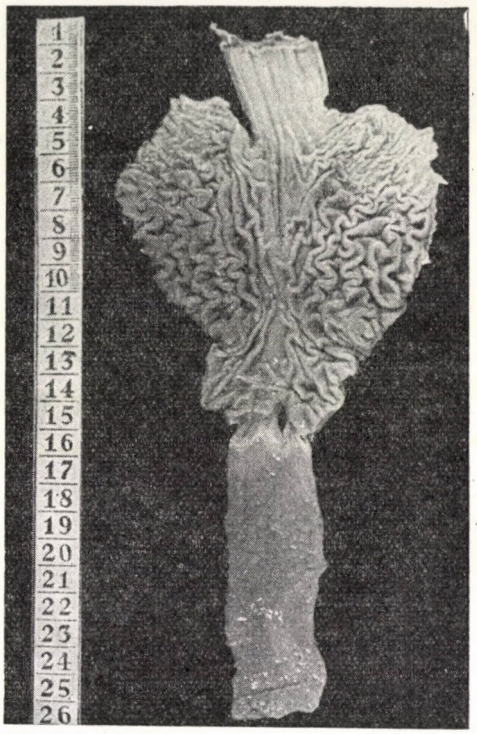

Fig. 7. Circular duodenal ulcer caused by prednisolone + cinchophen treatment. The animal died of perforation on the 17th day of experiment

ing and more or less regular change of the gastric and/or duodenal mucosa was its haemorrhagic tendency (haemorrhagic gastritis).

Cinchophen ulceration was greatly enhanced by the simultaneous administration of prednisolone. In this group there were three perforations and one death due to fatal haemorrhages; such complications did not occur in the control cinchophen ulcer group. In some cases giant ulcers were visible, similar to those found by us in earlier experiments in vagotomized and cinchophen treated dogs [4, 5]. The size of the ulcers can be seen in Figs 1, 2 and 3.

We distinguished two types of prednisolone + cinchophen ulcer.

Type 1. A large, deeply penetrating ulcer as if punched out.

Type 2. A less deep, but giant ulcer not accompanied by gastritis.

Progression of cinchophen ulcer is promoted by prednisolone not only in the stomach, but also in the duodenum. In this way circular penetrating duodenal ulcers may develop. A few ulcers are visible in Figs 4, 5, 6 and 7.

The adrenal glands were weighed at autopsy. In the prednisolone-treated group they weighed less than normal. The animals treated with both prednisolone and cinchophen displayed practically the same adrenal weight as those

treated with prednisolone alone (means, 0.077 and 0.072 g per kg body weight, respectively). Both these groups showed a highly significant difference from the group treated only with cinchophen (mean, 0.123). The statistical method applied was analysis of variance, the commont within-group S.D. 0.0327, ($P < 0.01$).

The control group with its mean of 0.099 differed from the three treated ones. The difference formally proved only borderline cases. Nevertheless, the economic efficiency of experimentation forbade to double the number of animals just to prove again facts well established in the literature.

Discussion

The ulcerogenic effect of prednisolone may be explained in various ways. Possible pathogenic factors are as follows:

1. Prednisolone increases the production of free hydrochloric acid and pepsin. BECK et al. [1] did not observe any significant change in gastric secretory activity in various patients and Heidenhain pouch dogs treated with high doses of prednisolone.

2. Prednisolone may slow down the regeneration of gastric mucosa. Using ^{3}H-thymidine, MYHRE [7] studied the healing of wounds of the gastric mucosa and the reaction of the intact mucosa in cortisone-treated rats. Autoradiography showed that the dynamics of cellular repair in the cortisone-treated animals lagged behind the control ones.

3. Iatrogenic adrenal atrophy. Prednisolone is known to reduce adrenal weight considerably, obviously by the negative feed-back mechanism. It is also known that peptic ulcer is common in patients suffering from Addison's disease. In our earlier experiments DOCA was found to prevent the development of cinchophen ulcer in the duodenum and to diminish it considerably in the stomach [2, 3]. According to our hypothesis prednisolone treatment gives rise to a iatrogenic hypomineralocorticism, which may be one of the factors in prednisolone-induced ulcerations.

If we compare our present results with those obtained in earlier experiments concerned with the effect of cortisone + cinchophen in the dog [2, 3], the ulcerogenic effect of prednisolone appears to surpass that of cortisone. We do not consider the question settled, because the experiments have not been carried out simultaneously.

From our results we have drawn the practical conclusion that prednisolone treatment should be preceded by X-ray examination of the patient's stomach and all those showing an ulcer in the stomach or duodenum should be excluded from such treatment. When higher doses are prescribed, the patient should be kept under close control. Prednisolone by itself may induce ulcer,

though its ulcerogenic effect is far less potent than that of cinchophen. In 3 weeks only 20% of the dogs developed ulcers, which were small and superficial. These results raise the hope that the prednisolone-induced ulcer may be detected in time, if the patients treated with large doses are subjected to X-ray examination at 3 week intervals. If there is ulceration, treatment must be discontinued immediately. As a result of this practice, we had no case of gastrointestinal bleeding or perforation at our Department during the past few years. As signs of iatrogenic adrenal insufficiency following the sudden interruption of steroid treatment we have observed headache, vertigo, weakness, tremor, tachycardia, anorexia and nausea. To prevent the interruption syndrome we suggest the administration of low doses of cortisone, which is less ulcerogenic. Experience has proved that daily 20 to 50 mg of cortisone injected intramuscularly is a suitable dose.

KONJETZNY [6] claimed that in man peptic ulcer would develop on the grounds of inflammation. His view has been adopted by many authors. In response to treatment with prednisolone and cinchophen giant ulcers may develop without gastritis. On the other hand, in other experiments we have observed the development of severe haemorrhagic gastritis without ulcer. In an overwhelming majority of cases prednisolone alone gives rise to haemorrhagic gastritis only. Earlier we found that in the dog DOCA inhibited the ulcerogenic action of cinchophen, but had no influence on gastritis and duodenitis [2, 3]. All these suggest that peptic ulcer and gastritis develop on the basis of two independent mechanisms. The relationship between the two diseases may be similar to that existing between arterial hypertension and arteriosclerosis. They often occur together, but may be present alone, too.

Recently, an increasing number of reports have described giant ulcers in old age. The pathogenesis of the condition is not clear. Earlier, HÁMORI et al. [4, 5] reported on the development of giant gastric ulcers in cinchophen-treated dogs following vagotomy. In the present study, similar changes have been observed following prednisolone + cinchophen treatment. We are fully aware of the fact that the results of model experiments cannot be applied directly to man, yet they offer two possible explanations. One of them is that the vagal nerve ensures eutropism of the stomach. In old age the vagus does not participate to a sufficient extent in the defensive mechanism of the gastric mucosa. Thus, ultimately, the senile giant ulcer may be considered a trophic one. The other explanation may be a significant role of endocrine factors in the pathological process. More closely: there may be a shift in the blood glucocorticoid and mineralocorticoid levels in favour of the glucocorticoids.

*

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REFERENCES

1. Beck, I. T., Fletcher, H. W., McKenna, R. D., Griff, H.: Gastroenterology **38,** 740 (1960).
2. Hámori, A., Nemes, T., Illyés, T.: Excerpta med. Int. Congr. Ser. **51,** 259 (1962).
3. Hámori, A., Nemes, T., Illyés, T.: Magy. belorv. Arch. **17,** 370 (1964).
4. Hámori, A., Scossa, K., Hetényi, G.: Acta med. Acad. Sci. hung. **1,** 120 (1950).
5. Hámori, A., Scossa, K., Hetényi, G.: Magy. belorv. Arch. **4,** 102 (1951).
6. Konjetzny, G. E.: Ergebn. inn. Med. Kinderheilk. **37,** 184 (1930).
7. Myhre, E.: Arch. Path. **70,** 476 (1960).
8. Van Wagoner, F. H., Churchill, T. P.: J. Amer. med. Ass. **99,** 1859 (1932).

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INVESTIGATIONS ON THE EFFECT OF LIVER PREPARATIONS

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The effect of some current liver preparations has been studied in rats. Histology and BSP retention values appeared to show that the preparations prevented and mitigated the severity of carbon tetrachloride induced liver injury. Both in acute and in chronic experiments the effect manifested itself mainly in stimulating regeneration; this was also observed in experimental cirrhosis of the liver.

Extracts of rat liver supposedly regenerating from carbon tetrachloride injury were more effective on carbon tetrachloride poisoned livers than were extracts of intact livers, indicating the existence of a humoral factor stimulating hepatic regeneration.

Since stimulation of regeneration is not always beneficial, the preparations were studied for effects on liver metabolism. Acute and chronic experiments have shown that the liver preparation Solcohepsyl administered both *in vivo* and *in vitro* increases O_2-consumption by both intact and injured liver tissue.

In the treatment of chronic liver diseases the physician can choose between four approaches. One is protection and care of the liver. This includes diet, bed-rest, various lipotropic substances of questionable value, vitamins and antibiotics. Another approach is the therapy with steroids which, due to its influence on connective tissue formation, inflammatory and autoimmunization processes, is in most cases a beneficial symptomatic treatment. The third approach is immunosuppressive treatment with certain cytostatics, chiefly 6-mercaptopurine and azathioprine coming into consideration. The value, however, of this method has not yet been definitely established. Lastly, the physician may apply liver extracts and hydrolysates to which many reports attribute the capacity of stimulating liver regeneration. Despite the long use of such preparations, there is no agreement concerning their value. In some countries, such as the U.K. and the U.S.A. their application is uncommon, while in others, for instance in West Germany, they are widely recommended on account of their diuretic and tonic effect and their ability to increase the serum albumin level and their supposed ability to enhance liver regeneration.

Since in clinical material it is hardly possible to establish objectively the effect of the lastly mentioned preparations, in the last few years we tried to obtain data on the effect of liver extracts and hydrolysates as well as preparations manufactured by both methods, in animal experiments. In our experiments we applied the home-made Sirepar and two Swiss preparations: Ripason and Solcohepsyl. The first can be regarded as a hydrolysate, the second as an

extract and the third as an "extralysate" (produced using hydrolysis and extraction).

In a preliminary experiment, 30 rats weighing 150 to 200 g each received a subcutaneous injection of 0.1 ml of carbon tetrachloride at four-day intervals. From the tenth day on, 10 of these animals were given daily a subcutaneous injection of 0.5 ml of Ripason, and another 10 animals, 0.5 ml of Sirepar. At the end of the third week, all the animals in this experiment were injected intravenously with 0.5 mg per 100 g body weight of bromsulphophthalein (BSP) and killed 45 minutes later by decapitation when blood was collected to determine BSP retention. The liver was examined histologically [9].

The liver of the animals treated only with carbon tetrachloride showed necrosis and fatty degeneration. The histological picture typical of carbon tetrachloride poisoning differed strikingly from that of the animals treated with the liver preparations; in these latter rats the liver was nearly normal, containing no or little fat and displaying no disintegration and necrosis. No appreciable histological difference was detected between the livers treated with liver extract and hydrolysate. At the end of the experiment, mean BSP retention was 33.7% for the control group, and 9.5% and 9.1% respectively, for the treated groups. It was 5.3% for intact rats.

These data showed that both liver hydrolysate and aqueous liver extract are of great value in protecting the liver against carbon tetrachloride injury.

In a second series of experiments 10 animals pretreated with Ripason and another 10 pretreated with Sirepar were poisoned each with 0.1 ml of carbon tetrachloride. A third group of 10 rats received only carbon tetrachloride, to serve as a control. Of each group 5 animals were killed 24 hours, and 5, 48 hours after the carbon tetrachloride intoxication, and the livers were subjected to histology.

This experiment yielded results similar to the first one. The liver of the control rats showed a high degree of hyperaemia, excessive venous dilatation, haemorrhages, degeneration, necrosis, and breakdown of the lobular structure. In the treated animals the histological picture was less severe in that at 24 hours necrosis was less extensive in degree, and at 48 hours it was absent. At 24 and 48 hours, respectively, BSP retention was 56.8% and 26.8% in the control group, 27.5% and 13.6%, respectively, in the Ripason-treated and 17.6% and 6% in the Sirepar-treated rats.

These findings showed that both liver extract and liver hydrolysate afford a certain degree of protection against carbon tetrachloride poisoning.

In the next experimental series [1, 8] were involved 14 control rats, 25 animals treated with carbon tetrachloride alone (0.5 ml/100 g administered 48 hours before measuring BSP retention), and 35 animals treated with carbon tetrachloride plus an 0.5 ml intravenous injection of Solcohepsyl. Fig. 1 shows the BSP retention values as determined by the method of Salingson et al.

The differences between the 0.31 mg% value for the control and the 1.78 mg% and 1.13 mg% values for the two treated groups, respectively, were statistically significant ($p < 0.01$).

In a further series of experiments we studied the liver preparations for their effect in chronic hepatic injury [8].

45 animals were each given 0.1 ml of carbon tetrachloride twice weekly for nine weeks. 15 of them were then treated with 0.5 ml of Ripason and 15

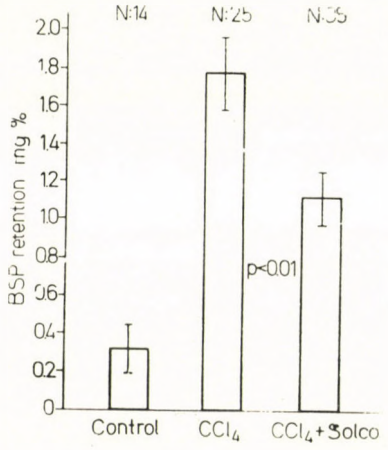

Fig. 1

with the same amount of Sirepar, on a total of 42 occasions. The remaining 15 animals served as controls. Eleven control rats, of all the Sirepar-treated, and 14 of the Ripason-treated animals survived the experiment.

Histology revealed in Group 1 no or slight changes; in group 2, moderate changes; in group 3, severe changes, mostly of degenerative character.

Table I presents the group distribution of the animals.

These investigations appear to show that, as seen in carbon tetrachloride-induced chronic liver injury without cirrhosis, the liver preparations under study were about equally effective in restoring normal conditions.

Table I

	Number of animals treated with		
	carbon tetrachloride	Sirepar	Ripason
Group I	5	9	8
Group II	3	4	4
Group III	3	2	2
Total	11	15	14

In 60 rats cirrhosis was induced by prolonged exposure to carbon tetrachloride. Each animal received 0.1 ml of the drug subcutaneously, twice weekly for four months. Eighteen animals died. The liver of nine of them showed severe cirrhosis, in the rest there was degeneration and diffuse necrosis. The 42 surviving animals were divided into three groups: one was left to serve as control, one received 0.5 ml of Ripason, and 0.5 ml of Sirepar, twice weekly for four months, all in all on 31 occasions [1].

Histology revealed that after carbon tetrachloride had been discontinued, definite hepatic regeneration set in. Although four months after the cessation of carbon tetrachloride treatment accumulation of connective tissue was still seen in seven control animals, the livers of the other seven rats were practically intact. In the Ripason-treated group 11 animals displayed an intact liver; in three cases there was connective-tissue accumulation with round cell infiltration, but much slighter than in the control rats. In the group treated with Sirepar eight livers were intact and in the rest, connective-tissue accumulation was slight.

Table II

No. of animals	Treatment	BSP retention, per cent
14	Carbon tetrachloride	22.8
14	Carbon tetrachloride followed by Ripason	9.9
14	Carbon tetrachloride followed by Sirepar	7.7

These findings permit the conclusion that liver hydrolysates or extracts accelerate and stimulate liver regeneration.

In the last-described experimental series aqueous liver extract and liver hydrolysate were found considerably to reduce BSP retention (Table II).

Accordingly, our findings seem to point to the presence in the tested liver preparations of some unidentified, probably humoral [4, 5, 7] factor capable of stimulating liver regeneration. This is supported by experiments [5, 12, 13] in which such a factor was revealed in the serum and the hepatic venous blood of partially hepatectomized animals, in which the regenerative response is known to be considerable. With a view of establishing the regenerative action of this factor we compared the therapeutic effect of extracts prepared from regenerating livers of carbon tetrachloride-poisoned animals with that of an extract prepared from intact livers.

Chronic liver injury was induced in 80 rats with 0.1 ml of carbon tetrachloride twice weekly, for eight weeks. Nineteen animals died during the experimental period. The remaining 61 rats continued to receive carbon tetra-

chloride. Of these, 11 animals served as control; 25 animals were given the extract prepared from regenerating livers; and another 25 animals, the extract prepared from intact livers.

Histological examination [2, 8] revealed that the extract prepared from the regenerating liver of poisoned animals was considerably more effective in enhancing regeneration than that prepared from intact livers.

Similar experiments were then carried out on a larger scale [3]. 150 rats were each given 0.1 ml of carbon tetrachloride twice weekly for seven months. Sixty-eight animals survived. Eight animals continued to receive carbon tetrachloride, and the rest was divided into three groups of 20. One group was given the extract prepared from intact livers, the other group an extract from intact livers to which carbon tetrachloride was added during hydrolysis, and the third group received an extract prepared from the liver of animals pretreated with carbon tetrachloride.

Histological evaluation of the liver injury, degree of regeneration, of connective-tissue accumulation together with the number of animals lost in the individual groups in the course of treatment, showed that the preparation made with carbon tetrachloride afforded the least protection. A considerably higher protective effect was seen from the extract of intact livers, and the highest, from livers of animals that had been exposed to carbon tetrachloride poisoning for three months [11].

Fig. 2 represents, according to the liver damage suffered, the number of control animals; that of animals treated with extract made of intact livers ("A"); that of animals treated with hydrolyzed intact liver preparation and carbon tetrachloride added to it ("B"); and that of animals treated with extract made of the liver of rats poisoned with carbon tetrachloride ("C").

Even though the experiments so far described seemed to leave little doubt about the ability of liver extracts and hydrolysates to enhance the regenerative process after liver injury. It remained, however, a problem whether it is advisable to stimulate regeneration by therapeutic means. The major role played in portal hypertension by the regenerative nodules compressing the small branches of the hepatic vein, is generally recognized. It is also common knowledge that primary hepatic cancers which develop in the regenerated areas of the cirrhotic liver, are increasing in incidence with lengthening of survival. Thus, the development of both portal hypertension and primary hepatoma seem to some extent be related to liver regeneration. But if with liver preparations we merely stimulate regeneration, shall we actually attain our therapeutic purpose?

Thus, we have studied whether the liver preparations affected the organ in some way in addition to stimulating hepatic regeneration.

In rats, acute poisoning was brought about by a single 0.5 ml/100 g dose of carbon tetrachloride, and chronic poisoning by 0.15 mg/100 g of carbon

tetrachloride dissolved 3 to 10 in oil, administered daily for three weeks. The animals were killed and slices of their liver were placed in Krebs–Ringer phosphate medium. To part of the slices 0.02 ml of Solcohepsyl were added. O_2-consumption was then measured during 60 minutes in a Warburg apparatus.

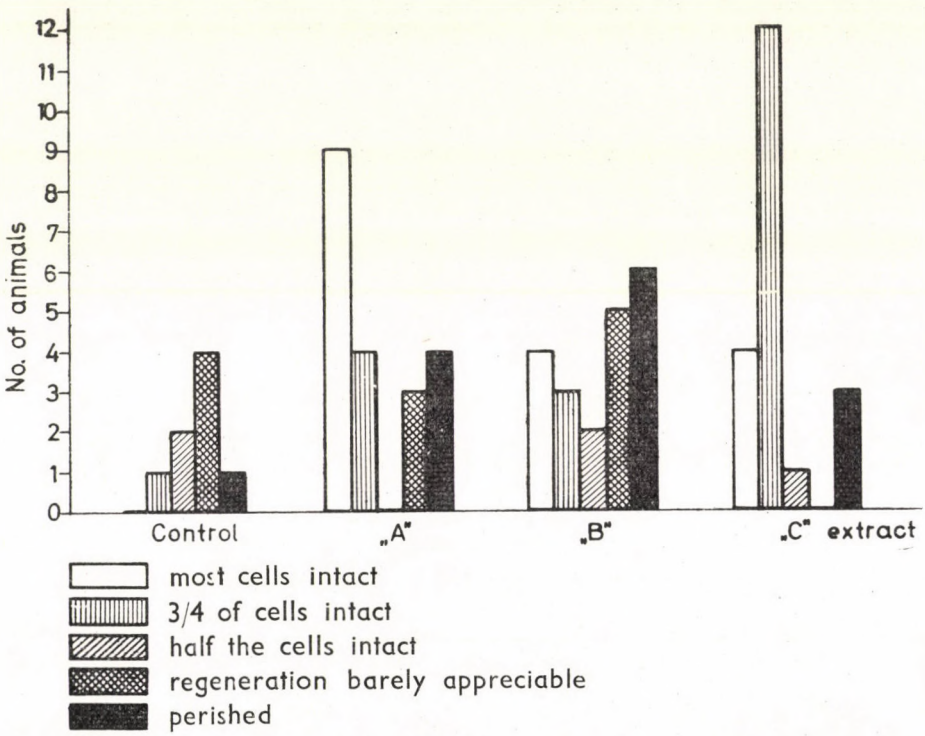

Fig. 2

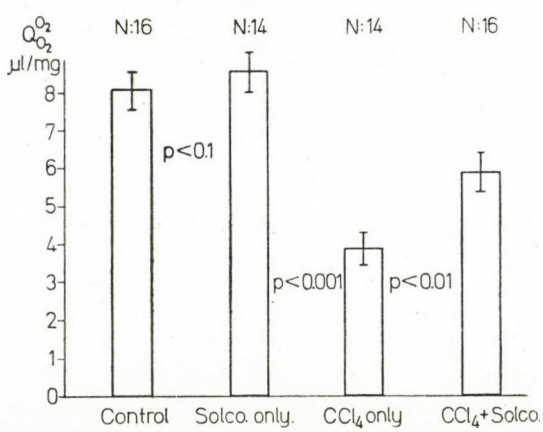

Fig. 3

Solcohepsyl was found to increase O_2-consumption by intact liver tissue. Under the influence of carbon tetrachloride poisoning, O_2 consumption decreased by 50%, but Solcohepsyl significantly increased O_2 consumption by slices obtained from livers with acute or chronic carbon tetrachloride poisoning (Fig. 3).

In another trial [6] rats were given 0.05 ml of Solcohepsyl daily while being subjected to chronic carbon tetrachloride poisoning. O_2 consumption by slices of their liver showed a significant increase (Fig. 4). Hence, Solcohepsyl was found to increase hepatic O_2-consumption both *in vivo* and *in vitro*, in addition to enhancing liver regeneration.

The present results [6] cannot of course be employed unconditionally in the treatment of patients. If for no other reason, then because the 0.5 ml dose of the liver preparations applied to animals in the regeneration experiments, would correspond to about 200 ml for a person of 60 kg body weight; and even the dose of 0.05 ml/100 g administered in the O_2-consumption experiments would mean a 30 ml human dose. Such quantities cannot, of course, be applied in humans. The results may, however, enhance efforts to isolate the active ingredient or ingredients of the liver preparations in pure or at least concentrated form.

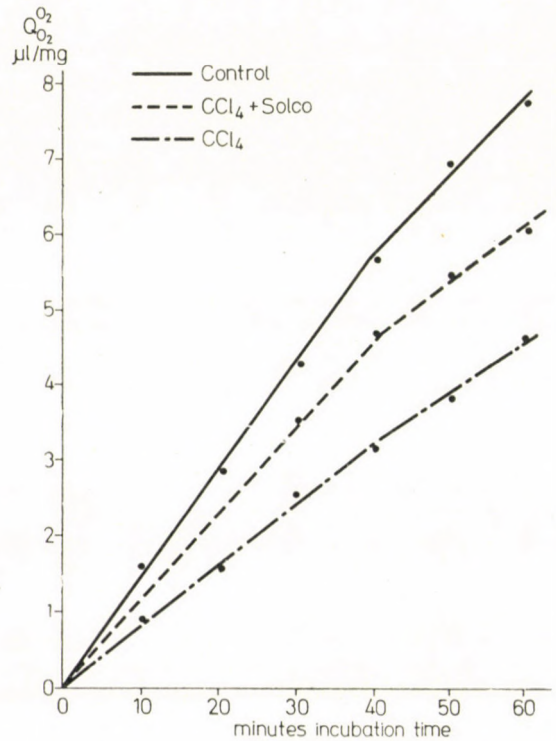

Fig. 4

REFERENCES

1. Balázs, M., Bán, I., Magyar, I., Richter, R. Vécsei, A.: Vizes májkivonat és májhydrolysatum hatásának vizsgálata patkányok széntetrachloriddal létrehozott krónikus májbántalmában. Orv. Hetil. **104,** 342 (1963).
2. Balázs, M., Magyar, I., Richter, R., Valu, J.: Effect of extract prepared from regenerating liver on liver regeneration in rats poisoned with carbon tetrachloride. Gastroenterologia (Basel) **105,** 214 (1966).
3. Balázs, M., Magyar I., Richter, R., Valu, J.: Regenerálódó májból készített kivonat hatása a máj regenerációjára széntetrachloriddal mérgezett patkányokon. Kísérl. Orvostud. **18,** 79 (1966).
4. Fischer, A., Kerekes, E., Bóna, A.: Májregenerációt serkentő humorális tényező kimutatása. Orv. Hetil. **102,** 66 (1961).
5. Glinos, A. D.: Humoral factors in the induction of liver regeneration in the rat. Proc. Soc. exp. Biol. (N. Y.) **80,** 421 (1952).
6. Kovács, Á., Kisfaludy, S.: Effect of liver extract on the metabolism of liver cells. Int. Congr. Gastroenterology, Prague 1968.
7. Leevy, C. M.: Liver regeneration. Arch. intern. Med. **105,** 966 (1960).
8. Magyar, I., Richter, R., Balázs, M., Vécsei, A., Bán, I.: Über die Wirkung von wässrigem Leberextrakt und Leberhydrolysat bei mit Tetrachlorkohlenstoff vergifteten Ratten. Acta hepato-splenol. (Stuttg.) **11,** 267 (1964).
9. Magyar I., Richter, R., Vécsei, A., Bán, I.: Vizes májkivonat és májhydrolysatum hatása patkányok akut és krónikus széntetrachloridmérgezésében. Magy. belorv. Arch. **16,** 37 (1963).
10. Magyar, I., Balázs, M., Bán, I., Richter, R.: Wirkung von Leberextrakten aus geschädigten und gesunden Organen auf die Leberregeneration der mit Tetrachlorkohlenstoff ver gifteten Ratten. Z. Gastroent **1,** 150 (1963).
11. Magyar, I., Richter, R., Balázs, M.: New trends in the therapy of liver cirrhosis. Rec. Advanc. Gastroenterology, Tokyo 1966. Vol. 131, pp. 665.
12. Smythe, R. L., Moore, R. O.: A study of possible humoral factors in liver regeneration in the rat. Surgery **44,** 561 (1958).
13. Torelli, G., Manzotti, M.: Fattori umorali nel processo epato-rigenerativo. Boll. Soc. ital. Biol. sper. **36,** 1785 (1960).

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FATTY ACID POISONING AS A POSSIBLE FACTOR PRODUCING THE SYNDROME OF FAT EMBOLISM

By

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1. The amount of free fatty acid lethal to rabbits was practically the same when administered in a single dose or divided doses. This is indicative of a cumulation of the toxic effect.

2. After administration of 0.50 ml/kg of triolein, no increase in lipase activity of lung tissue was demonstrable.

3. The same dose of neutral fat caused no elevation of the free fatty acid content of blood plasma, lung, liver and brain tissue.

4. When administered intravenously, 0.15 ml/kg of free fatty acid disappeared rapidly from the circulation and was carried for the greatest part to the lung. The concentration of fatty acids in the tissues soon decreased to the normal level.

5. Moderate doses of free fatty acid and unemulsified neutral fat administered intravenously induce considerable oedema and inflammatory reaction in the lung. Free fatty acids elicited these signs immediately while neutral fats gradually. The changes culminated when the majority of neutral fat had already disappeared from the lung.

6. These pulmonary reactions interfere with gas exchange as reflected by the reduction of arterial blood O_2 saturation.

7. The secondary pulmonary changes in the syndrome of human fat embolism are ascribed to the gradual release of free fatty acids from embolized neutral fat in the lung.

8. The consequential arterial hypoxia is regarded as the essential factor leading to the syndrome of fat embolism.

Arterial hypoxia arising in consequence of certain pulmonary changes [11, 18, 19] plays a decisive part in the development of the syndrome of fat embolism. It is, furthermore, known that non-esterified fatty acids are definitely toxic, for instance rabbits rapidly succumb to doses of 250 to 300 mg/kg [10, 15]. The syndrome is characterized by a copious pulmonary exudate indicative of severe damage to the lung capillaries. At autopsy, the lung weighs several times more than normal and there is excessive alveolar and interstitial accumulation of fluid containing numerous erythrocytes [7, 8]. In fat embolism, 70 to 80% of the fat reaching the blood is intercepted by the pulmonary capillaries [17], but it practically disappears from the lungs in a week [16, 17, 22], probably after having been split by the lipase present in pulmonary tissue [6, 1]. The first step of this enzymatic breakdown is obviously hydrolysis of neutral fat, yielding non-esterified fatty acids. Intrapulmonary hydrolysis of fats is proceeding rapidly at a constant rate. For instance of the LD_{50} of triolein in the rabbit, i.e. of 1.0 ml/kg [17], at least 30 to 40% is hydrolyzed

in the first 24 hours. The total amount of free fatty acids presumed to result from the process does, however, barely attain the quantity lethal in a single dose. It has therefore been studied whether the above amount was equally lethal on slow continuous administration or on that divided in several doses. Next, the enzymatic activity of the tissues was studied, to clarify whether the tissues from which mos tof the neutral lipids reaching the blood stream may be recovered show sufficient activity for the hydrolysis of the intercepted fat and whether the activity of these enzymes was enhanced in fat embolism? The subsequent aim was to verify the presence or the possible accumulation of free fatty acids in the lung and brain in experimental fat embolism. Having found that in these organs the free fatty acid content was not affected in experimental fat embolism, we examined the rate at which intravenously injected free fatty acids disappeared from the same organs as a result of enzymatic breakdown or of transport by the blood stream. The last issue of our studies concerned the effect of free fatty acids on lung tissue and their influence on gas exchange.

Material and methods

Rabbits of both sexes weighing between 2 and 4 kg were used. In the first series, 0.07 ml/kg of hydrolyzed bone fat ("Elain", made by Research Institute of Plant Oils and Detergents) was administered intravenously every six hours until the death of the animals. The preparation contains non-esterified fatty acids of which 90% are oleic acid, the rest prevalently palmitic and a small fraction stearic acid. In the second series, the animals were given 0.50 ml/kg (0.516 mM) triolein (half of the LD_{50}) intravenously. Blood samples were taken at 2, 24 and 72 hrs, and then the animals were killed, the individual organs weighed, the free and esterified fatty acid contents estimated in blood plasma, brain and lung. Lipase activity was estimated in lung and liver tissue.

A further group of animals received 0.15 ml/kg of Elain, corresponding to 0.47 mEq/kg of free fatty acid, intravenously. Blood samples were drawn at 5 and 30 minutes, 2, 6, 24 and 72 hours. The animals were killed in successive groups and the free fatty acid content of the blood samples, the lung and liver was determined. In a number of these animals under pentobarbital anaesthesia (25 mg/kg) a polythene catheter was inserted into the exposed femoral artery. After the recovery from anaesthesia the fatty acid was injected and arterial blood samples were taken for O_2 estimation at predetermined times.

The last group of animals received 0.15 ml/kg of ^{131}I-labelled Elain. Attempts at tagging of the hydrolyzed fatty acids by various methods yielded more or less unsatisfactory results. A procedure was finally adopted by which a material was produced containing 8 to 10% of free ^{131}I unattached to fatty acids. Moreover, the physical properties, particularly the viscosity of the material, had suffered some alterations by the labelling procedure, therefore the reliability of the results must be considered as dubious unless corroborated by those obtained in experiments with unlabelled fatty acids.

Chemical methods

The total esterified fatty acid content of blood plasma and tissues was determined by the ferrihydroxamate method of BAUER and HIRSCH [2] with minor modifications. The tissues were minced, homogenized in a glass homogenizer, extracted in a mixture of ether and alcohol, evaporated on a water bath, the residue was redissolved in absolute ethyl ether. Hydroxylamine-HCl was added so as to form hydroxylaminic acid with the esterified fatty acids; this gives with ferriperchlorate a complex of a characteristic red colour suited for colorimetry.

The free non-esterified fatty acids were determined by the method of DOLE [4]. The fatty acids were extracted from the tissue homogenates by means of n-heptane–isopropyl alcohol. In the interest of comparability the fatty acid concentration will be expressed through-

out this study in mEq/kg wet tissue and in mEq/L plasma. The free and esterified fatty acid content of lung tissue per unit body weight (μEq/kg) was also calculated.

For the estimation of tissue lipase the method described by COLOWICK and KAPLAN [3] was adapted to the present requirements. Accordingly, the organs were sliced, dehydrated in acetone, pulverized and added to the reaction mixture in quantities of 50, 100, 150 and 200 mg. Owing to the lack of adequate data in respect to tissue lipase activity, i.e. to the kinetics of the reaction, at least two different amounts were incubated from each sample in a water bath of 37°C, under continuous shaking for 4 hours. The reaction mixture was composed of 1 mM triolein emulsified with polyvinyl alcohol, the pH was adjusted to 7.8 with McIlvaine's phosphate-citrate buffer. After incubation a mixture of acetone and alcohol was added to prevent further hydrolysis. The released fatty acids were titrated with 0.05 N NaOH and the results were expressed in ml alkali used up. Arterial O_2 saturation was determined by means of a Kipp-type Haemoreflector apparatus.

Radioisotope procedures

Following ^{131}I-Elain administration, the tissue samples were weighed, digested with 20% NaOH and the activity was estimated by a well-type scintillation detector. Anorganic ^{131}I was determined from trichloroacetic acid extracts of the tissues. In addition, occasional activity determinations were made from tissue extracts obtained with n-heptane–isopropyl alcohol.

The amount of ^{131}I-fatty acid present in the tissues and in blood plasma were computed from the results and expressed in mEq oleic acid per kg tissue and μEq per kg body weight (i.e. the product of the former value and the weight of the respective tissue per unit body weight).

Results

Lethal doses of fatty acid. In preliminary experiments we found in agreement with PELTIER [10] that 0.28 ml/kg (250 mg/kg) free fatty acid consisting of more than 90% oleic acid killed the animals in a short time.

The features of oleic acid intoxication observed in the present study—blood-stained, frothy nasal discharge, tonic-clonic twitches and convulsions, terminal coma—are in agreement with those described by DE RUITER [12]. Autopsy revealed gross swelling of the lungs and filling of the alveolar and bronchial lumen with abundant haemorrhagic exudate. Subsequently, 6 rabbits were given 25% of the above dose at six-hour intervals on several occasions. All animals died, one after the third (190 mg/kg), and the last after the ninth dose (Fig. 1). The average dose was 343 mg/kg oleic acid. The

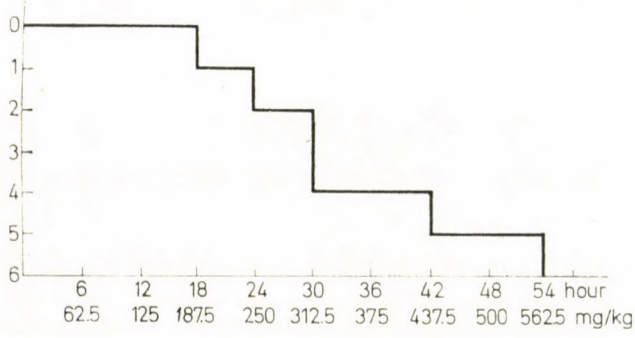

Fig. 1. Effect of free fatty acids administered in divided doses. Abscissa: Number of deaths. Ordinate: Doses of Elain and elapsed times

animals died 10 to 60 minutes after the last dose; the symptoms induced in this manner were similar to those elicited by an undivided lethal dose.

Tissue lipase activities after administration of neutral fat. A significant lipase activity was demonstrated in normal rabbit lung and liver; in 4 hours 600 μEq of free fatty acid was released per 1 g dry lung tissue. The dry weight of lung tissue corresponded to 21.98% (S.D. 3.81) of fresh tissue (33 animals). Lipase activity in the liver was slightly more intensive. While the activity of lung tissue 2, 24 and 72 hours following the intravenous injection of triolein was practically unchanged, activity in liver tissue exhibited a slight increase, for instance a 15% one in the 72-hour sample ($p < 0.05$; Table I).

Table I

Lipase activity (ml 0.05 N NaOH) after intravenous administration of 0.50 ml/kg of triolein

	Lung			Liver		
	100	150	200	100	150	200
	mg acetone dried tissue			mg acetone dried tissue		
Control	(9) 1.17±0.33	(9) 1.80±0.57	(6) 2.38±0.39	(10) 1.58±0.38	(9) 2.19±0.42	(10) 2.76±0.51
At 2 hours	(13) 1.14±0.25	(8) 1.58±0.37	(8) 2.01±0.50	(15) 1.63±0.46	(10) 2.18±0.52	(12) 2.80±0.56
At 24 hours	(7) 1.31±0.19	(4) 1.95±0.27	(4) 2.41±0.57	(8) 1.83±0.46	(8) 2.47±0.47	(8) 3.09±0.52
At 72 hours	(10) 1.32±0.36	(5) 1.96±0.37	(8) 2.34±0.39	(10) 1.97±0.37	(10) 2.46±0.34	(11) 3.03±0.36

(Figures in brackets indicate the number of animals.)

Esterified and free fatty acids in tissues and plasma. In the 10 control animals the total esterified fatty acid content of blood plasma was 6.05 (S.D. 2.95) mEq/L. Practically the same value was found at 2 and 24 hours after intravenous administration of 0.50 ml/kg (i.e. 0.516 mM of triolein), indicating that non-emulsified fat rapidly disappears from the blood stream. The administered amount of fat was recovered practically *in toto* from the lung. The control lungs contained 58 mEq/kg esterified lipids (expressed in triolein) on the average, whereas 2-hour samples revealed 296 mEq/kg. Referred to unit body weight this gives a surplus of 1.64 mEq as compared to the administered 1.55 mEq/kg. The slight difference may be partly ascribed to the determinations having been made in two separate animal groups, partly to a possible inaccuracy of the procedure, the lungs having been weighed together with the bronchi and large vessels. Moreover, slicing of tissues may involve

some fluid loss, particularly in presence of oedema resulting from fat embolism or fatty acid poisoning. Since the lipid content of oedema fluid is presumably low, this would produce an increase in tissue lipid concentration.

At 24 hours there was a considerable reduction in the fatty acid content of the lung. The excess was 43% less than at 2 hours and disappeared by the end of 72 hours. The cerebral tissue exhibited a moderate, statistically not significant ($p < 0.30$) increase in esterified fatty acids at 2 hours (Table II).

There was no significant elevation of titratable acidity in the heptane extracts of blood plasma and of tissues at 2, 24 and 72 hours. In the 13 controls the free fatty acids in the plasma amounted to 619 (S.D. 234) μEq/L. The slight rise at 72 hours averaging 160 μEq/L was not significant ($p < 0.20$). In the lung the free fatty acid content showed no rise, but as reckoned per

Table II

Total triglycerides after intravenous administration of 0.50 ml/kg of triolein

	Blood serum	Lung		Brain
	mEq/L	mEq/kg	μEq/kg body weight	mEq/kg
Control	(10) 6.05±2.95	(12) 58.02±18.25	227.6±103.4	(12) 73.17±19.10
At 2 hours	(13) 6.19±2.47	(14) 296.00±71.35	1868.0±176.6	(14) 115.50±33.61
At 24 hours	(9) 7.24±3.67	(12) 194.00±79.26	1210.0±605.0	(9) 89.90±14.11
At 72 hours	(11) 5.63±2.00	(11) 85.04±23.60	783.8±226.5	(11) 75.43±9.62

(Figures in brackets indicate the number of animals.)

Table III

Free fatty acid content after intravenous administration of 0.50 ml/kg of triolein

	Blood serum		Lung			Brain	
	No.	μEq/L	No.	mEq/kg tissue	μEq/kg body weight	No.	mEq/kg tissue
Control	13	619±234	8	12.04±1.59	55.8± 8.9	7	30.27±5.56
At 2 hours	8	577±201	8	11.14±2.25	75.7±25.3	8	35.28±5.56
At 24 hours	6	581±165	6	9.58±2.14	70.5±13.5	6	32.88±5.89
At 72 hours	6	783±230	6	12.79±1.67	123.0±25.5	6	36.33±2.37

kg body weight it showed a certain elevation at 2 hours owing to the increase in lung weight (Table III).

Fate of free fatty acids after introduction into the organism. After the intravenous injection of 0.15 ml/kg of Elain (approximately 470 μEq oleic acid) this soon disappeared from the organism. Thirty minutes later the plasma contained only 500 μEq/L more of free fatty acid than in the controls, and at 2 hours no elevation was demonstrable.

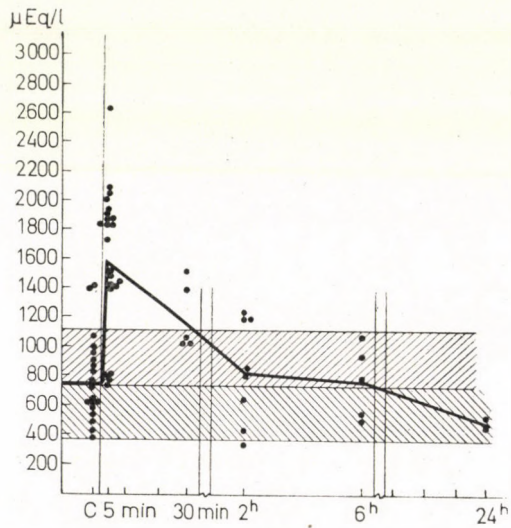

Fig. 2. Effect of 0.47 μEq/kg oleic acid on plasma free fatty acid levels (μEq/l). C: Control values. Horizontal line: Average control values. Shaded area: ± 1 S.D.

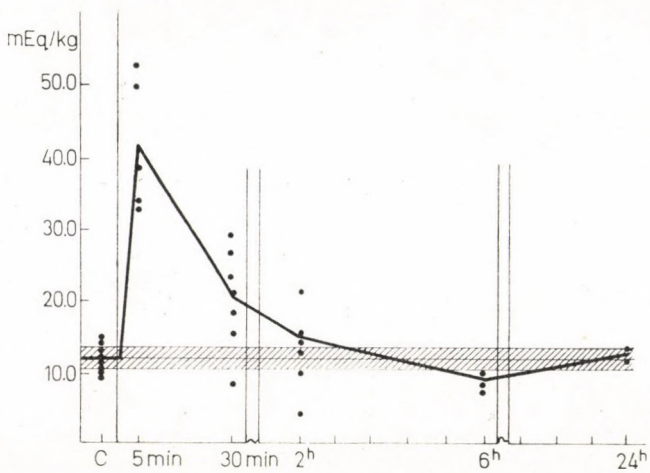

Fig. 3. Effect of 0.47 μEq/kg oleic acid on free fatty acid content of lung tissue (mEq/kg) C: Control values. Horizontal line: Average control values. Shaded area: ± 1 S.D.

Non-esterified lipids introduced into the blood are rapidly intercepted by the lung. The free fatty acid content of lung tissue shows a more than three-fold incrase five minutes after injection as against the controls. Calculated from these values and the relative lung weights, the average amount which may be recovered from the lung from 0.470 mEq/kg free fatty acid injected into the blood is 0.317 mEq/kg (67.4%). In the liver and brain no change in fatty acid concentration was seen. Even from the lung the excess of free fatty acid disappeared fast and by the 30th minute it fell from the average 5-minute value of 28 mEq to 8 mEq and at 2 hours it was barely higher than in the control animals (Table IV).

Studies with ^{131}I-oleic acid revealed comparable figures. The total activity found in blood plasma 5 minutes after administration of 0.15 ml/kg ^{131}I-Elain corresponded to 1292 μEq/L free fatty acid. However, 697 μEq

Table IV

Elain content in mEq/kg after intravenous administration of 0.15 ml/kg

	Blood serum		Lung		Liver	
	No.	μEq/L	No.	mEq/kg	No.	mEq/kg
Control	18	751±370	8	12.04±1.59		
At 5 minutes	19	1595±584	5	41.70±9.41	5	13.77±1.31
At 30 minutes	6	1070±358	7	20.14±7.14	6	14.34±1.55
At 2 hours	9	850±325	5	14.64±8.57	4	11.77±1.94
At 6 hours	4	778±287	3	8.22±1.13		
At 24 hours	2	600	2	12.00		

of this could be recovered from the trichloroacetic acid extract, in other words, it was anorganic iodine. Calculated from the actual fatty-acid-bound (heptane-extractable) activity, the plasma level of ^{131}I oleic acid did not exceed 381 μEq/L and even this decreased to 225 μEq/L by the end of 30 minutes. The radioactive fatty acids were thus cleared from the blood stream perhaps even more rapidly than the unlabelled ones. The predominant part of the introduced activity was again found in the lung. Total activity in this organ corresponded to 0.272 mEq oleic acid, i.e. to 58% of the introduced dose and the fraction extractable with trichloroacetic acid accounted for less than 10% of that amount. At 30 minutes the activity in the lung had fallen by more than 50%.

Ten per cent of the administered dose of ^{131}I-Elain was recovered from the liver, where the labelled fatty acid concentration was approximately 1/20 of that demonstrable in the lung. From the kidney no more than 2 to 2.5% of the activity was recovered, but more than 70% of it was extractable with trichloroacetic acid, indicating the renal excretion of anorganic iodine. Activity

was insignificant in the muscles, brain and thyroid. Activity in brain corresponded to 0.073 mEq/kg (S.D. 0.018) of oleic acid, and of this 0.058 mEq/kg (S.D. 0.041) was extractable with trichloroacetic acid (Table V).

Thus, free fatty acids disappear soon from the blood stream to be taken up by the lung. On the other hand, the pulmonary concentration of fatty acid declines fast, probably in consequence of enzymatic breakdown.

Table V

Activity after intravenous administration of 0.15 ml (0.47 μEq) of ^{131}I-Elain expressed in mEq per kg tissue and μEq per kg body weight of oleic acid

	at 5 minutes				at 30 minutes			
	No.	Total	TCE	HP	No.	Total	TCE	HP
Lung								
mEq/kg	8	35.13	3.74	22.41	8	16.92	2.62	14.60
S.D. ±		11.52	0.71	0.99		9.00	0.80	0.91
μEq/kg	8	272.30	25.53	153.66	8	149.06	19.49	190.83
S.D. ±		12.53	4.25	5.78		91.16	7.18	20.85
Liver								
mEq/kg	8	1.705	0.890	1.383	8	1.232	0.449	1.117
S.D. ±		0.339	0.141	0.360		0.760	0.164	0.197
μEq/kg	8	47.27	24.76	38.66	8	40.40	14.09	37.00
S.D. ±		9.63	3.60	7.25		15.39	6.17	11.03
Kidney								
mEq/kg	8	2.682	1.514	1.032	8	1.489	0.943	0.525
S.D. ±		0.108	0.089	0.395		0.670	0.324	0.278
μEq/kg	8	10.23	7.44	5.32	8	7.95	4.60	2.71
S.D. ±		8.94	0.77	1.37		3.27	1.90	0.77

TCE : trichloroacetic acid extract
HP : n-heptane—isopropyl alcohol extract

It is improbable that significant amounts of free fatty acids should reach the brain whether directly from the administered dose or indirectly from the lung.

Effect of fat embolism and fatty acid poisoning in the lung. In earlier studies we have observed in experimental fat embolism a substantial enlargement and increased density of the lung. The microscopical finding was typical

Table VI

Plasma ^{131}I-fatty acid (as μEq/L oleic acid) level after intravenous injection of 0.15 ml/kg (0.47 mEq/kg) of ^{131}I-Elain

	No.	Total	TCE	HP
At 5 minutes	8	1292±444	697±350	381± 69
At 30 minutes	6	964±305	670±467	225±110

of congestion with significant, prevalently interstitial oedema and patchy intraalveolar accumulation of fluid. After administration of a lethal dose of fat, massive oedema developed all over the lung. In the present study the lung, liver and brain were weighed in animals killed 2, 24 and 72 hours after administration of 0.50 ml/kg triolein (corresponding to 50% of the LD_{50}) and in untreated controls. At 2 hours there was a considerable swelling of the lungs, the average lung weight per kg body weight had nearly doubled. This process continued throughout the experiment, so that for instance at 72 hours the relative lung weight was 9.21 g/kg as against 3.81 g/kg in the controls.

Pulmonary swelling was also significant after the administration of 0.15 ml/kg free fatty acid. At 5 minutes the lungs had doubled their weight, the maximum of 10.76 g/kg was noted at 2 hours. There was microscopic evidence of a haemorrhagic intrapulmonary exudate.

The liver and brain did not change significantly in weight after administration of neutral fat or free fatty acids (Table VI).

Administration of neutral fat or free fatty acids is thus followed by a significant intraalveolar and interstitial fluid accumulation in the lung. At 5

Table VII

Effect of 0.50 ml/kg of triolein on organ weights

	Animal weight (kg)	Lung (g)	Lung (g/kg)	Liver (g/kg)	Brain (g/kg)
Control (n= 13)	3.22 (2.1—4.5)	11.81±1.61	3.81±0.84	29.75±6.91	3.12±0.59
2 hrs (n = 12)	3.35 (2.0—4.2)	20.67±3.98	6.26±0.94	26.50±7.34	3.38±0.68
24 hrs (n = 9)	2.97 (2.0—3.6)	20.11±4.77	6.79±1.48	25.87±2.65	3.21±0.41
72 hrs (n = 11)	2.95 (2.1—4.0)	26.70±7.10	9.21±2.49	28.58±6.25	3.51±0.46

Effect of 0.15 ml/kg of Elain on organ weights

5 min (n = 5)	2.70 (2.3—3.0)	20.03±4.40	7.63±2.23	27.86±1.41	3.51±0.73
30 min (n = 12)	2.64 (2.1—3.0)	21.05±6.34	8.95±1.90	28.17±4.49	3.87±0.53
2 hrs (n = 6)	2.63 (2.1—3.0)	27.97±8.24	10.76±2.72		
6 hrs (n = 3)	4.2 (4.0—4.6)	39.0	9.24 (8.00—10.47)		
24 hrs (n = 2)	4.5 (4.5—4.6)	31.4	6.90 (6.28— 7.53)		

minutes after administration of 0.15 ml/kg Elain, microscopy revealed marked pulmonary oedema with prevalently peribronchial infiltration. The inflammatory changes proved progressive; by the 24th hour they had become predominant in addition to the exudative component.

In the animals sacrificed 72 hours after administration of 0.5 ml/kg triolein, an inflammatory reaction with patchy alveolar oedema was found, in agreement with observations on humans. It must be pointed out that in fatty acid poisoning as also in fat embolism oedema represents a specific tissue response rather than congestive oedema.

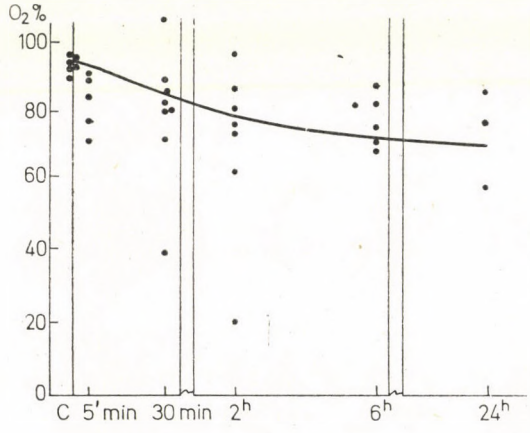

Fig. 4. Effect of 0.47 mEq/kg intravenous oleic acid on arterial O_2 saturation

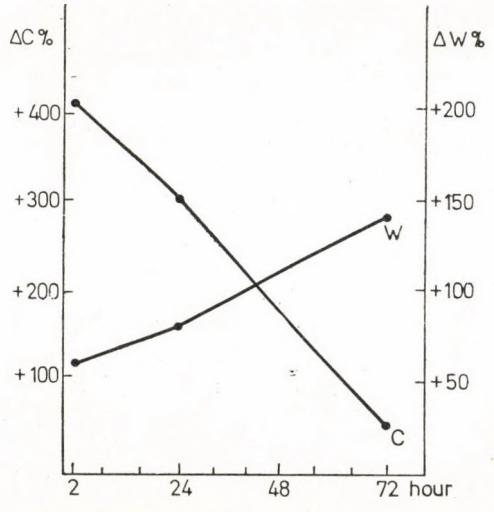

Fig. 5. Elevation of neutral fat content (ΔC) and of relative weight (ΔW) of lung tissue after intravenous injection of 0.50 ml/kg triolein

The effects of experimental fat embolism on gas exchange, especially arterial O_2 saturation, have been dealt with earlier [19]. In the present study the effect of fatty acids was examined.

Arterial hypoxia was demonstrable 5 minutes after fatty acid administration, without, however, attaining a severe degree (apart from a single instance), the average level of arterial blood oxygen saturation having remained above 70% throughout. It is to be noted that the animals had received only 50% of the lethal dose and survived the intervention without exception. Thus, the extent of hypoxia is of less significance than the invariable reduction of arterial O_2 saturation observed in all animals without exception (Fig. 4).

Discussion

The lethal dose of free fatty acid does not noticeably increase by being divided in several doses. As demonstrated earlier [1, 6, 5], the lung is adequately provided with lipolytic enzymes to cope with the accumulation of neutral fats. In opposition to the pulmonary tissue of rats [1], after the introduction of neutral fat that of rabbits failed to exhibit any increase in lipolytic activity. This was, however, of no major significance, since the lipase activity in normal tissues is more than adequate to this task. Thus, in the course of 24 hours following the administration of 0.50 ml/kg triolein the excess of neutral fat demonstrable in the lung was found to diminish by 43%. Under the present experimental conditions, presumably more free fatty acid than the lethal dose was produced during that period. However, neither the blood, nor the tissues including the lung exhibited any rise in free fatty acid content, indicating that the formed free fatty acids disappear rapidly probably as a result of enzymatic decarboxylation. This is consistent with the observation that the disappearance from the lung of intravenously administered free fatty acids is a very rapid process. The disappearance of exogenous fatty acids from the blood stream is no less rapid. As regards the brain, here no significant amounts of exogenous fatty acids were demonstrable, however large the introduced dose had been and whether labelled or not labelled lipid was administered.

Administration of moderate doses of neutral fat was followed by a gradual development of pulmonary oedema. However, considerably smaller doses of free fatty acid were found to induce pulmonary oedema of similar, if not greater, severity in a few minutes and culminating at 2 hours. After the administration of neutral fat, pulmonary oedema attained its maximum at 72 hours, when the greatest part of embolized fat had already disappeared from the lung (Fig5.). This is inconsistent with the interpretation ascribing the arisal of pulmonary oedema to haemodynamic factors, particularly to an elevation of pulmonary capillary pressure in consequence of obstruction. Injury to the pulmonary capillary bed by free fatty acids produced by hydrolysis would seem to offer

a more probable explanation, being also in accordance with the microscopical findings and it is by no means contradicted by the fact that in embolism induced by neutral fat the pulmonary non-esterified fatty acid content did not increase. The pulmonary non-esterified fatty acid content after intravenous administration (similarly to neutral fat, free fatty acid is prevalently taken up by the pulmonary capillaries) declined rapidly, and by the end of two hours the excess lipid practically disappeared from the lung. In pulmonary embolism resulting from the administration of neutral fat, hydrolysis by tissue lipase is gradual and the fatty acids being formed during this process are split at a sufficiently fast rate. Accordingly, an equilibrium is established between hydrolysis and decarboxylation, preventing any significant rise in the concentration of free fatty acid. (A dilution of fatty acids by the accumulating oedema fluid might also have a part in this.)

The released free fatty acids may without any conspicuous rise in tissue concentration damage the capillary wall. The toxic effect of free fatty acids administered in small divided doses has been shown to cumulate, despite the fact that by the onset of acute symptoms the first dose had obviously disappeared from the lung. At any rate, after the successive administration of divided doses the fulminating symptoms which invariably follow the administration of a single lethal dose of fatty acid have remained absent, the syndrome showed a more progressive course. Small doses of free fatty acids induced a syndrome quite similar to that observed after the administration of sublethal doses of neutral fat and characterized by interstitial and patchy alveolar oedema, the counterpart of which is found in human fat embolism. This process may impose more or less severe limitations to alveolar ventilation.

The amount of fatty acid applied in the present experiment caused a significant arterial O_2 desaturation in a short time. Arterial anoxia, on its part, is a decisive factor in the clinical syndrome of fat embolism [19]. In two of our patients, arterial O_2 saturation was as low as 60% at the time of the clinical manifestations.

In fat embolism, progressive lipolysis, i.e. cumulation of the toxic effect on the capillaries and accumulation of oedema fluid takes a certain length of time. This might account for the symptom-free interval of 24 to 48 hours preceding the clinical manifestations of fat embolism.

It does not seem likely that a secondary invasion of the brain by free fatty acids produced in the lung should account for the typical syndrome. On the other hand, intracerebral release of fatty acid resulting from hydrolysis of fat droplets in the cerebral capillaries may well be responsible for some of the symptoms. However, as pointed out earlier [19] arterial anoxia may in itself account for the neurological signs as well as for the typical microscopical pattern including the annular perivascular haemorrhages demonstrable in the brain.

In experimental and clinical fat embolism increased plasma lipase has been reported by several authors and it has been attempted to make a diagnostic use of this finding [5, 9, 14]. Lipase has been assumed to originate from the lung. No increase in pulmonary lipase activity was, however, found in the present study and no relationship could be demonstrated between the change in blood lipase level and fat embolism [16, 20]. Arterial O_2 saturation provides obviously a more reliable clue as regards the severity of the pulmonary process and its possible clinical consequences.

REFERENCES

1. Armstrong, H. J., Kuenzig, C., Peltier, L. F.: Proc. Soc. exp. Biol. (N. Y.) **124,** 959 (1967).
2. Bauer, F. C., Hirsch, E. F.: Arch. Biochem. **20,** 242 (1949).
3. Colowick, S. P., Kaplan, N. O.: Methods in Enzymology. Vol. 1. Academic Press, New York 1955.
4. Dole, V. P.: J. clin. Invest. **35,** 150 (1956).
5. Gomori, G.: Arch. Path. **41,** 121 (1946).
6. Hess, W.: Helv. chir. Acta **15,** 163 (1948).
7. Hirsch, E. F.: Arch. Path. **21,** 765 (1936).
8. Jefferson, N. C., Necheles, H.: Proc. Soc. exp. Biol. (N. Y.) **68,** 248 (1948).
9. Krönke, F.: Langenbecks Arch. klin. Chir. **283,** 466 (1956).
10. Peltier, L. F.: Surgery **40,** 665 (1956).
11. Peltier, L. F.: Surgery **62,** 756 (1967).
12. De Ruiter, H.: Traumatic Fat Embolism. Thesis. Amsterdam 1966.
13. Schüttemeyer, W.: Langenbecks Arch. klin. Chir. **270,** 50 (1951).
14. Schüttemeyer, W., Flach, A.: Chirurg **21,** 289 (1950).
15. Scuderi, C. S.: Surg. Gynec. Obstet. **72,** 732 (1941).
16. Sevitt, S.: Fat Embolism. Butterworths, London 1962.
17. Szabó, Gy., Jankovics, R., Magyar, Zs., Szabó, Á., Szepesházy, K.: Acta med. Acad. Sci. hung. **24,** 299 (1967).
18. Szabó, Gy., Magyar, Zs., Jankovics, R.: Magy. Seb. **21,** 94 (1968).
19. Szabó, Gy., Jankovics, R., Ritthnovszky, Gy.: In press.
20. Titze, A., Friess, W.: Mschr. Unfallheilk. **57,** 33 (1954).
21. Vance, B.: Arch. Surg. **23,** 426 (1931).

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RÖNTGENKINEMATOGRAMMETRISCHE ANALYSE DER BRONCHIALBEWEGUNGEN

Von

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Die Röntgenkinematographie hat zur Untersuchung der Bewegungsfunktionen, zur Analyse ihrer Gesetzmäßigkeiten und Mechanismen der wissenschaftlichen Forschung und auch der praktischen Anwendung der Diagnostik weite, exakt-methodische Perspektiven eröffnet.

Ziel der vorliegenden Arbeit war das röntgenkinematographische Studium der respiratorischen Bewegungserscheinungen des tracheobronchialen Systems in Tierversuchen. Es wurde festgestellt, daß die Gesetzmäßigkeiten der Bewegungen, der Charakter ihres physiologischen oder pathologischen Verlaufes an Hand der methodischen Analyse des Röntgenkinematogramms mit überraschender Ausführlichkeit erfaßbar sind. Ausdrücklich zu betonen ist, daß eine wiederholte Beobachtung der kontinuierlichen Bewegungserscheinungen am laufenden Filmstreifen keineswegs hinreichend ist, sondern eine unerläßliche Forderung daneben auch die metrische Analyse der auf Grund der Messung der Längen-, Kaliber- und Lokalisationsänderungen der Bronchien an vergrößerten Einzelbildern der Serie konstruierten Bewegungskurven darstellt.

In der Röntgendiagnostik hat sich von Anfang an parallel mit der Entwicklung der statischen Röntgenmorphologie und in engem Zusammenhang mit ihr eine dynamische Röntgenmorphologie herausgebildet: die die Röntgensymptomatologie der verschiedensten Bewegungserscheinungen umfassende sogenannte funktionelle Röntgendiagnostik. Beide zusammen bilden eine unzertrennliche Einheit. Die Möglichkeit der Untersuchung der Bewegungsfunktionen auf röntgenmorphologischer Grundlage ist von großer Bedeutung nicht nur in der diagnostischen Praxis, sondern auch auf dem Gebiete der wissenschaftlichen Forschung. Zahlreiche physiologische und pathophysiologische Motilitätsprozesse sind als Ergebnis röntgenologischer Untersuchungen zutage gefördert und ihr Wesen und Mechanismus geklärt worden.

Einen neuen Weg in den Möglichkeiten der Untersuchung und des Studiums der Bewegungsfunktionen haben vor rund anderthalb Jahrzehnten die auf elektronenoptischen Prinzipien fußenden modernen röntgentechnischen Konstruktionen eröffnet. Die Entdeckung des Röntgenbildverstärkers hat die Durchleuchtung bei wesentlich verringerter Strahlenbelastung am Bildschirm mit hoher Lichtstärke und dann am Fernseh-Monitor, sowie die Einführung der Röntgenkinematographie ermöglicht. Auf diese Weise sind auch Bewegungserscheinungen in ihrer Kontinuität gut sichtbar dem Studium zugänglich gemacht worden, die zuvor am klassischen Durchleuchtungsschirm verborgen blieben. Andererseits können sie mit Hilfe der Röntgenkinematographie am

Film oder mit Video-Telerekorder am magnetischen Band in ihren feinsten Einzelheiten festgehalten werden und sind beliebig oft reproduzierbar. Es gibt eine breite Skala von Möglichkeiten, die sich uns durch die Röntgenkinematographie nicht nur in der diagnostischen Praxis, sondern auch in der wissenschaftlichen Forschung darbieten.

Ausgehend von jener, sich für eine statisch-dynamische Einheit aussprechenden Anschauung, daß die morphologischen Veränderungen pathologisch-funktionelle Erscheinungen zeitigen können und umgekehrt, pathologische Funktionen Veränderungen des morphologischen Bildes nach sich ziehen können, haben wir uns in unseren Untersuchungen das Studium der Bronchusbewegungen zum Ziele gesetzt. In der Röntgenuntersuchung des tracheobronchialen Systems muß der Beobachtung und diagnostischen Beurteilung der funktionellen Röntgensymptome eine große Bedeutung beigemessen werden. Als eine entferntere Aufgabe hatten wir beabsichtigt das Studium und die Analyse der Erkrankungen der Bronchien, namentlich eventueller früher funktioneller Röntgensymptome des Bronchuskarzinoms, und als nähere Aufgabe, gewissermaßen als Vorstudien, die physiologischen Bewegungen und die künstlich hervorgerufenen, aphysiologischen Bewegungsänderungen des tracheobronchialen Systems zum Gegenstande eingehender Untersuchungen zu machen. Es war überraschend, wie gering die Zahl der sich mit der Röntgen-Bronchokinematographie befassenden Arbeiten in der Weltliteratur ist. Hervorzuheben sind darunter die Untersuchungen von BONSIGNORE, PICONE und BELLOMONTE [1, 2, 3, 4], YAMAMOTO und SASAKI [9], sowie die Mitteilungen von FRASER [5], HOLDERN und ARDRAN [6], MARSHALL und HOLDERN [8]. Diese Autoren haben über Beobachtungen an gesunden Menschen, meistens aber an Patienten mit verschiedenen bronchialen Erkrankungen berichtet. Sie betonen die Bedeutung der Röntgenkinematographie in der funktionellen Röntgendiagnostik der Erkrankungen des Bronchialsystems, doch stützen sich ihre Feststellungen meistens auf die laufende Beobachtung des Filmstreifens. Einer Analyse der aufeinanderfolgenden Bilder des Filmstreifens auf Grund von Messungen begegnen wir nur in den Arbeiten ganz weniger Autoren, wie z. B. bei FRASER, YAMAMOTO und SASAKI, MARSHALL und HOLDERN. Als Bahnbrecher im Ausbau der technischen und methodologischen Entwicklung der Röntgenkinematographie und u. a. in der Initiative der Broncho-Röntgenkinematographie ist JANKER [7] zu nennen.

Abweichend von den obigen Autoren haben wir versucht, auf tierexperimentellem Wege den Grundlagen des Mechanismus der Bronchienbewegungen näher zu kommen. Zu diesem Zweck haben wir bei Hunden in Pentobarbital-Narkose nach der bronchographischen Auffüllung mit Bariumsuspension oder Dionosil mit Hilfe eines Siemens-Bildverstärkers 7" mit der 35 mm Arriflex-Kamera kinematographische Aufnahmen angefertigt. Neuerdings führen wir die Untersuchungen mit einer 16 mm Arriflex-Kamera am Siemens Sirecon

duplex Bildverstärker durch. Neben den physiologischen Bewegungen haben wir die auf die intrabronchiale Verengerung eintretenden Bewegungsänderungen studiert. Die bronchokinematographischen Untersuchungen an den Kranken beschränkten sich namentlich auf die Beobachtung der tumorösen Bronchusstenosen und die durch schwere, deformierende Bronchitis bedingten funktionellen Röntgensymptome. Die Bewegungsanalysen wurden teils auf einem Montagetisch durch wiederholte Beobachtung der kontinuierlichen Bewegungserscheinungen und andererseits an Hand von Meßanalysen an vergrößerten Standbildserien der aufeinander folgenden Bewegungsphasen auf Photopapier, neuerdings am Documator-Lesegerät (Zeiss) durchgeführt.

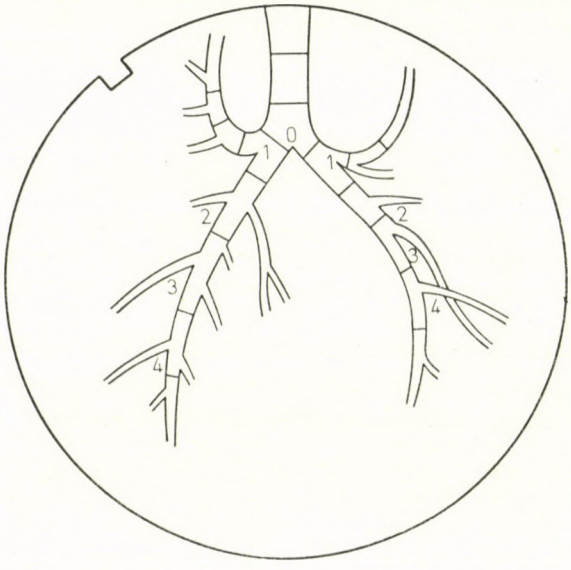

Abb. 1. Schema des tracheobronchialen Systems des Hundes

Im folgenden soll an einigen der Versuchsserie entnommenen Beispielen ein Bild über die von der Röntgenkinematographie gebotenen Möglichkeiten zur Analyse der Bronchusbewegungen dargestellt werden.

Abb. 1 veranschaulicht schematisch das tracheobronchiale System eines Hundes bei postero-anteriorer Strahlenrichtung im Blickfeld des Bildverstärkers. Die Bestimmung der Bronchienlänge und des Lumendurchmessers geschah durch Messung der Länge bzw. des Durchmessers der Strecken zwischen den mit Ziffern bezeichneten Abzweigungen. Nach Darstellung der Meßdaten im Koordinatensystem in Abhängigkeit von den Zeitpunkten der aufeinanderfolgenden Bewegungsphasen entstanden für die Inspirations- und Exspirations-Längen und Kaliberänderungen der Bronchien charakteristische Kurven. Die Bronchien als annähernd zylinderförmig aufgefaßt, wurde das Gebiet des

Lumen-Querschnittes berechnet und in Kurvenform dargestellt. An dem so erhaltenen Graphikon können die zeitlichen und Größenzusammenhänge der respiratorischen Bewegungsveränderungen festgehalten und objektiv analysiert werden.

Auf Grund der durchgeführten Tierversuche sollen vier Typen der röntgenkinematographischen Aufnahmeserien der respiratorischen Bronchienbewegungen und der davon hergestellten Bewegungskurven dargestellt werden.

Versuch 1. Physiologische Atmung. An der röntgenkinematographischen Aufnahmeserie (Abb. 2), wo jedes 8. Bild des mit 24/sec-Geschwindigkeit aufgenommenen Films darstellt, sind die inspiratorische Verlängerung der Bronchien und die Weitung ihrer Kaliber bzw. ihre exspiratorische Verkürzung und Verengerung deutlich sichtbar. Dies ist der typischste Vorgang der respiratorischen Bronchialbewegungen. Die Längen- und Kaliberänderungen sind an der rechten und an der linken Seite gleichen Ausmaßes. Neben diesen Bewegungen fällt noch besonders ausgesprochen der Lokalisationswechsel in seitlicher Richtung in den Bronchusästen des oberen Lappens ins Auge.

Das Graphikon der Bewegungskurven (Abb. 3) demonstriert überzeugend, wieviel größeren Aufschluß diese über die Einzelheiten des Vorganges der respiratorischen Bronchusbewegungen bieten, als die einfache Betrachtung der röntgenkinematographischen Aufnahmeserie. Die Säulen stellen die Länge der Bronchien bzw. deren Veränderung, die unteren Kurven den Wechsel des Kaliberdurchmessers und die oberen Kurven die Veränderungen des Flächenraumes des Lumenquerschnittes dar. Das Graphikon läßt feststellen, daß sich beim Maximum des Inspiriums die Bronchuslänge um 35% vergrößert, die Lumenerweiterung mehr als das Doppelte des Exspiriumsdurchmessers und die Vergrößerung des Querschnittes das 5-fache erreicht hat. Es ist auch ersichtlich, daß unter physiologischen Verhältnissen das Maß der Bewegungsveränderungen und der zeitliche Ablauf an der rechten und linken Seite völlig identisch sind.

Versuch 2. Verengerung des rechten Hauptbronchus. Die Verengerung erfolgte durch Einführen einer mit einem Bleiknopf versehenen Sonde. Die röntgenkinematographische Aufnahmeserie (Abb. 4) ließ feststellen, daß an der eingeengten Seite neben der inspiratorischen Verlängerung der Bronchien (schwarze Säule) eine Lumenerweiterung nicht zustandekommt. Gleichzeitig ist an der intakten Seite nur eine Lumendilatation — ohne Bronchusverlängerung — wahrnehmbar. Die Trachea wird während des Inspiriums im Bogen zur verengten Seite gedrängt, während die Bronchien des unteren Lappens sich lateralwärts heben.

Am Graphikon der Bewegungskurven (Abb. 5) sind diese Erscheinungen metrisch analysierbar. Es kommt während des Atemprozesses zur mäßigen inspiratorischen Verlängerung der Bronchien, zur Verringerung des Kalibers und noch eher des Querschnittes. An der intakten Seite ist zu Beginn des

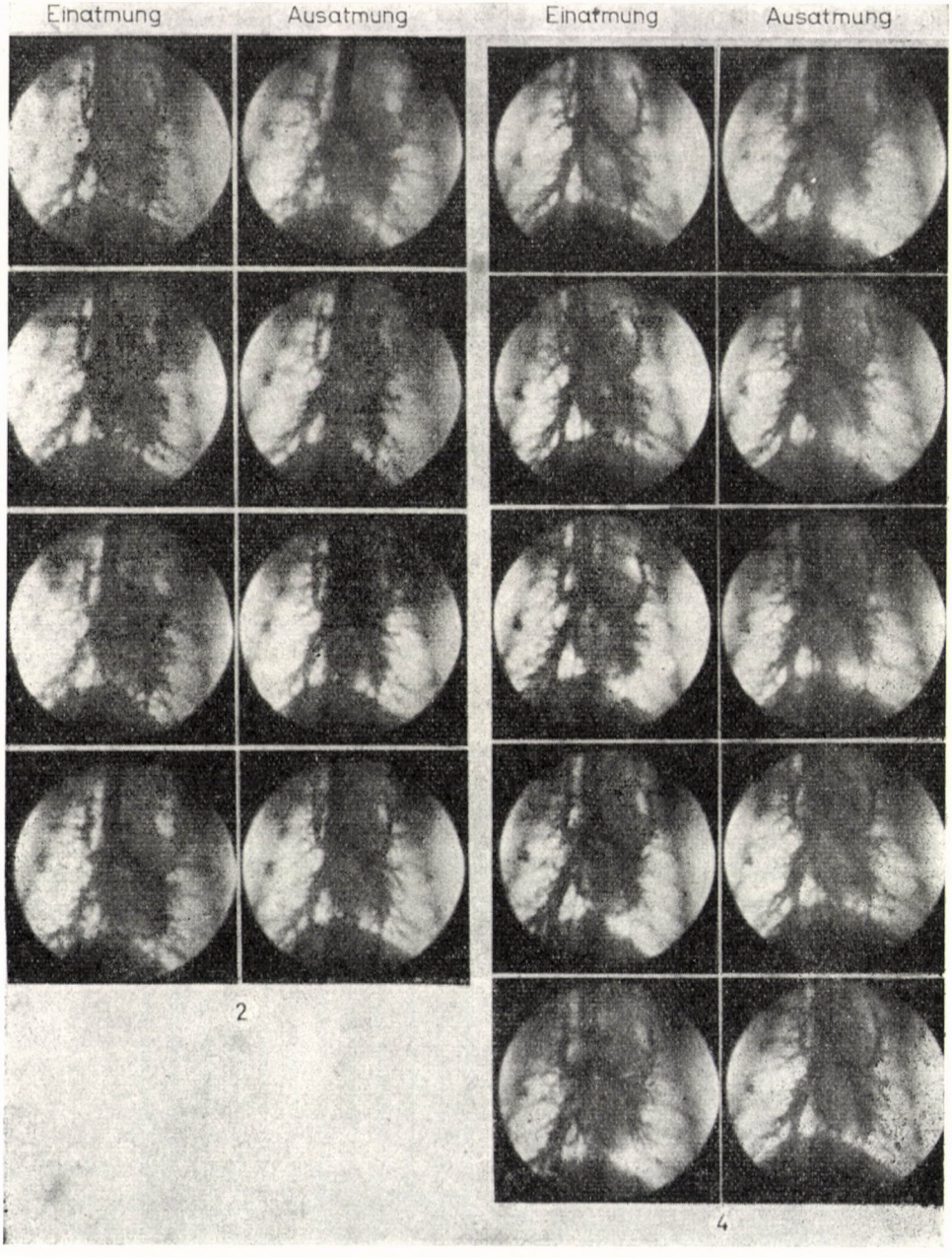

Abb. 2. Röntgenkinematogramm. Zwischenzeiten 1/3 sec. Normale Atmung
Abb. 4. Röntgenkinematogramm. Zwischenzeiten 1/3 sec. Verengerung des rechten Hauptbronchus

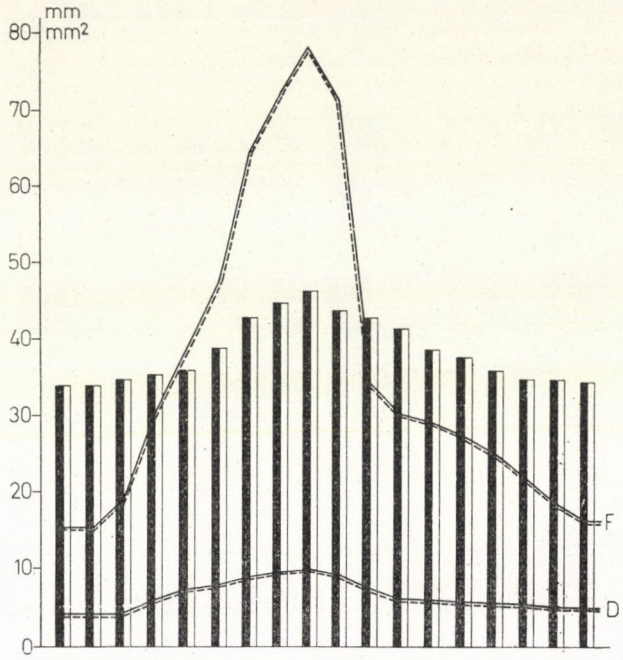

Abb. 3. Respirationskurve des Röntgenkinematogramms in Abb. 2. Zwischenzeiten 1/6 sec. (Bezeichnungen: D: Durchmesser des Bronchus; F: Durchschnittsfläche des Bronchus; Säulen: Länge des Bronchus; ————————: rechte Seite, — — — — —: linke Seite)

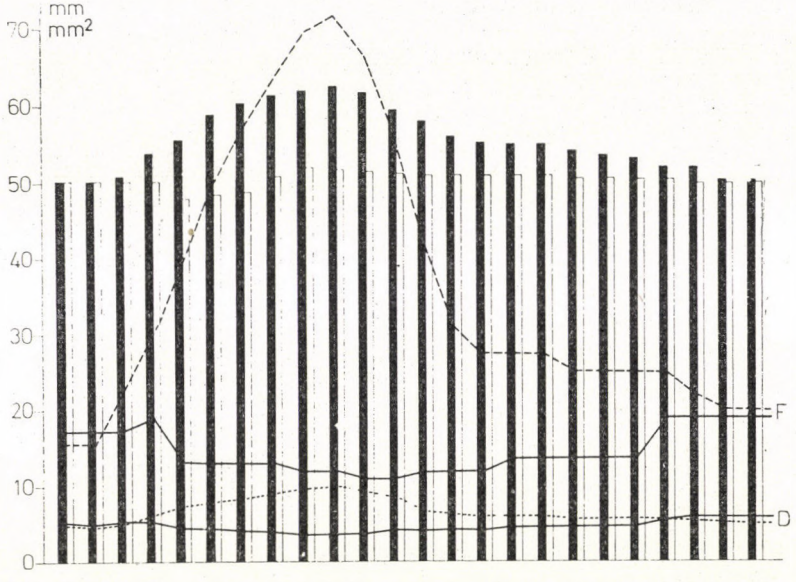

Abb. 5. Respirationskurve des Röntgenkinematogramms in Abb. 4. Zwischenzeiten 1/6 sec.

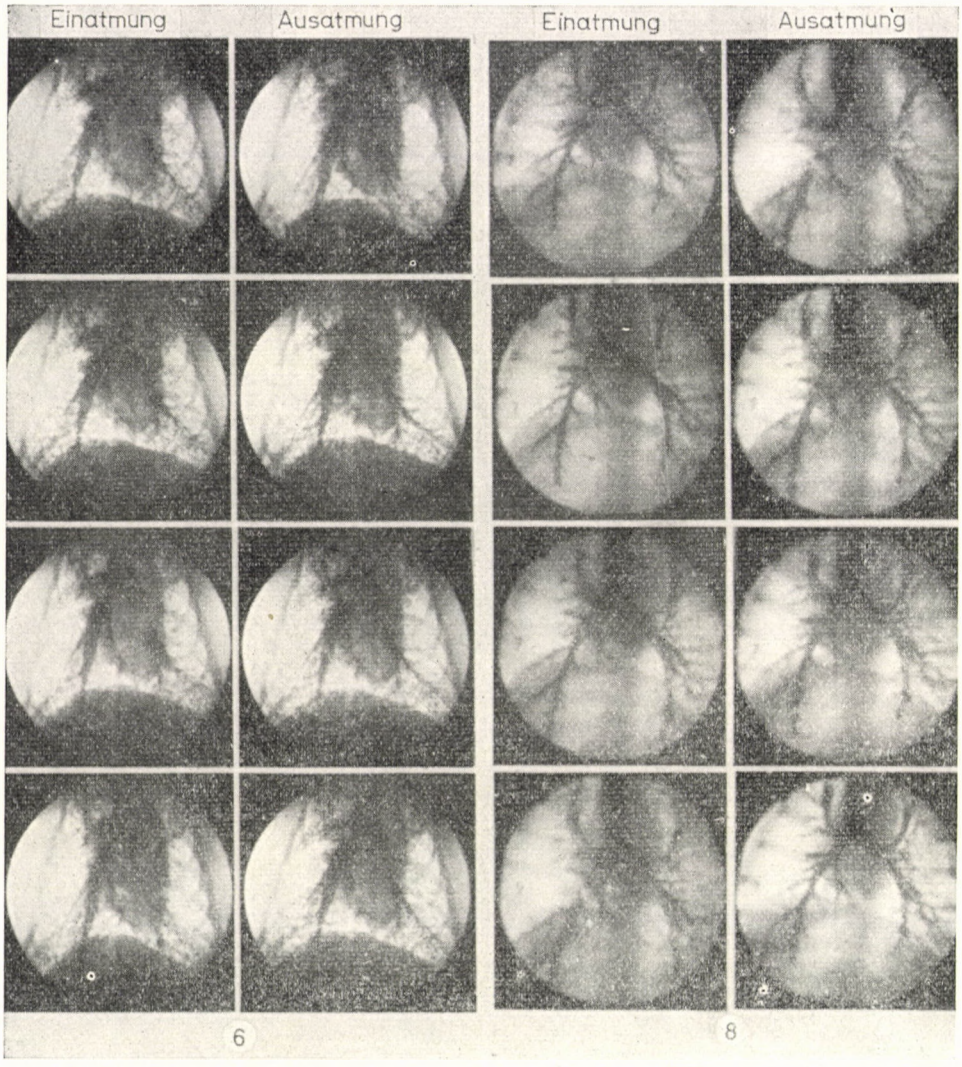

Abb. 6. Röntgenkinematogramm. Zwischenzeiten 3/16 sec. Verschluß des rechten Hauptbronchus

Abb. 8. Röntgenkinematogramm. Zwischenzeiten 1/3 sec. Verengerung der Trachea

Inspiriums eine geringgradige Verkürzung und dann eine leichte Verlängerung des Bronchus nachweisbar. Während die Lumenerweiterung einen zweifachen Wert erreicht hatte, war der Querschnitt auf das 4,5-fache gestiegen.

Versuch 3. Verschluß des rechten Hauptbronchus. Der Schattenausfall im Bronchus-Intermedium (Abb. 6) in der röntgenkinematographischen Aufnahmeserie zeigt, daß nur der Bronchus des unteren Lappens verschlossen, der obere Bronchus aber frei ist. Beim Einatmen erfährt der verschlossene

rechte untere Bronchusast überhaupt keine Erweiterung und eine minimale Längsdehnung; der rechte obere Bronchus erweitert sicht gut. An der intakten Seite ist eine geringgradige inspiratorische Bronchusverlängerung und eine sehr bedeutende Lumenerweiterung zu beobachten.

Am Graphikon der Bewegungskurven (Abb. 7) sind diese Erscheinungen in metrischer Darstellung analysierbar. Augenfällig ist die Lumendilatation

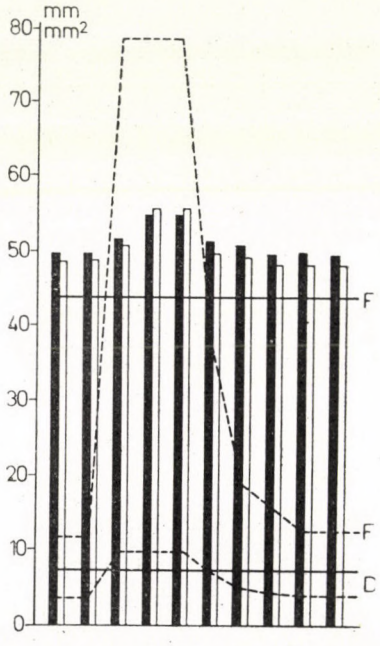

Abb. 7. Respirationskurve des Röntgenkinematogramms in Abb. 6. Zwischenzeiten 3/16 see.

des Bronchus der intakten Seite und die Querschnittvergrößerung auf das 6,7-fache.

Versuch 4. Trachea-Verengerung. Die Verengerung geschah durch Abklemmen eines in die Luftröhre eingebundenen Gummirohres. In der röntgenkinematographischen Aufnahmeserie (Abb. 8) fällt die symmetrische Verengerung des Bronchussystems im Vergleich zur Norm sowie die beträchtliche, — aber die Normalwerte nicht überschreitende — ebenfalls symmetrische Längsdehnung der Bronchien auf. Ein interessanter Befund ist noch die ausgesprochene Vergrößerung des Bifurkationswinkels und die Divergenz der Bronchusäste der Gegenseite. Beim Einatmen bewegt sich die Bifurkation tief abwärts.

Die Bewegungskurve (Abb. 9) zeigt ein interessantes Bild. Während des Inspiriums erfolgt die Verlängerung allmählich und protrahiert, um bei dessen

Maximum plötzlich in die Verkürzung während des Exspiriums umzuschlagen, die dann ebenfalls protrahiert wird. Die Erweiterung der Lumendurchmesser tritt dagegen schneller ein, und die Periode der maximalen Dilatation ist anhaltend verlängert. Noch deutlicher tritt dies an der Querschnitt-Kurve zutage.

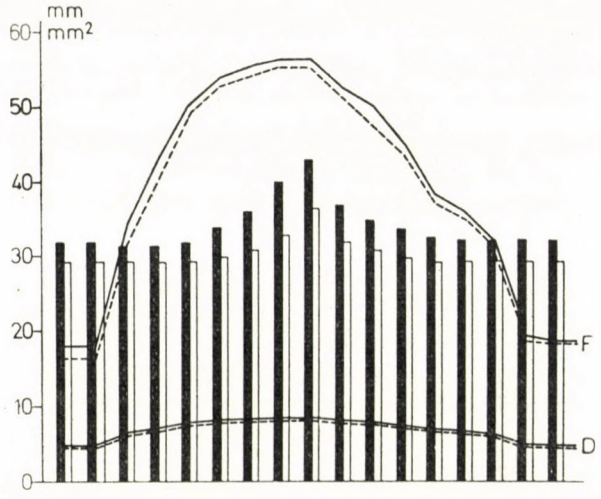

Abb. 9. Respirationskurve des Röntgenkinematogramms in Abb. 8. Zwischenzeiten 1/6 sec.

Tabelle 1

Die Werte der beim Maximum des Inspiriums beobachteten bronchialen Bewegungsänderungen (Prozent und Multiplikationszahl)

	Verlängerung des Bronchus		Erweiterung des Bronchus		Vergrößerung des Bronchusquerschnittes		Erweiterung der Trachea
	Rechte Seite	Linke Seite	Rechte Seite	Linke Seite	Rechte Seite	Linke Seite	
Normal	+35% 1,35	+35% 1,35	+120% 2,2	+120% 2,2	+400% 5,0	+400% 5,0	minimal fast 0
Verengerung des rechten Hauptbronchus	+25% 1,25	−5% +5% 0,95 1,05	−20% 0,8	+100% 2,0	−35% 0,65	+350% 4,5	+10% 1,1
Verschluß des rechten Hauptbronchus	+10% 1,1	+15% 1,15	0% 1,0	+160% 2.6	0% 1,0	+570% 6,7	+25% 1,25
Verengerung der Trachea	+35% 1,35	+35% 1,35	+80% 1,8	+80% 1,8	+210% 3,1	+210% 3,1	+20% 1,2

Die Konklusionen unserer Untersuchungen lassen sich folgendermaßen zusammenfassen:

1. Die experimentelle Bronchokinematographie hat sich als eine höchst geeignete Methode zum Studium sowohl der physiologischen, als auch der pathologischen Bronchusbewegungen erwiesen.

2. Die Auswertung der röntgenkinematographischen Aufnahmen mittels einfacher visueller Beobachtung kann hinsichtlich der Feinheiten kein befriedigendes Ergebnis liefern, weil die Charakteristika der schnell entfliehenden und ineinander übergehenden Bewegungen und ihre Veränderungen mit dem Auge kaum zu verfolgen sind und so nicht entsprechend bewertet werden können. Zur Analysie der Gesetzmäßigkeiten der respiratorischen Bronchienbewegungen ist außer der mehrmaligen Beobachtung des laufenden Filmstreifens die ausführliche metrische Analyse der aufeinander folgenden Bewegungsphasen mit Hilfe der auf Grund der Ausmessung der jedes 3., 4. oder 5. Bild der röntgenkinematographischen Aufnahmen enthaltenden, vergrößerten Bildserie eine unerläßliche Forderung.

Unseres Erachtens beweisen die geschilderten vier Versuchstypen mit hinreichender Überzeugung die Bedeutung der Röntgenkinematographie und besonders den Wert der Kinematogrammetrie im Nachweis der physiologischen oder pathologisch veränderten Bewegungsphasen und in der Erforschung ihres Mechanismus.

*

Die experimentellen Untersuchungen wurden unter Mitarbeit von J. Ökrös durchgeführt.

LITERATUR

1. Bonsignore, G., Picone, F., Bellomonte, F.: Riv. sicil. Tuberc. **14**, 53—71 (1960).
2. Bonsignore, G., Picone, F., Bellomonte, F.: Riv. sicil. Tuberc. **15**, 195—222 (1961).
3. Bonsignore, G., Picone, F., Bellomonte, F.: Riv. sicil. Tuberc. **16**, 112—123 (1962).
4. Bonsignore, G.: Riv. sicil. Tuberc. **15**, 173—187 (1961).
5. Fraser, R. G.: J. Canad. Ass. Radiol. **12**, 102—112 (1962).
6. Holdern, W. S. und Ardran, G.: Kongreßber. 2. Tg. med. wiss. Ges. Röntgenol. DDR, S. 116—118 (1958).
7. Janker, R.: Röntgenologische Funktionsdiagnostik, Girardet, Wuppertal-Elberfeld 1954.
8. Marshall, R. und Holdern, W. S.: Thorax **18**, 54—58 (1963).
9. Yamamoto, K. und Sasaki, T.: Dis. Chest **41**, 79—84 (1962).

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POSTIRRADIATION CHANGES IN ULTRASTRUCTURE AND ENZYME CYTOCHEMISTRY OF RAT THYMUS

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Cytochemical changes induced in the rat thymus by sublethal X-ray irradiation have been studied by means of light and electron microscopy and starch-gel electrophoresis.

The reticular cells were found to exhibit different acid phosphatase and non-specific esterase activity ratios. These ratios were not affected by the irradiation, though the intensity of the individual activities increased.

On the evidence of starch-gel electrophoresis X-ray irradiation was found to affect the isozyme spectrum of the thymus qualitatively as well as quantitatively.

Electron microscopic studies revealed that the dense bodies of the mesenchymal reticulum as well as the moniliform material of the epithelial reticulum are sites of acid phosphatase activity and, by this fact, identifiable as lysosomes.

The mesenchymal reticular cells accumulate large amounts of lipid subsequent to irradiation.

The origin and functions of the individual cell elements making up the framework of the thymus are still open to controversy. A static approach to these questions by the study of the normal gland provides little information unless combined with observation of the gland under various experimental conditions. It is a well established fact [3, 10, 12, 18, 19, 20, 21, 22] that total body irradiation in rats is followed by typical changes in the thymus; destruction of the majority of thymocytes occurs within 24 to 48, but often in the first hours. The place of these cells is occupied by the more resistant reticular elements which will preserve fragments of phagocyted thymocytes for a long time.

Irradiation of rats provides optimal conditions for the study of cells making up the framework of the thymus, not only because depletion of thymocytes brings the cells of the basic structure of the gland into prominence but also because under the stimulus of disintegration these cells, still capable of endocytosis, exhibit maximum activity. Under such conditions the reticular cells with different function are expected to reveal distinctive features.

These considerations have prompted us to submit thymuses of rats having received total body irradiation to electron microscopic, enzyme-histochemical and isoenzyme studies.

Material and methods

Female albino Wistarrats ranging in weight from 70 to 80 g were given a sublethal radiation dose of 450 r (180 kV, 4 mA, 0.5 Cu filter, 50 cm STD), and were killed successively at determined intervals between the fourth hour and the eleventh day after irradiation.

For light-microscopical enzyme-histochemical studies the glands were fixed overnight in cold neutral 4% formaldehyde containing 0.22 M sucrose, washed twice each time for one hour in 0.22 M cold sucrose, placed for 24 hours in Holt's gum-sucrose, frozen by means of carbon dioxide, then cryostat sections of 6μ were cut.

For the demonstration of acid phosphatase activity naphthol-AS-BS-phosphate was used as a substrate and freshly diazotized 4-chloro-2-toluidine as the coupler in Michaelis's veronal acetate buffer pH 5. The nonspecific esterase incubating medium contained naphthol-AS-D-acetate as the substrate and Fast Blue 2B stabilized diazonium salt as the coupler in 0.1 M tris-buffer, pH 7.1. The specimens were incubated at room temperature. The two enzymes were demonstrated in the same preparation. Acid phosphatase activity was indicated by a red, that of nonspecific esterase by a blue end product. Details of the method were described earlier [1, 2, 21].

Lipids were demonstrated in cryostat sections by Fettrot staining. A saturated isopropyl alcoholic solution of Fettrot dye was diluted with water at 1 : 1 prior to use, allowed to stand for 2 hours and filtered. The sections mounted on slides were rinsed with 50% isopropyl alcohol, stained for 20 minutes, rinsed again with 50% isopropyl alcohol, washed in tap water for 20 minutes and rinsed with distilled water.

For electron microscopic studies thymus specimens of 1 cu.mm were fixed in 4.5% glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.4 at 4°C for 2 hours, then washed for 24 hours in cacodylate buffer containing 0.22 M sucrose and postfixed with 1% osmium tetroxide in Millonig's buffer for 1.5 hours.

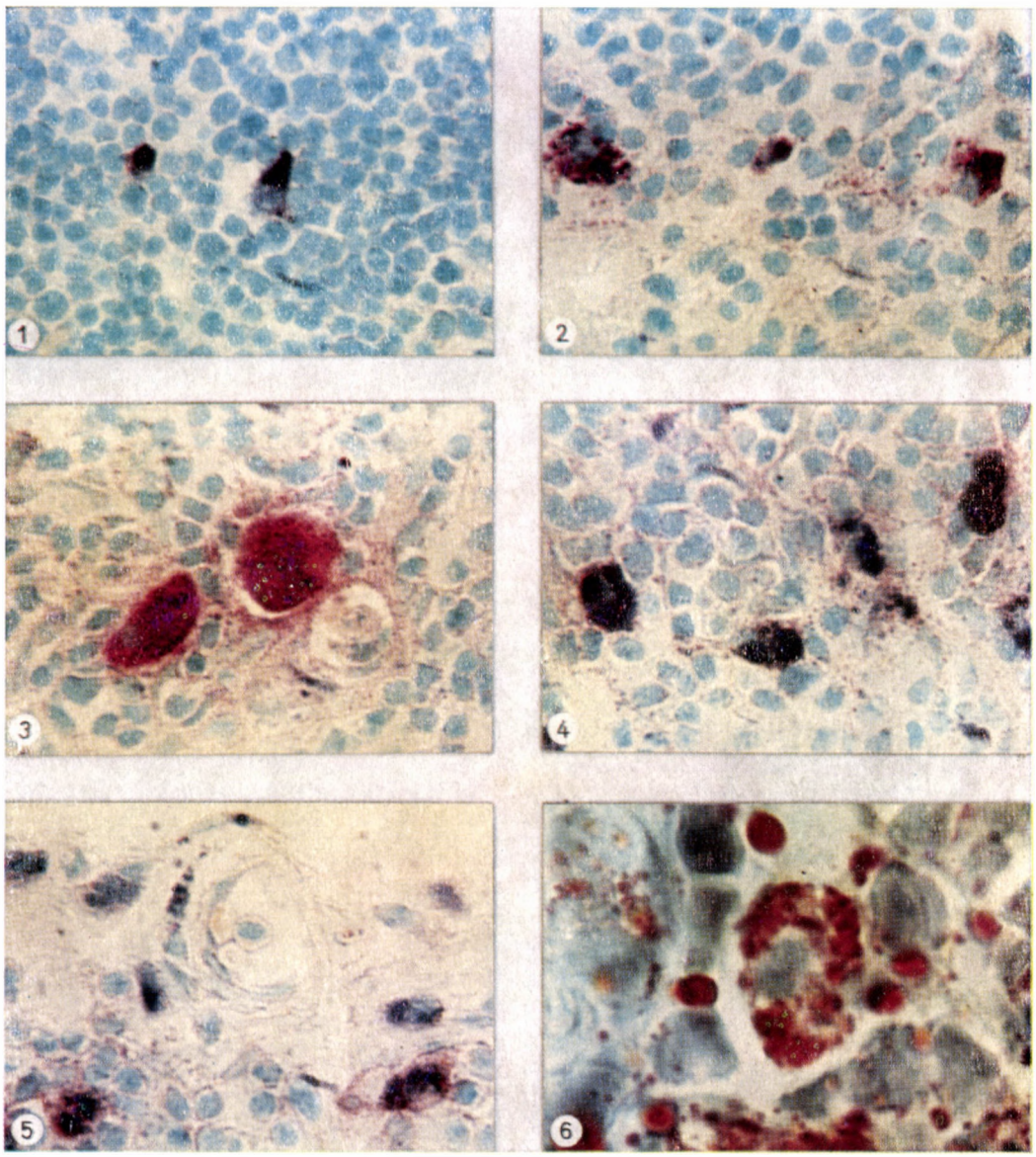
For the localization of acid phosphatase activity the specimens prepared in the above manner were frozen with carbon dioxide, cryostat sections of 40 μ were floated onto an 0.05 M acetate buffer pH 5 for 15 minutes and then incubated for 30 minutes at 37°C. The incubating medium differed from that of GOMORI [6] by containing 0.08% lead nitrate only. It was used immediately after preparation and filtration so as to avoid nonspecific precipitates liable to occur otherwise. The specimens were then dehydrated in an alcoholic series and after propylene oxide treatment embedded in Durcupan–ACM. Sections were cut by means of a Porter-Blum I-type microtome, treated with uranyl acetate, and, if required in the interest of contrast, allowed to react once again with lead nitrate, finally studied under an electron microscope of the JEM-6-AS type.

For esterase isozyme studies by starch-gel electrophoresis the thymuses were weighed and by addition of thrice the amount of water homogenized, centrifuged, and the supernatant was examined. The starch-gel electrophoresis was performed in a discontinuous tris-citrate-borate buffer-system at room-temperature. Filter paper strips were saturated with the homogenate and fed into the system. By the aid of our technique [23] parallel, 1 mm thick sheets were cut from the starch blocks, fixed in 4% cold formaldehyde for 30 minutes and washed in running water for another 30 minutes. The sheets were incubated for 2 hours at room temperature. The incubating medium was the same as that used for histochemical demonstration of esterase activity, with the difference that the substrate was α-naphthyl-acetate instead of naphthol-AS-D-acetate.

Results

The radiation reaction of the thymus was distinct 4 hours after irradiation, and reached its peak on the 4th day. The normal microscopical pattern of the gland was practically restored by the end of 11 days. The time relations of the microscopic changes will not be discussed here in detail since they were consistent with the findings of other authors [3, 5, 10, 12, 18, 19, 20, 21, 22].

Light microscopical histochemical reaction. In the thymus of untreated controls the ratio between acid phosphatase and nonspecific esterase activity was not uniform throughout the reticular cell population. In certain cells



Figs 1—6. Nonspecific esterase (blue) and acid phosphatase (red) reactions. Counter-staining with methylene-green. ×800. *Figs 1—2.* Normal thymus. *Figs 3—4.* Thymus 24 hours after irradiation. *Fig. 5.* Thymus four days after irradiation. *Fig. 6.* Thymus four days after irradiation. Fettrot; haemalaun counter-staining. ×1600

which were particularly numerous in the cortical area, nonspecific esterase activity was predominant. Though exhibiting a marked esterase activity these cells also showed a faint acid phosphatase reaction (Fig. 1).

In another group of reticular cells which was found to predominate at the corticomedullary boundary both enzyme reactions were intensive (Fig. 2). In the medulla — particularly in connection with Hassal's corpuscles — there were occasional acid phosphatase-active reticular cells with faint traces of nonspecific esterase activity (Fig. 3). The capsular and septal cells were negative.

In qualitative respects irradiation left the enzyme reactions of the reticular cells unaffected. The ratio between acid phosphatase and nonspecific esterase activity was distinctive of three reticular cell types. The cells belonging to the first two groups were generally increased in size, and contained phagocyted cellular fragments mostly being in digestive vacuoles as indicated by the cumulation of enzyme activity characteristic to each cell type (Fig. 4). The majority of the capsular and septal fibrocytes exhibited nonspecific esterase reactions (Fig. 5). The first, just demonstrable, changes appeared 4 hours after irradiation and culminated by the 4th day. On the 11th day the general appearance of the gland no longer differed from that of the controls.

In the control thymuses there were only occasional cells taking up lipid stains. In contrast, in the irradiated animals numerous reticular cells with lipid droplets were found 4 hours after irradiation. Subsequently, a gradual increase in the number of lipid-containing cells as well as in the amount of intracellular lipid was observed. There were some large cells heavily packed with lipid droplets, others with less, but finely dispersed lipid (Fig. 6). There were still large numbers of lipid-laden cells on the 11th day.

Electron microscopic observations. In the cortical cell population of the thymus of nonirradiated controls, two types of reticular cells were represented. The first type, the prevalent one, was identified as epithelial reticular cell on the evidence of its tonofilaments, desmosomes, and vacuoles containing characteristic material. The second type, marked by numerous dense bodies and digestive vacuoles, was regarded as mesenchymal reticular cell. The walls and contents of the vacuoles characteristic of the epithelial reticular cells as well as the dense bodies and digestive vacuoles of the mesenchymal reticular cells revealed acid phosphatase activity. Now and then we could see a lysosome in the cytoplasm of some lymphocytes (Fig. 7).

On the first day after irradiation there was a massive destruction of thymocytes. The places of these cells were still unoccupied by the apparently unaffected reticular cells and the majority of cellular debris was still outside the cells (Fig. 8). The digestive vacuoles of the epithelial and mesenchymal reticular cells also contained considerable amounts of cellular debris. Phagocytic activity became more and more marked and culminated by the 4th day.

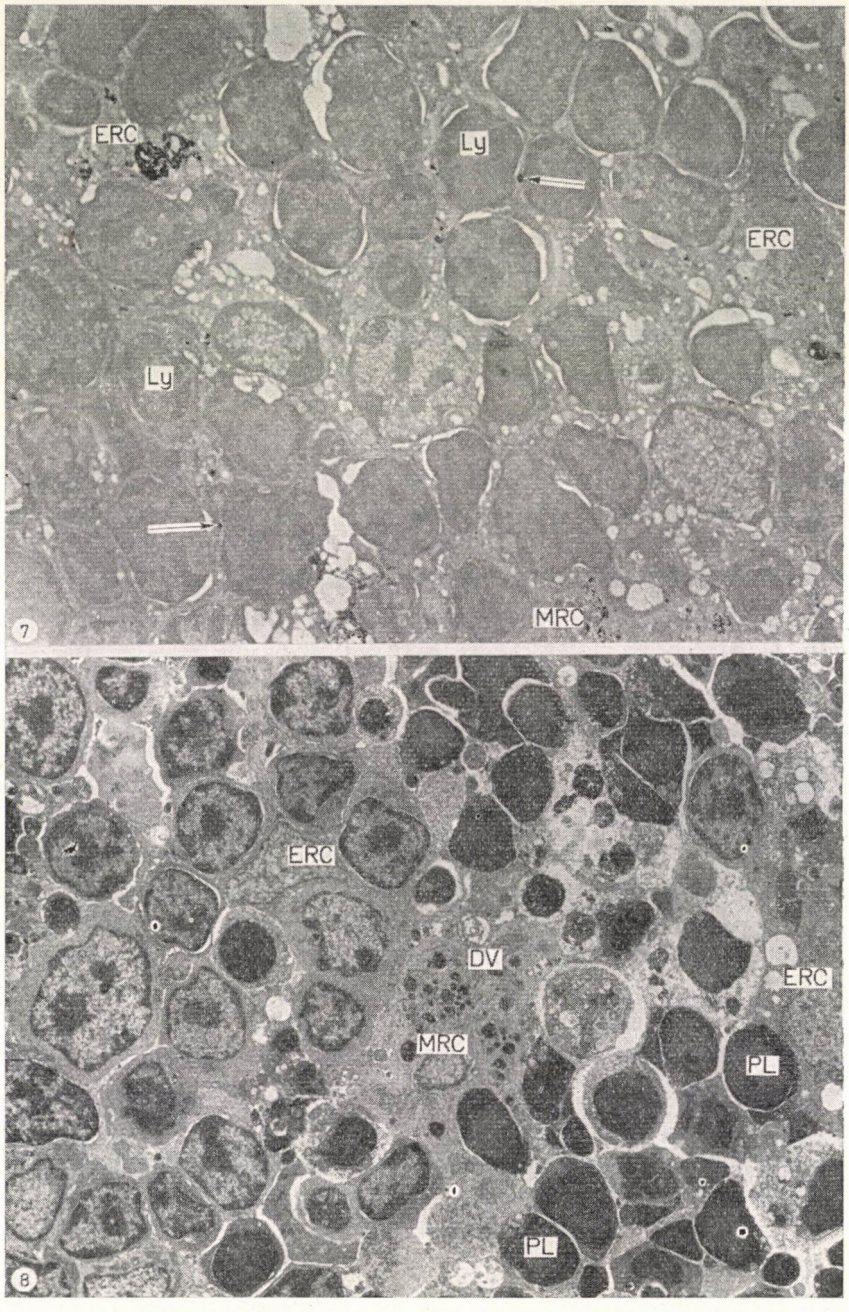


Fig. 7. Control thymus, acid phosphatase reaction. ERC = epithelial reticular cell. MRC = mesenchymal reticular cell. Ly = lymphocyte. The arrows point at lysosomes. ×3400

Fig. 8. Thymus 4 hours after irradiation. The pycnotic lymphocytes (PL) are prevalently of extracellular site. DV = digestive vacuole. ×3400

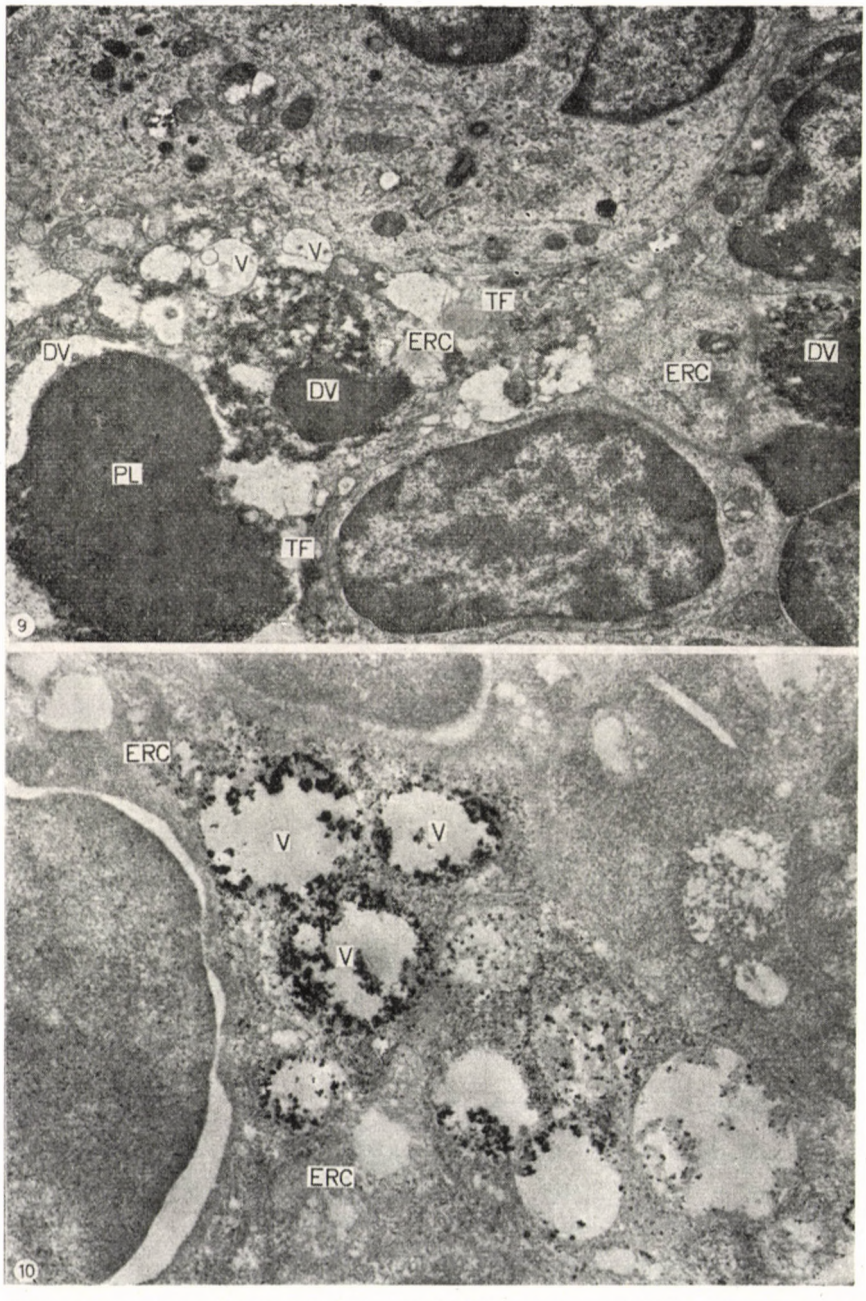


Fig. 9. Thymus one day after irradiation. Epithelial reticular cell. DV = digestive vacuoles with lymphocyte debris representing intermediate forms of typical vacuoles (V) of the epithelial reticulum. TF = tonofilament. ×9000

Fig. 10. Thymus 11 days after irradiation. Acid phosphatase reaction in the epithelial reticular cells. ×20 000

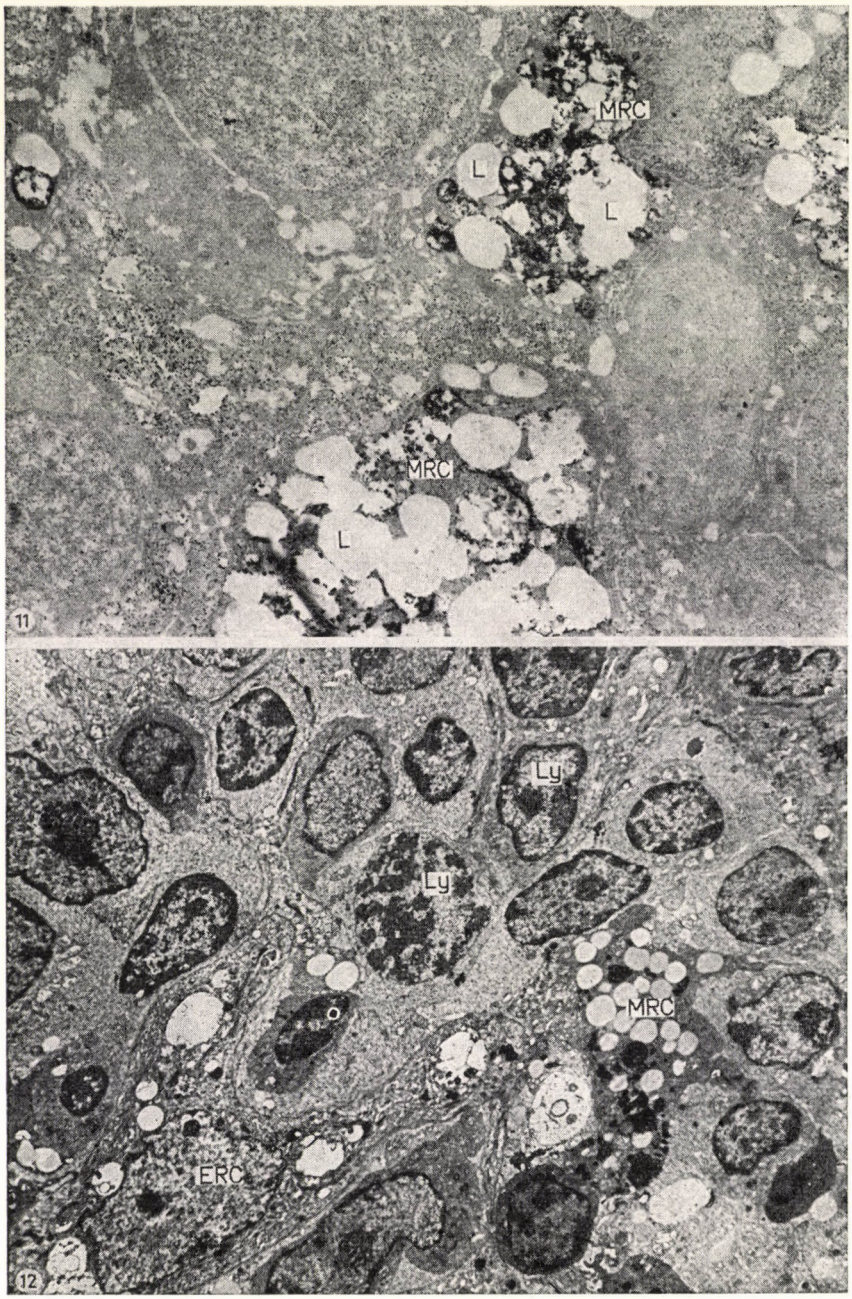
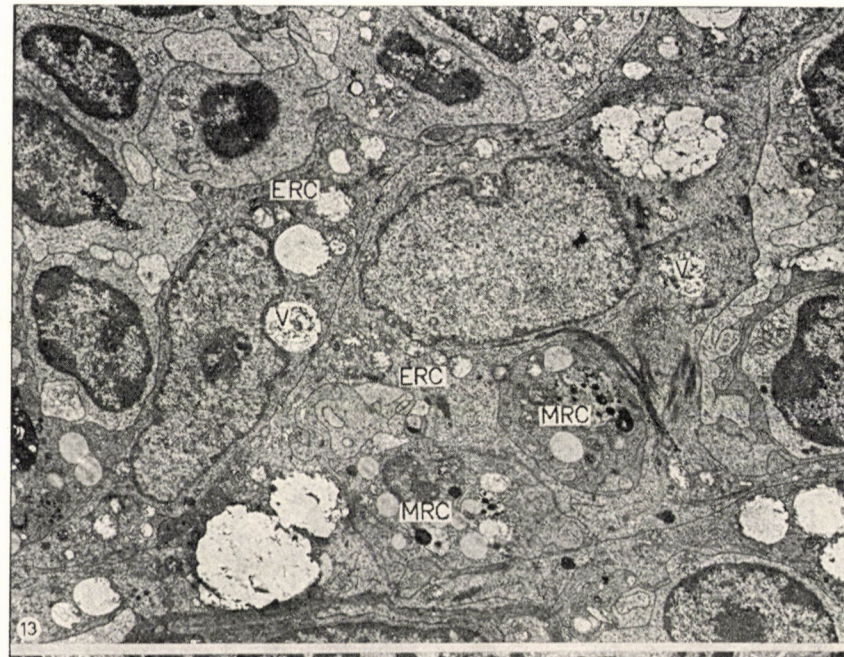


Fig. 11. Thymus 4 days after irradiation. Acid phosphatase reaction. L = lipid. ×8000
Fig. 12. Thymus 4 days after irradiation. ×3900



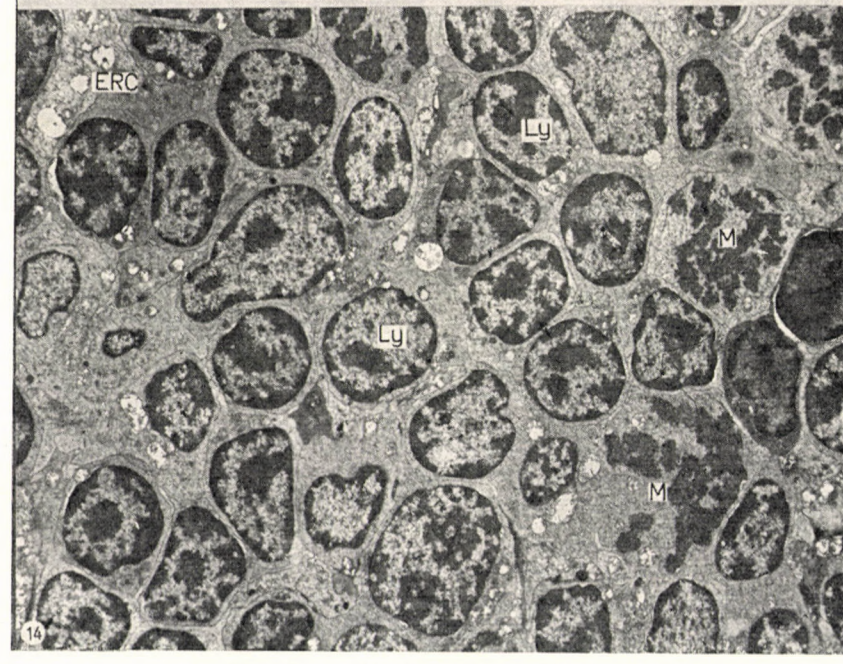


Fig. 13. Thymus 11 days after irradiation. ×5200

Fig. 14. Thymus 11 days after irradiation. M = mitosis. ×3740

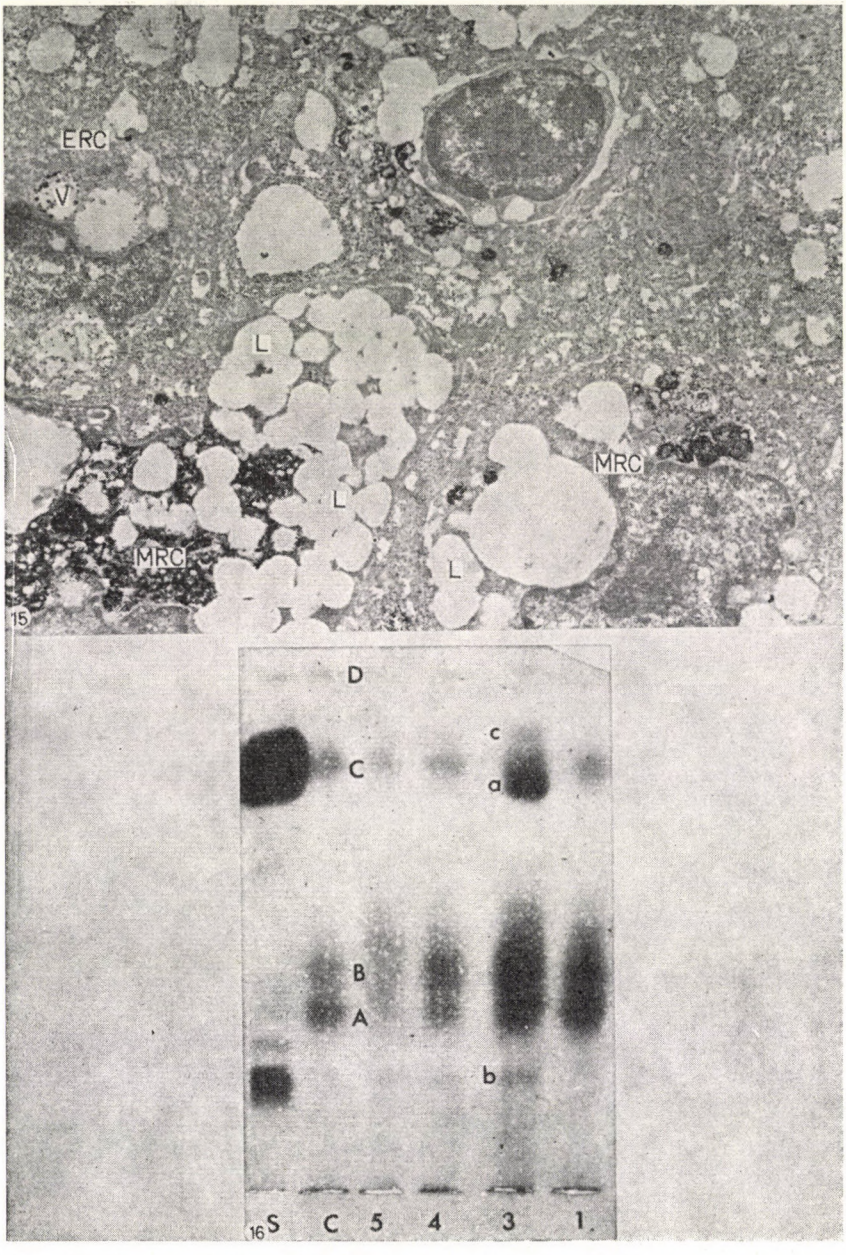


Fig. 15. Thymus 11 days after irradiation. Acid phosphatase reaction. ×7000

Fig. 16. Esterase zymogram of thymus homogenates on starch-gel electrophoresis. S = normal rat serum. C = control rat thymus homogenate. 1, 3, 4, 5 = days after irradiation

At that time the thymus was prevalently made up of reticular cells. The typical vacuoles of the epithelial reticulum had increased in number. Various intermediate forms between digestive vacuoles filled with lymphocytic debris and with moniliform inclusions were seen (Fig. 9). All these vacuoles showed a marked acid phosphatase activity (Fig. 10). The cytoplasm contained scattered, moderately dense lipid droplets, 1 to 2 μ in size. The mesenchymal reticular cells exhibited large numbers of vacuoles in various phases of digestion. The digestive vacuoles as well as the lysosomes unrelated to these displayed a considerable acid phosphatase activity (Fig. 11). In the vicinity of the digestive vacuoles and dense bodies there were numerous lipid droplets of moderate density, definitely larger than those of the epithelial elements, occasionally even as large as 3 or 4 μ (Fig. 12). In a number of mesenchymal reticular cells the greatest part of the cytoplasm was occupied by agglomerated fat droplets. Cytoplasmic extensions of the mesenchymal cells were found to project in many places into the epithelial reticular cells (Fig. 13). The first signs of repopulation of the thymus by lymphoid elements were seen on the 4th day (Fig. 12). By the 11th day the proportion between thymocytes and reticular cells was nearly normal (Fig. 14). At this time the mesenchymal reticular cells were still packed with lipid droplets (Fig. 15).

Esterase isozymes. The zymograms of thymus homogenates of controls and irradiated animals are shown in Fig. 16. X-ray irradiation was found to induce quantitative and qualitative changes. Activity of isozymes A, B and C, present also in the control homogenates, was found to increase until the 3rd day after irradiation and to return to the control value by the end of the 5th day. On the 3rd day the activity corresponding to the band of isozyme B in the controls exhibited greater increase than did A and C, while activity of band D had definitely declined and was even absent in some of the cases. A very intensive enzyme band — *a* — had appeared subsequent to band C and two new faint bands were seen, the first — *b* — directly after, and the second, — *c* — before, band C.

Discussion

It has long been known from biochemical studies [15, 16] that X-ray irradiation induces an increase in acid hydrolase activity per tissue unit. This might, however, be interpreted by the fact that in the course of involution the thymus becomes depleted of the very cell population in which these enzymes are scarce, therefore the increase in activity might be merely relative. Though this would explain the finding that the activity of three out of the four isoenzymes had increased but could not account for the fact that this increase was not uniform, and that a new band of high activity appeared in addition to two moderately active new bands. To explain this we not only

have to assume that the originally existing enzymes have increased in concentration but also that new ones have been formed or activated, a possibility consistent with the observation that in the individual reticular cells, as opposed to the controls, there could be seen an increase in the esterase activity demonstrable by light microscopy, and in the activity of acid phosphatase, as revealed by light and electron microscopy alike.

The fall in the activity of an individual isozyme despite the general increase in isozyme activity suggests that this particular isoenzyme might be prevalently produced by the lymphocytes sensitive to radiation. This would be in harmony with our light and electron microscopical findings of lysosomes in the thymocytes as revealed by their enzyme activity (Fig. 7).

The finding of the two isozyme bands *b* and *c* of faint activity is still open to the interpretation that these enzymes need not have been newly formed but might have been present in the control thymuses of considerably larger volume and owing to this had remained unidentifiable. As regards, however, the appearance of the high-activity band *a* there can be no doubt whatsoever about its production under the influence of irradiation. Though there may be certain relationships between this isoenzyme and the isozyme fraction of the same electrophoretic mobility present in the serum, there can be no question of a mere contamination, since in that case the other isozymes of the blood would also have to be present.

The vacuoles of the epithelial reticular cells with characteristic inclusion material have been known for years [4, 9] but it was not attempted to explain the origin of the material; later, contradictory interpretations were published [8, 11, 12]. Our findings seem to support the theory of Klug, not only because of the evidence of transitional types between digestive vacuoles filled with lymphocyte fragments and these vacuoles, but also in view of the acid phosphatase activity exhibited by the membrane as well as the contents of the vacuole.

The lysosome character of the dense bodies in the interior of the mesenchymal cells has been confirmed by their intensive acid phosphatase activity. The digestive vacuoles also exhibited a marked acid phosphatase activity.

One of the most conspicuous post-radiation findings was an excessive increase in the lipids of mesenchymal reticular cells. On the grounds of light and electron microscopic evidence we feel justified in connecting the intensive light microscopic lipid reaction with the presence of lipids in the mesenchymal cells. Massive breakdown and metabolic transformation of cellular debris would account for this finding. There may, however, be additional factors. Total body irradiation is known to stimulate the activity of the pituitary-adrenocortical system. There is evidence [13, 14, 17] that certain biochemical changes demonstrable in the thymus are unrelated to any direct radiation effect on the gland, but arise, in all probability, indirectly by a pituitary-

adrenocortical mechanism. VAN HAELST [7], on studying acute thymic involution consecutive to hydrocortisone treatment connected the finding of numerous lipid inclusions with hydrocortisone metabolism. KLUG, in his work on electron microscopical postradiation changes [11, 12] does not even refer to the behaviour of lipids, nor do his microphotographs reveal any noteworthy amount of lipid in the mesenchymal cells. But then, his evidence has been mainly derived from adrenalectomized rats, therefore it does not invalidate the possibility of a relationship between increase in lipids and steroid metabolism.

It has been found that the two hydrolytic enzymes were not represented at the same ratio in the reticular cells of the normal thymus. The fact that the same discrepancy was demonstrable after irradiation and in the course of massive phagocytosis, would suggest that the different enzyme concentrations represent different kinds of cell rather than different functional phases of the same cell type. Evaluation of light microscopic preparations is rendered difficult by the electron microscopic finding that the cytoplasmic extensions of the two cell types often overlap. Giving due consideration to all light and electron microscopic observations, it would seem that the cells combining an intensive esterase activity with a fainter acid phosphatase activity correspond to mesenchymal cells while those in which both enzyme types are equally represented — obvious allowance being made for the time relationships of incubation — represent epithelial elements. This claim awaits, however, further support.

*

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REFERENCES

1. BÁCSY, E., RAPPAY, GY.: Acta biol. Acad. Sci. hung. **17,** 394 (1966).
2. BÁCSY, E., VADÁSZ, GY., FAZEKAS, I., RAPPAY, GY.: Morph. és Ig. Orv. Szle **8,** 216 (1968).
3. BRAUN, H.: Strahlentherapie **126,** 236 (1965).
4. CLARK, S. L.: Amer. J. Anat. **112,** 1 (1963).
5. GHOSSEIN, N. A., AZAR, H. A., WILLIAMS, J.: Amer. J. Path. **43,** 369 (1963).
6. GOMORI, G.: Arch. Path. **32,** 189 (1941).
7. VAN HAELST, U.: Z. Zellforsch. **80,** 153 (1967).
8. HOLLMANN, K. H., VERLEY, J. M.: Z. Zellforsch. **78,** 47 (1967).
9. HOSHINO, T.: Z. Zellforsch. **59,** 513 (1963).
10. KHUSSAR, YU. P.: Arkh. Anat. Gist. i Embriol. **51,** 109 (1966).
11. KLUG, H.: Experientia (Basel) **21,** 129 (1965).
12. KLUG, H.: Z. Zellforsch. **68,** 43 (1965).
13. MAOR, D., ALEXANDER, P.: Int. J. Radiat. Biol. **6,** 93 (1963).
14. MAOR, D., ALEXANDER, P.: Nature (Lond.) **205,** 40 (1965).
15. RAHMAN, Y. E.: Proc. Soc. exp. Biol. (N. Y.) **109,** 378 (1962).
16. RAHMAN, Y. E.: J. Cell. Biol. **13,** 253 (1962).
17. SCAIFE, J. F.: Canad. J. Biochem. **42,** 623 (1964).

18. SMITH, C.: Proc. Soc. exp. Biol. (N. Y.) **93,** 310 (1956).
19. SMITH, C., KIEFFER, D. A.: Proc. Soc. exp. Biol. (N. Y.) **94,** 601 (1957).
20. SMITH, C., WHARTON, T. J., GERHARDT, M. A.: Anat. Rec. **131,** 369 (1958).
21. TÖRŐ, I., RAPPAY, GY., BÁCSY, E.: Ann. Histochim. **12,** 91 (1967).
22. TROWELL, O. A.: Int. J. Radiat. Biol. **8,** 239 (1964).
23. VADÁSZ, GY., BÁCSY, E.: In the press.

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EFFECT OF ACTH ON TARGET-ORGAN BLOOD FLOW, WITH SPECIAL REFERENCE TO AN EXTRA-ADRENAL EFFECT

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Heated thermocouple measurements have shown that intravenous ACTH increases in the first 1 or 2 minutes both the ovarian and the adrenal blood flow in the dog. As synthetic ACTH exerts this effect also on the ovary of the adrenalectomized dog, it is definitely extra-adrenal. Given as an infusion to the wakeful animal, even small doses of ACTH have this effect, indicating that it probably obtains also under physiological, and particularly, pathological conditions. A study of the mechanism of action involving several vasoactive substances, pointed to histamine as a possible mediator.

An investigation of the part possibly played by the synthesis of rapid-turnover protein, using cycloheximide which is known to inhibit this process, produced evidence that ACTH increased blood flow, at least in some measure, independently of its corticosteroidogenic action: blood flow continued to rise when corticosteroid secretion no longer increased, and cycloheximide influenced, i.e. stopped or reduced, the two actions with a lag in time between them. ACTH raises ovarian blood flow also in the cat.

Measurements made with the ^{86}rubidium method of Sapirstein showed that the hormone likewise increased ovarian blood flow in the hamster and, though not significantly, in the rat.

The physiological significance of this extra-adrenal effect of ACTH has been discussed.

The close relationship between blood supply and functional activity of tissues has long been established. It is of obvious importance to know if and how the trophic hormones affect the supply of blood to their respective target organs; the more so as according to our present knowledge these hormones are regulators of the metabolic processes occurring in them. The trophic hormones are governing the metabolic processes which ensure normal growth, weight and structure of the glands, and where this is beneficial to the organism, develop hypertrophy. Many workers have sought to clarify the effect of the trophic hormones on target-organ blood supply, and to establish its underlying mechanism(s). Nevertheless, until quite recently it has been doubtful whether exogenous gonadotrophic hormones, thyrotrophic hormone, and ACTH increased gonadal, thyroidal, and adrenal blood flow, respectively.

The present paper deals, first, with the effect of ACTH on adrenal blood flow; secondly, with a recently detected extra-adrenal effect on ovarian blood flow; thirdly, with experiments concerned with the mechanism by which ACTH increases blood flow.

Many of the data published on the effect of ACTH on adrenal blood flow, are contradictory. Some workers [1, 2] found that ACTH increased

adrenal blood flow, others [3, 4] that it did not. The discrepancy is due partly to differences in the methods used, and partly to the inadequacy of some of them. For instance, the formerly common method of cannulating the adrenal vein may in itself alter the flow, particularly in chronic experiments. The experiments of SAPIRSTEIN and GOLDMAN [5] using the ^{86}Rb method brought the problem a step nearer to its solution. These authors found that ACTH significantly increased adrenal blood flow. In addition to the ^{86}Rb and the direct method, we measured adrenal blood flow using heated thermocouples, which procedure made it possible to compare the values obtained before and after handling, and the repeated use of the same animal, and also of conscious animals.

A series of experiments was performed to determine whether adrenal blood-flow changes, if any occur at all, preceded or followed the corticosteroidogenic response to ACTH and whether they were identical in conscious animals and animals under anaesthesia.

Methods

The salient features of the methods employed were as follows. About 250 dogs and a few hamsters and rats were used. In acute experiments, the dogs were laparotomized and a heated thermocouple, as modified by BETZ et al. [6], was embedded into the adrenal. Temperature changes were recorded with a two-channel Fluvograph. In experiments on conscious animals, after laparotomy performed under sterile conditions, a thermocouple was introduced into the left adrenal. The heating and measuring wires were fixed to the neck of the animal [7]. Post-operative blcod-flow measurements were made in the wakeful state. Changes in blood flow were expressed on a percentage basis, where resting flow values were considered to be 100, and values recorded at the instance of killing regarded as zero flow. Evaluations were based on Hensel's formula. Blood pressure was recorded from a femoral artery, using a Statham transducer and a Hellige electromanometer with a Hellige multiscriptor. Breathing was registered by means of a respiration counter with a thermistor connected to the multiscriptor. In a number of experiments blood flow was measured by the method of SAPIRSTEIN [8], injecting the ^{86}Rb two minutes after ACTH. Cardiac output was determined by the method of HAMILTON et al. [9], and corticosteroids by paper chromatography as described earlier [10].

Results and discussion

Effect of ACTH on adrenal blood flow

In anaesthetized animals intravenous ACTH produced maximum blood flow in 1 to 2 minutes as measured by heated thermocouples. The flow decreased to its pre-ACTH value in 10 to 40 minutes, depending on the dose applied. Each subsequent ACTH injection given when the effect of the preceding one had worn off, induced a new rise in adrenal blood flow. Renal flow remained unchanged (Fig. 1).

In agreement with published data [11] and our earlier findings [10], the applied dose of exogenous ACTH failed to raise the corticosteroid level in

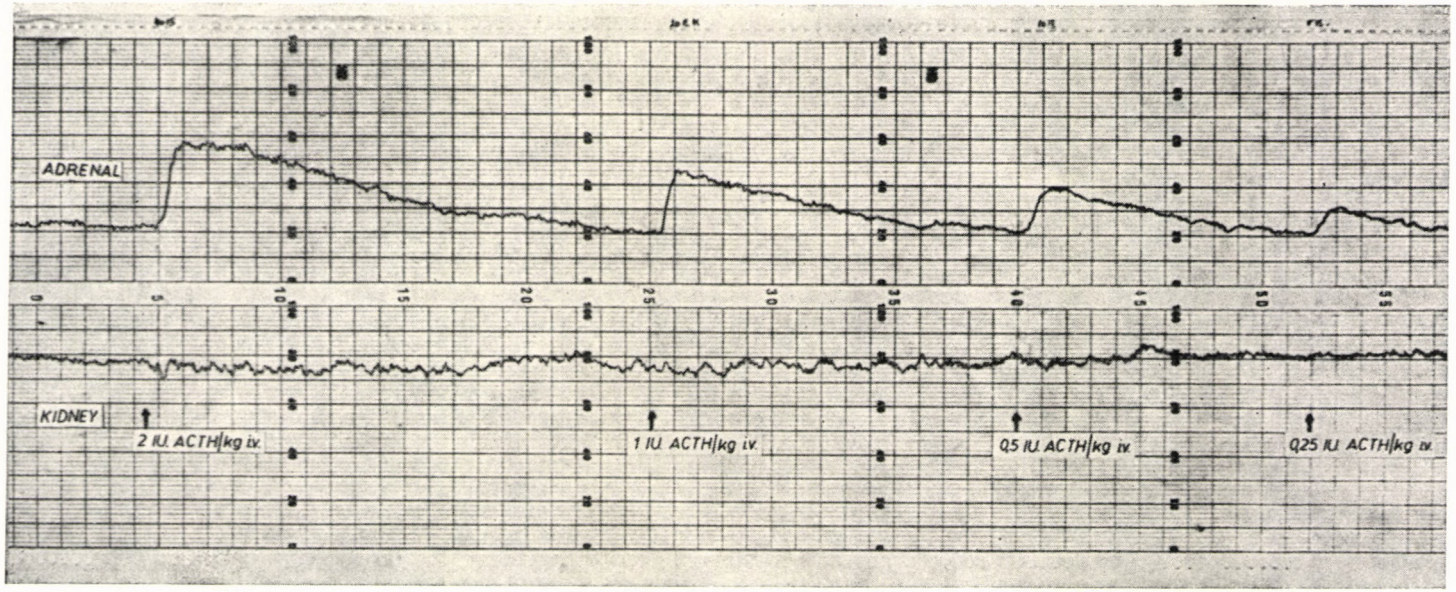

Fig. 1. Effect of graded ACTH doses on adrenal blood flow in the anaesthetized dog

the peripheral blood of animals laparotomized under anaesthesia, but increased adrenal blood flow (Fig. 2).

In anaesthetized dogs, ACTH significantly increased adrenal blood flow [12], as it did in rats in the experiments of SAPIRSTEIN [8]. On the other hand, even large doses of ACTH failed to affect splenic, hepatic, thyroid and pancreatic blood flow. The dose effective on the adrenal, had no effect on systemic blood pressure.

In about 15% of the anaesthetized animals the intravenous injection of ACTH was not followed by an increase in blood flow; in these animals the thermocouple had not been embedded adequately or haemorrhage had occurred in the adrenal.

In conscious animals, on the second and subsequent postoperative days much smaller doses sufficed to raise adrenal flow to the level recorded imme-

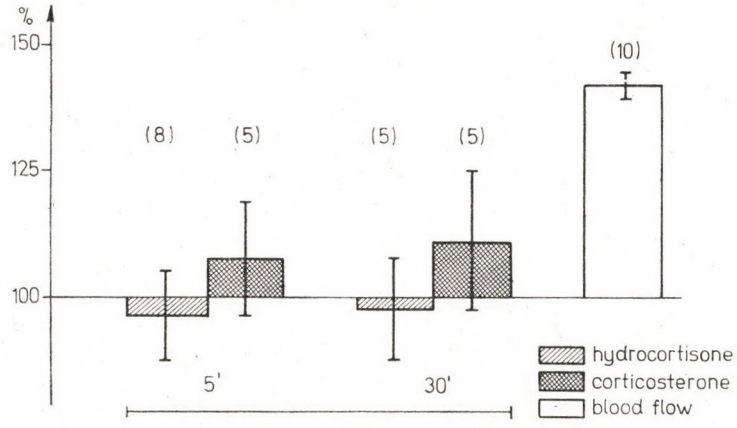

Fig. 2. Changes in peripheral corticosteroid levels and adrenal blood flow after 2 IU/kg of ACTH

diately after surgery. The probable cause of this difference had been discussed in an earlier paper [12].

The question arose whether ACTH was effective if, to approximate physiological conditions, it was infused by the intravenous route.

Adrenal blood flow began to increase in the first 2 or 3 minutes of a 5-minute infusion of ACTH (Fig. 5).

All these results are in contrast with those of HARRISON and HOEY [13], since in our experiments ACTH took only one to two minutes to raise adrenal flow and the rise was preceded or was concomitant to, but never followed, the increase in corticosteroid secretion.

When given repeatedly, ACTH repeatedly increased blood flow even after it had ceased to increase corticosteroid secretion. NICHOLS and RICHARDSON [14] made a similar observation. Two explanations are possible; either

ACTH acts on the blood vessels directly or it sets free one or more metabolites in the adrenal which locally dilate the vessels and thereby increase blood flow. The second explanation is based on the assumption that after repeated doses of ACTH some limiting factor blocks the corticoidogenic process at a time when the vasodilator substance is increased, or perhaps released. The amount of corticosteroid accumulating in the adrenal might be this limiting factor, as suggested by FERGUSON et al. [15].

The question now is, what exactly is the metabolic process which is repeatedly initiated by ACTH, and the intermediary product(s) of which are increasing blood flow.

Extra-adrenal effect of ACTH on ovarian blood flow

After SUTHERLAND et al. [16] had discovered the key role played by cyclic 3'5'-adenosine monophosphate (AMP) in the action of adrenaline, HAYNES et al. [17] and SAVARD et al. [18] demonstrated that this nucleotide played an important part in the steroidogenic effect of ACTH and LH, respectively.

AMP enhances both adrenal and ovarian steroidogenesis, and both ACTH and LH significantly increase the amount of 3'5'-AMP in its target organ [18]. Bearing this in mind, together with the fact that ACTH increases adrenal blood flow even after it has ceased to increase corticosteroid secretion,

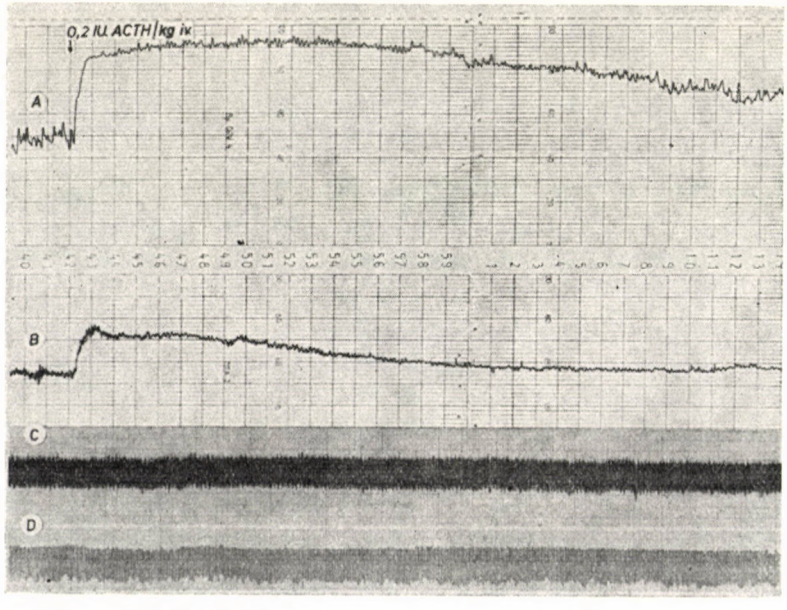

Fig. 3. Effect of naturally occurring ACTH on ovarian (A) and adrenal (B) blood flow, blood pressure (C) and respiration (D) in the dog. Time scale = 1 min

it was supposed that other trophic hormones acting on other steroid-producing glands might similarly affect adrenal blood flow, and, on the other hand, ACTH might increase the flow of blood through the target gland of the other hormones. This might occur by initiating metabolic processes which, although they do not result in organ-specific hormone production, are common to the various steroid-producing organs. We carried out experiments using the same methods as in the case of adrenals. Female dogs weighing 10 to 20 kg were used, and in most dogs adrenal and ovarian blood flow were measured, and systemic blood pressure and respiration were recorded simultaneously. In pre-

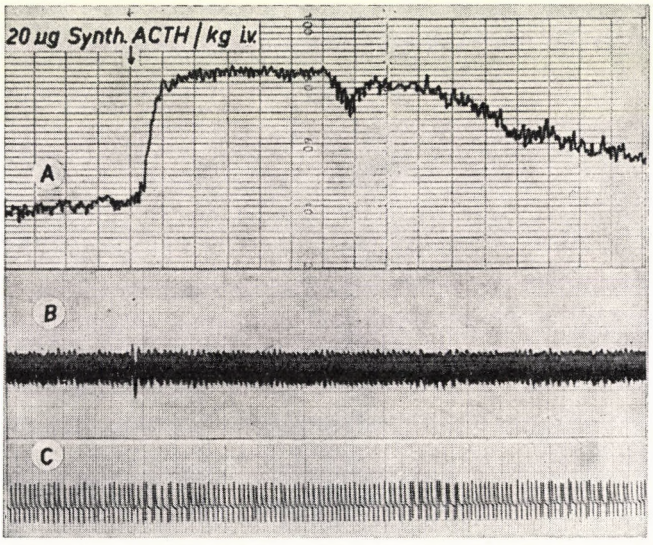

Fig. 4. Effect of synthetic ACTH on ovarian (A) blood flow in the dog, blood pressure (B) and respiration (C)

liminary experiments FSH or LH failed to induce a rise in adrenal blood flow during an observation period of one hour. ACTH, however, significantly increased both adrenal and ovarian blood flow (Fig. 3). That ACTH should exert its effect on the ovary by the mediation of some humoral substance set free in the adrenal, seemed improbable since the hormone took as much time to increase ovarian as adrenal blood flow. Nevertheless, we re-investigated the point in adrenalectomized animals, discarding those whose blood pressure fell to less than 90 mm Hg. Three hours after the operation, ACTH significantly elevated ovarian blood flow.

To exclude a contaminant in natural ACTH as the cause of this effect, we repeated the experiments with synthetic ACTH and found that this, too, raised ovarian blood flow (Fig. 4). Like in the case of the adrenal, this effect of ACTH proved to be dose-dependent, whether the compound was infused (Fig. 5) or injected intravenously. Increasing doses not only produced higher

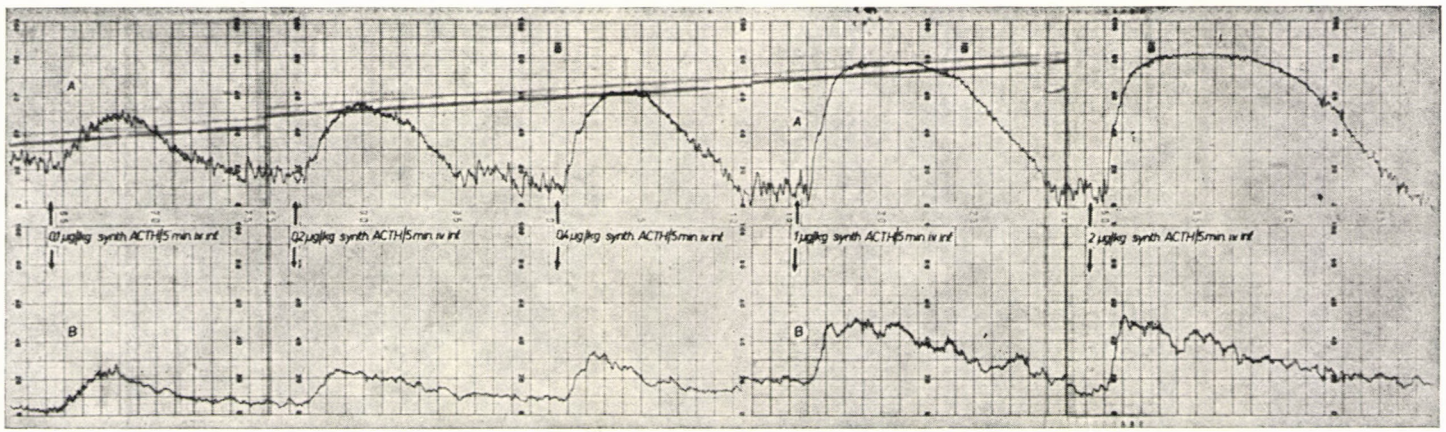

Fig. 5. Effect of graded infusions of ACTH on ovarian (A) and adrenal (B) blood flow in the anaesthetized dog. Time scale = 1 min

peaks of flow, but also prolonged the time during which flow was supernormal. The dose–effect relationship mostly varied from one animal to the other. Since we used the same preparation throughout, such variations were probably due to individual sensitivity. The possible effect of the phase of the sexual cycle was not studied.

The many experiments gave the impression that the same dose of ACTH administered per kg of body weight to the anaesthetized animal immediately after operation, induced a greater rise in ovarian than in adrenal blood flow.

ACTH increased ovarian blood flow in the cat as well.

To check the results obtained by internal calorimetry, blood flow was also measured by a direct method in dogs a polyethylene cannula was in-

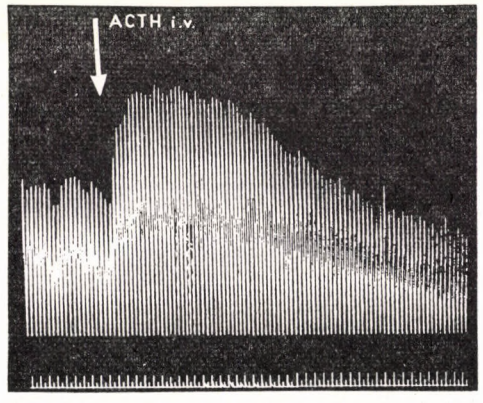

Fig. 6. Changes in volume of blood flowing from the ovarian vein of a dog in response to ACTH (0.2 IU/kg). Scale indicates minutes

Table I.

Effect of ACTH on endocrine blood flow (ml/min/kg) in the Syrian hamster and the rat

	Treatment	Adrenal	Ovary	Testis	Epididymis	Kidney
Hamster ♀	Control (5)	2.9 ±0.5	1.0±0.2			10.0±1.8
	2 IU ACTH/kg (5)	5.8 ±0.3*	2.4±0.3*			13.4±0.8
Hamster ♂	Control (7)	0.73±0.05		0.026±0.005	0.083±0.15	
	2 IU ACTH/kg (7)	0.90±0.05*		0.024±0.006	0.097±0.007	
Rat ♀	Control (8)	2.82±0.20	1.20±0.05			4.29±0.33
	2 IU ACTH/kg (10)	4.83±0.48**	1.52±0.19			4.55±0.50

Means ± standard errors
( ) number of animals
* $p < 0.05$
** $p < 0.01$

serted into the ovarian vein, the outflowing blood was measured with a Jacquet-type piezoelectric drop-counter and registered kymographically. With this method, ACTH was found to increase ovarian blood flow (Fig. 6).

ACTH raised adrenal and ovarian blood flow in the hamster and the rat, but in the rat the rise of ovarian flow was not significant statistically (Table I).

Mechanisms by which ACTH increases adrenal and ovarian blood flow

Vasoactive substances

Since sympathomimetic amines are causing vasodilatation by the mediation of beta-receptors [19], experiments were carried out to test if ACTH would increase adrenal blood flow in animals pretreated with propranolol. The results clearly showed that blocking of beta-receptors did not inhibit the flow-raising effect of ACTH.

The data of SZEGO and her group [20–23] and of others [24, 25] prompted us to study the effects of serotonin and histamine on adrenal and ovarian blood flow [26]. Two groups of experiments were performed. In one group the animals received the compound in the form of a rapid intravenous injection, in the other group in a 2-minute infusion given at a blood pressure stabilized by the method of ENGELKING and WILLING [27].

The results obtained without the stabilization of blood pressure were difficult to interpret because serotonin induced a significant rise (54 ± 10 mmHg) and histamine a fall (70 ± 5 mmHg) in blood pressure. Fig. 7 shows that serotonin infused at stabilized blood pressure, after an initial rise reduced both ovarian and adrenal blood flow. Histamine significantly raised the blood flow of both glands. Blood pressure had stabilized in 1 to 2 minutes. These findings indicate that serotonin has probably no part in the ACTH-induced rise of blood flow; histamine, on the other hand, increases both these flows. Our data support the view of SZEGO [21] that ovarian hyperaemia due to gonadotrophic hormones is mediated by histamine which, in our view, is presumably a mediator of the ACTH effect. It should be noted that WURTMAN [24] reported histamine to increase ovarian blood flow, and according to WRIGHT [2] it increased the amount of blood flowing through the transplanted adrenal gland of sheep.

The fact that both ACTH and histamine increased adrenal blood flow does not necessarily mean that ACTH increases adrenal blood flow by liberating histamine. Experiments concerning this point are in progress.

It should be noted that comparatively large doses of infused synthetic corticosteroids had no effect on the ability of ACTH to increase blood flow [28].

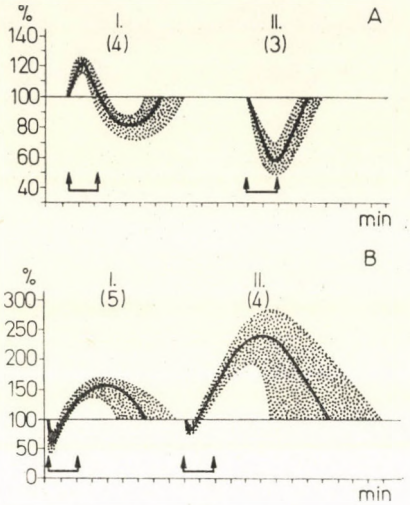

Fig. 7. Effect of intravenous infusion of 40 μg/kg/min of serotonin (A) and of 25 μg/kg/min of histamine (B) under conditions of stabilized blood pressure. Arrows indicate time of infusion. ( ) = number of experiments

Cycloheximide

ACTH was found to enhance corticosteroid synthesis immediately after its administration; as many reports indicate, this is achieved by the activation of specific enzymes [29–32]. In recent years it has been shown that puromycin, cycloheximide, and chloramphenicol inhibit the steroidogenic action of ACTH [33–35]. On this ground it has been assumed that the effect of ACTH is bound to the synthesis of proteins of a rapid turnover. This seemed to offer a new angle of approach to the relationship between blood flow and corticosteroidogenesis. As repeated doses of ACTH repeatedly increased adrenal blood flow without continuing to increase corticosteroid production, we wished to find out if the flow-raising effect of ACTH was nevertheless bound to the synthesis of proteins of rapid turnover. If ACTH increases the blood flow in the ovary by a mechanism similar to that operating in the adrenal, and if its effect is bound to protein synthesis inhibitable with cycloheximide, then this compound must inhibit the flow-raising effect in both organs.

After registering the blood flow-raising effect of ACTH (Synacthen, Ciba) in the adrenal and the ovary by the heated thermocouple method, the animals were given 50 mg/kg of cycloheximide intravenously.

Thirty, 60 and 120 minutes after cycloheximide the animals again received ACTH. As early as 1 hour after its administration cycloheximide was found to have substantially weakened the effect of ACTH, nearly suppressing it by the end of the second hour. Cycloheximide reduced adrenal flow and particularly the ovarian resting flow, and reduced blood pressure by an average

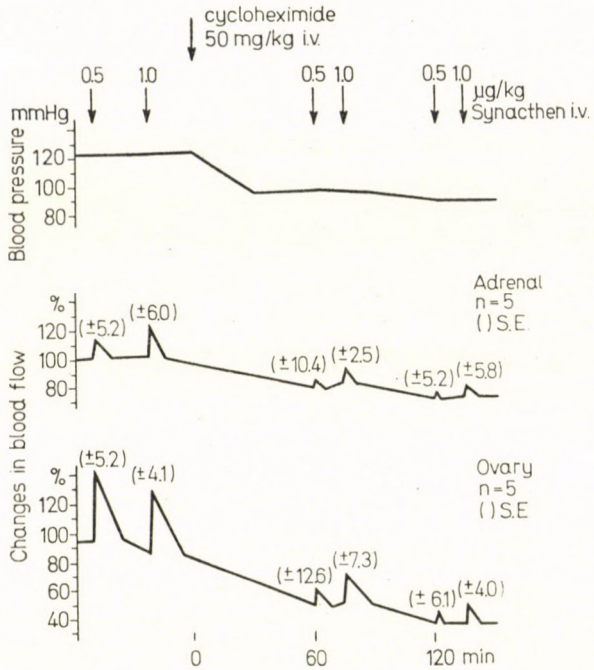

Fig. 8. Effect of ACTH (Synacthen) on ovarian and adrenal blood flow before and after cycloheximide without previous stabilization of blood pressure. n = number of experiments; ( ) = S.E.

of about 25 mmHg (Fig. 8). In both organs, pressor substances produced similar blood-flow changes in treated and non-treated animals.

The possibility arose that cycloheximide inhibited the increase in blood flow by reducing systemic blood pressure. Against this argues the fact that the compound exerted its inhibitory action in animals whose blood pressure had been stabilized (Fig. 9).

Cycloheximide took much longer to inhibit the action of Synacthen in increasing adrenal and ovarian blood flow than in increasing corticosteroid production (Fig. 10). Working with rats, STAEHELIN et al. [33] found that in the first hour cycloheximide did not inhibit the blood flow-raising effect of ACTH. We observed inhibition, but only when ACTH had been administered at least one hour after cycloheximide; when administered two hours after it, inhibition was much more pronounced.

ACTH increased adrenal blood flow in the first two minutes. This shows that any rise precedes, and by no means follows, the increase in corticosteroid production. But why is it that cycloheximide decreased [34] or, as in our experiments, inhibited corticosteroid synthesis induced by ACTH in a few minutes whereas it decreased the blood flow-raising effect of ACTH only after a considerably longer period of time?

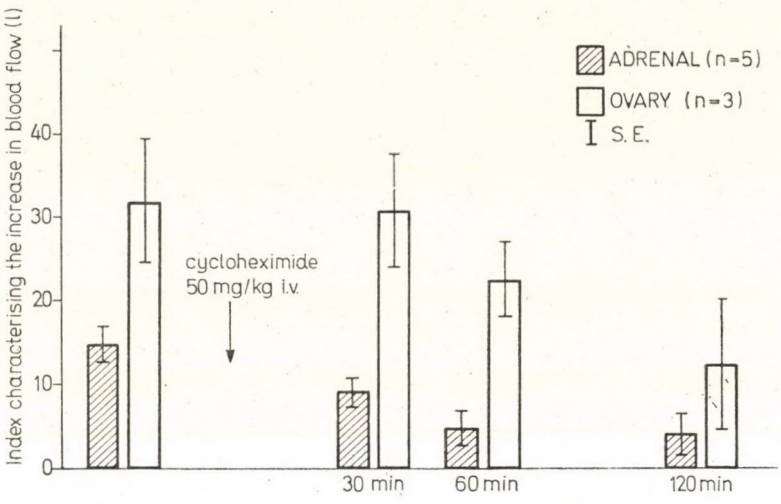

Fig. 9. Effect of ACTH (Synacthen) on ovarian and adrenal blood flow before and after cycloheximide at stabilized blood pressure. The index of increase was computed as follows. (I) = $\sqrt{\text{(maximum increase, per cent) (duration of increase, min)}}$.

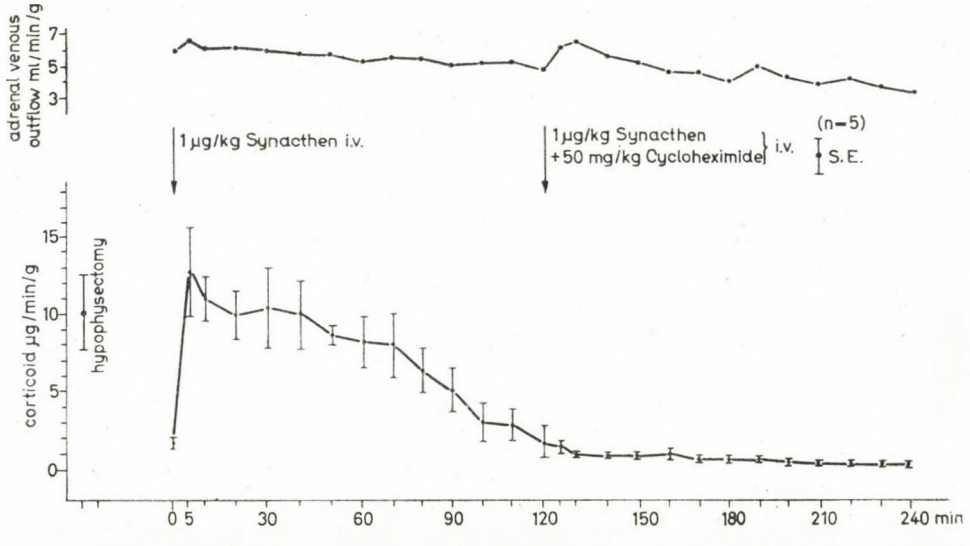

Fig. 10. Effect of ACTH (Synacthen) on corticoid production, as measured by µg/min/g output in adrenal venous blood, before and after cycloheximide in hypophysectomized dog

Earlier we assumed [35] that the rise in adrenal and ovarian blood flow was brought about either by the metabolic products causing an increase of cyclic AMP, or by an intermediary product of the AMP-induced metabolic process. GRAHAME-SMITH et al. [36] showed that cycloheximide inhibits those of the proteins involved in steroidogenesis, which act at a site past adenyl cyclase. DAVIS and GARREN [37] demonstrated that cycloheximide does not prevent cleavage of cholesterol esters, and so an intermediary product released from them might be responsible for the local vascular action of ACTH. One such product may be prostaglandin [38], which continues to be released when cholesterol is no longer converted into Δ-5-pregnenolone; a step which requires that protein synthesis be inhibited by cycloheximide. The fact that cycloheximide nevertheless inhibits the blood flow-raising effect of ACTH after one hour, seems to indicate that this effect is dependent on the presence of a cycloheximide-sensitive protein, which is not identical with the rapid-turnover protein participating in corticoidogenesis. It might as well be that some unknown, slowly developing (non-specific?) effect of cycloheximide inhibits ACTH from increasing blood flow. ACTH takes the same length of time to increase ovarian and adrenal blood flow and increases them as rapidly as it increases the adrenal AMP level. Cycloheximide inhibits the blood flow-raising action of ACTH in the two organs in nearly the same manner. These data together with the results obtained with histamine, appear to suggest that the increase in blood flow caused by ACTH in the two organs is brought about by the same mechanism in which the metabolic process that results in an increase of cyclic AMP or by an intermediary product of the AMP-induced metabolic process, may be involved.

We once more emphasize the possibility that in the two steroid-producing organs there exist vascular structures sensitive to ACTH [35], and that cycloheximide acts on them.

KAR et al. [39] have shown that ACTH increases ovarian weight and accelerates follicular maturation in both the intact and the adrenalectomized immature animal. These authors, however, used unpurified ACTH. CHRISTIAN [40] demonstrated that ACTH inhibited sexual maturation of the infantile mouse; mature follicles were absent and ovarian and uterine weights were decreased. According to ENGEL [41] two criteria must be applied in the appraisal of the effect of a trophic hormone on an extra-target organ; the biological effect must be exerted even in the absence of the target organ, and the hormone preparation used must be a protein or a peptide of the highest possible purity. In our investigation synthetic ACTH has been shown to increase ovarian blood flow in the adrenalectomized animal. *This means that a hitherto unknown extra-adrenal effect of ACTH has been detected* [35, 42]. Recently CHRISTIAN [43] reported that in the adrenalectomized animal both synthetic ACTH and naturally occurring ACTH induced extraadrenal changes in the ovary.

The physiological and pathological significance of the action of ACTH on the ovary is unknown. It is left for future studies to throw light on its possible effect on physiological, and perhaps pathological, ovarian function.

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REFERENCES

1. Balfour, W. E.: J. Physiol. (Lond.) **122,** 59 (1953).
2. Wright, R. D.: Endocrinology **72,** 418 (1963).
3. Hartman, F. A., Brownell, K. A., Tsung, Y. L.: Amer. J. Physiol. **180,** 375 (1955).
4. McDonald, I. R., Reich, M.: J. Physiol. (Lond.) **147,** 33 (1959).
5. Sapirstein, L. A., Goldman, H.: Amer. J. Physiol. **196,** 159 (1959).
6. Betz, E. D., Brasch, D., Hensel, H.: Arzneimittel-Forsch. **11,** 333 (1961).
7. Papp, M., Stark, E., Ács, Zs., Varga, B.: Endokrinologie **46,** 280 (1964).
8. Sapirstein, L. A.: Amer. J. Physiol. **193,** 161 (1958).
9. Hamilton, W. E., Moore, J. W., Kimsman, I. M., Spurling, R. G.: Amer. J. Physiol. **99,** 534 (1932).
10. Stark, E., Papp, M., Fachet, J., Mihály, K.: Acta physiol. Acad. Sci. hung. **21,** 347 (1962).
11. Hechter, O. L., Macchi, A., Korman, H., Frank, E. D., Frank, H. A.: Amer. J. Physiol. **182,** 29 (1955).
12. Stark, E., Varga, B., Ács, Zs., Papp, M.: Pflügers Arch. ges. Physiol. **285,** 296 (1965).
13. Harrison, R. G., Hoey, M. J.: The adrenal circulation. Blackwell, Oxford 1960. P. 41.
14. Nichols, J., Richardson, A. W.: Proc. Soc. exp. Biol. (N. Y.) **104,** 539 (1960).
15. Ferguson, J. J., Morita, Y., Mendelsohn, L.: Endocrinology **80,** 521 (1967).
16. Sutherland, E. W., Rall, T. W.: J. Amer. chem. Soc. **79,** 3608 (1957).
17. Haynes, R. C., Sutherland, E. W., Rall, T. W.: Recent Progr. Hormone Res. **16,** 121 (1960).
18. Savard, K., Marsh, J. M., Rice, B. F.: Recent Progr. Hormone Res. **21,** 285 (1965).
19. Ahlquist, R. P.: Amer. J. Physiol. **153,** 586 (1948).
20. Szego, C. M., Gitin, E.: Nature (Lond.) **201,** 682 (1964).
21. Szego, C. M.: Fed. Proc. **24,** 1343 (1965).
22. Clayton, J. A., Szego, C. M.: Endocrinology **80,** 689 (1967).
23. Szego, C. M., Roberts, S.: Recent Progr. Hormone Res. **8,** 419 (1953).
24. Wurtman, R. J.: Endocrinology **75,** 927 (1964).
25. Sobin, S. S., Frasher, W. G.: Physiologist **3,** 146 (1960).
26. Varga, B., Ács, Zs., Papp, M., Stark, E.: Endocr. exp. **1,** 173—179 (1967).
27. Engelking, R., Willing, F.: Pflügers Arch. ges. Physiol. **267,** 306 (1958).
28. Stark, E., Varga, B.: Unpublished data.
29. Haynes, R. C., Berthet, L.: J. biol. Chem. **225,** 115 (1957).
30. McKerns, K. W.: Biochim. biophys. Acta (Amst.) **90,** 357 (1964).
31. Stone, D., Hechter, O.: Arch. Biochem. **51,** 457 (1954).
32. Karaboyas, G. C., Koritz, S. B.: Biochemistry **4,** 462 (1965).
33. Staehelin, M., Maier, R.: Excerpta med. Found. Int. Congr. Series No. **161,** 193–195 (1968).
34. Garren, L. D., Ney, R. L., Davis, W. W.: Proc. nat. Acad. Sci. (Wash.) **53,** 1443 (1965).
35. Stark, E., Varga, B., Ács, Zs.: J. Endocr. **37,** 245 (1967).
36. Grahame-Smith, D. G., Butcher, R. W., Ney, R. L., Sutherland, E. W.: J. biol. Chem. **242,** 5535 (1967).
37. Davis, W. W., Garren, L. D.: Biochem. biophys. Res. Commun. **24,** 805 (1966).

38. Shaw, J. E., Ramwell, P. W.: In: Prostaglandins (Nobel Symposium 2), Almquist and Wiksell, Stockholm 1967. P. 293. cit. 33.
39. Kar, A. B., Karkun, J. N., Roy, S. K.: Acta endocrin. (Kbh.) **15,** 101 (1954).
40. Christian, J. J.: Endocrinology **74,** 669 (1964a).
41. Engel, F. L.: Vitam. and Horm. **19,** 189 (1961).
42. Varga, B., Ács, Zs., Papp, M., Stark, E.: Acta physiol. Acad. Sci. hung. **30,** Suppl. 326 (1966).
43. Christian, J. J.: Acta endocrin. (Kbh.) **55,** 62 (1967).

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DATA ON THE ELECTRON MICROSCOPY OF THE BONE MARROW IN LATE EMBRYONAL AND ADULT RATS

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The cytology of the bone marrow has been compared by light and electron microscopy (fixation, glutaraldehyde + osmium; embedding, Durcupan ACM, shadowing, uranyl acetate + lead citrate) in 21-day-old embryos and young Wistar rats weighing 80 to 120 g.

At the end of intrauterine life, (1) the incidence of undifferentiated elements (stem cells, haemocytoblasts), megakaryocytoblasts and basophilic granulocytes was higher, that of the eosinophilic cells was lower, than in the adult animals.

(2) In the myeloid series, the mature granulocytes and basophilic cells were similar, while the intermediate precursors different in structure, displaying many mitochondria and nuclei of medium maturity, few small granules and a dense cytoplasm as compared with the postnatal state.

(3) In general, the embryonal bone marrow cells contained more mitochondria than the adult elements of identical type.

For a more exact determination of the time of onset of myelopoiesis and of the endogenous repopulation, electron microscopic studies of the bone marrow and the blood of younger (16 to 20-day-old) embryos should be carried out.

In previous studies, differentiation of the myeloid cells of the bone marrow was analysed after sedimentation by means of light microscopy [3] and in ultrathin sections by means of electron microscopy [2].

On the basis of the findings it seemed interesting to compare the data for haemopoiesis (myelograms and haemograms) obtained simultaneously in adults and in the late embryonal phase.

In the rat haemopoiesis begins on the 11th day of foetal life [6] with the extramedullar appearance of haemocytoblasts. The erythroid and megakaryocytoid precursors develop first, in the liver on the 13th [5, 8], and in the spleen on the 17th [7] day. Likewise, lymphoid development, e.g. in the thymus, starts early [4]. At that time many immature forms enter the blood stream, for example on the 14th—15th days there is one erythroblast to each 10 nucleated erythrocytes, then an ontogenetic shift to the right is observable [9, 10]. It is only on the 18th to 20th days that proliferation [11] with myelopoiesis starts in the bone marrow. The appearance of neutrophilic and lymphoid cells is followed by an increase in the number of eosinophilic granulocytes and erythroblasts [1].

Materials and methods

In the present investigation 8 embryos on the 21st day of intrauterine life and 8 normal Wistar rats, weighing 80 to 120 g, were used.

The age of the embryos was determined by taking into consideration the 12-hour mating time and the light microscopic appearance of the vaginal smear. On the 21st day the mothers were killed by decapitation, the embryos were removed, decapitated, then their femur was incised under a stereomicroscope, and fixed for light microscopic study in Helly's fluid and stained according to Pappenheim or with haematoxylin-eosin.

From the adult animals the bone marrow plug was removed after cutting off the epiphysis. For electron microscopy, both the embryonal and the adult tissue specimens were fixed in 4.6% glutaraldehyde buffered to pH 7.4 with 0.1 M cacodylate. Elution was carried out likewise with 0.1 M cacodylate buffer; for postfixation similarly buffered 1% OsO_4 pH 7.4 was used. After dehydration in ethanol the specimens were embedded in Durcupan ACM and cut by means of a Reichert Om U2 ultramicrotome. The sections were put on copper grid covered by carbon-stabilized Formvar film and were shadowed with uranyl acetate + lead citrate. The electron micrograms were made by a JEM 6 AS electron microscope with a magnification of 3 500 to 80 000.

Results

In the 21-day embryos, ossification was advanced, occupying the diaphyseal two-thirds of the bone. The bone marrow cells were found between the ossification lamellae in a loose arrangement, forming islets. Many blast cells, myeloid, lymphoid, megakaryocytoid and erythroid cells and few eosinophilic elements could be differentiated (Fig. 1).

At the end of prenatal growth, the mature forms of the erythroid, myeloid, lymphoid and megakaryocytoid series were precisely identified by electron microscopy. In this paper the cytology of myeloid elements will be dealt with in the first place.

Structurally, some embryonic cells were similar to, while other types different from the granuloid cells of the adult animals. The similarity was especially obvious with the initial and final forms of the myeloid elements. Both embryonic and adult myeloblasts (Fig. 2) were characterized by a light nucleus, thin heterochromatin layer, giant nucleolus and by the presence in the cytoplasm of many polysomes and mitochondria. Similarly, the mature granulocytes displayed a segmented, heterochromatin-rich nucleus with a halo around it and a cytoplasm with strongly electro-dense basal tone, many neutrophilic granules, dense core, vacuoles, but few azure granules and mitochondria poor in structure (Fig. 3).

There were few intermediate myeloid precursors in the embryonal bone marrow and the distribution of their organelles differed from what was usually found in adults (Fig. 4). The structure of the nucleus and the considerable number of mitochondria corresponded to the grade of maturity of adult myelocytes. In the latter, however, the granules were abundant and homogeneous (Fig. 5) and the cytoplasm was rich in endoplasmic reticulum. From the point of view of maturity, the embryonal intermediate precursors may be considered

to be mixed cells. This is illustrated by Fig. 6 in which this "intracellular heterochronia" is celarly visible, when we compare the cytoplasm of a younger cell (promyelocyte) with that of a more differentiated one (granulocyte).

According to our observations, embryonal bone marrow cells are richer in mitochondria than are the adult ones of similar maturity. In addition to the myeloid elements this was particularly obvious in the megakaryocytoblasts

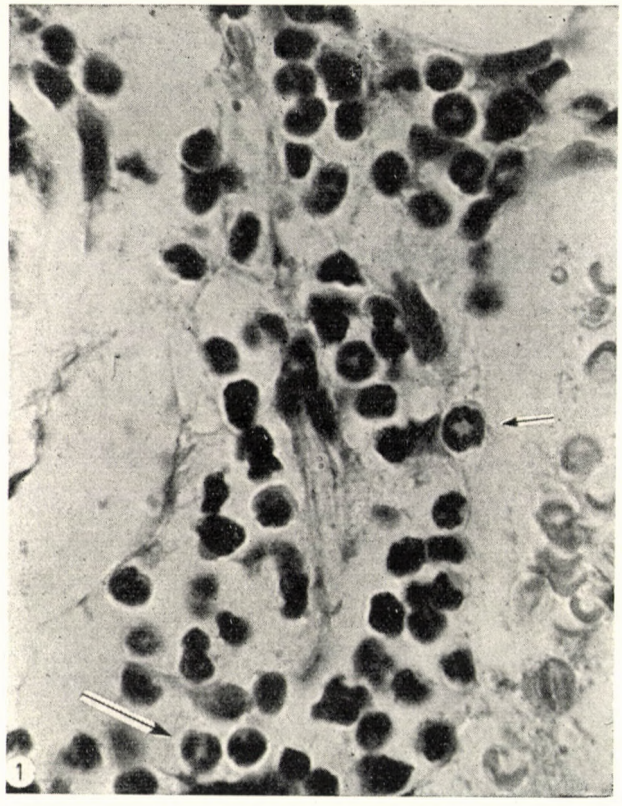


Fig. 1. Light microscopic appearance of the femoral bone marrow of a 21-day-old rat embryo. Haematoxylin-eosin, ×800

(Fig. 7); beside the few α and δ granulomeres there were masses of regular oval mitochondria.

As to the quantitative relations, there were conspicuously numerous megakaryocytes, basophilic granulocytes and mast cells to be seen. In general, the two latter had the same structure as in the adult animals (Fig. 8). The mature (homogeneous dense) basophilic granules were similar in size and structure; only the immature or degenerating granules showed some differences (Fig. 9).

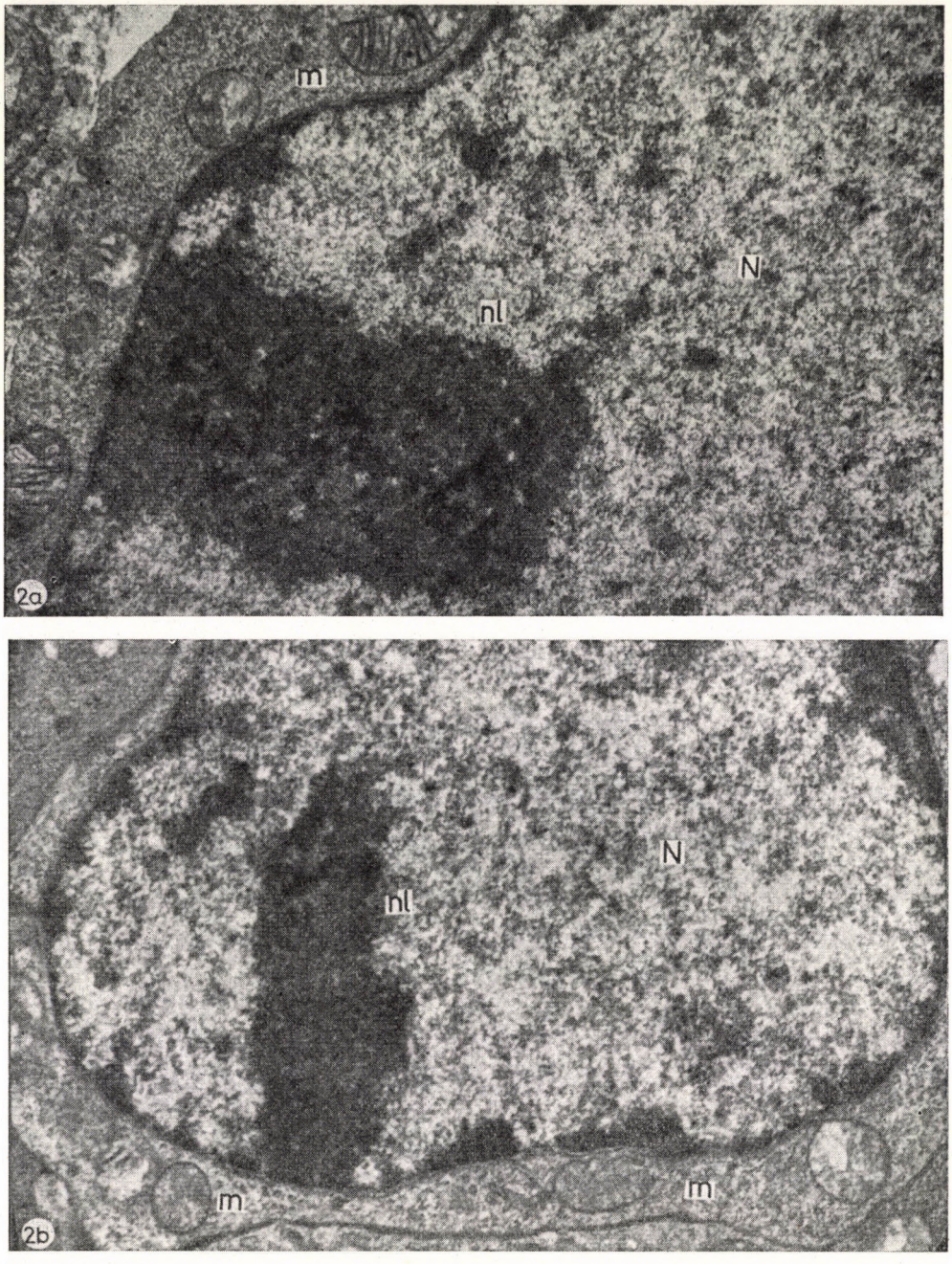


Fig. 2. Myeloblast with developed nucleolus, many polysomes and mitochondria. Bone marrow from a) 21-day-old embryo, b) adult rat. ×14 700

N = nucleus, nl = nucleolus, m = mitochondria, g = granule, G = Golgi apparatus, er = endoplasmic reticulum, h = halo

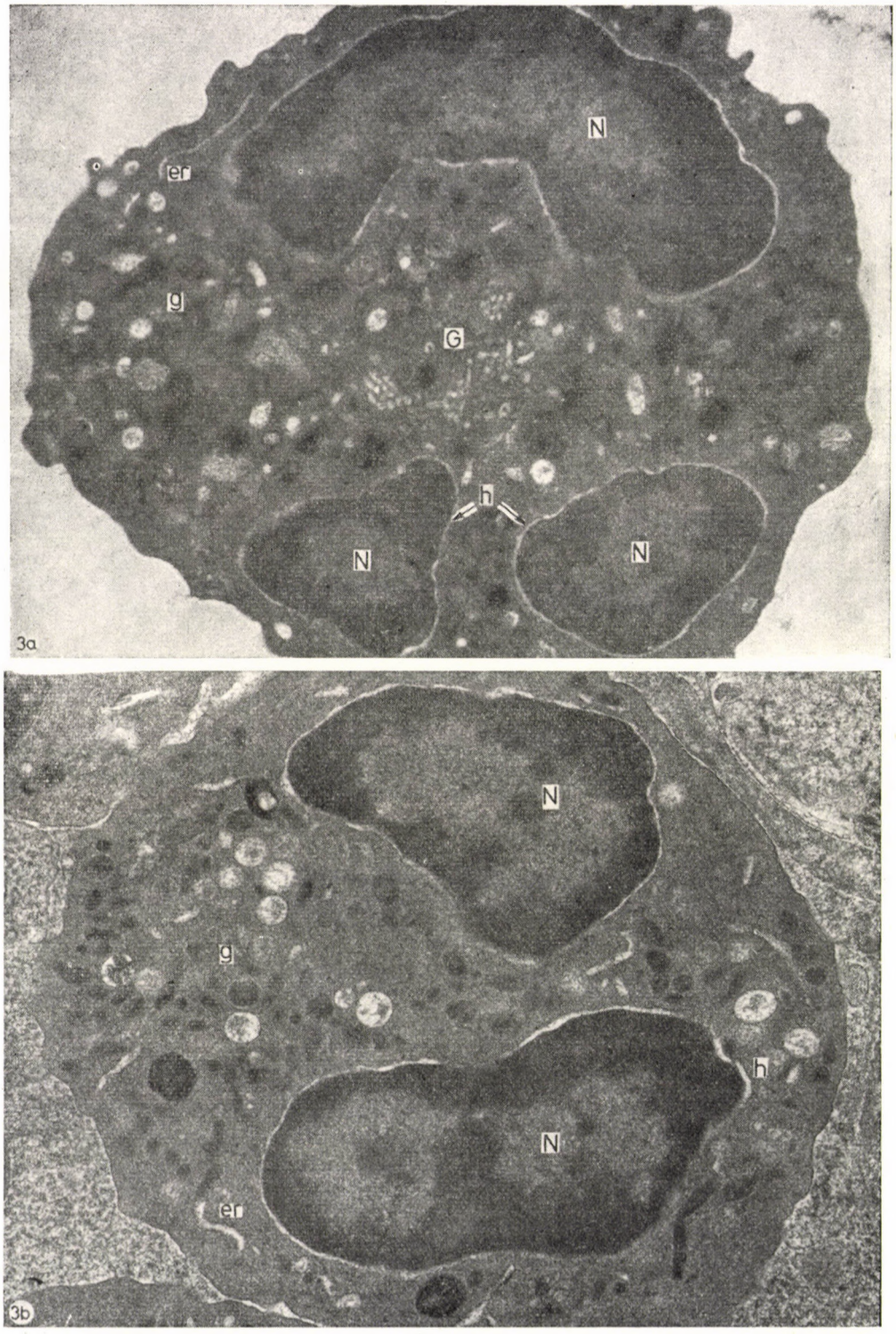


Fig. 3. Mature granulocytes, with similar ultrastructure, from the femur of a) 21-day-old embryo, b) adult rat. ×17 500

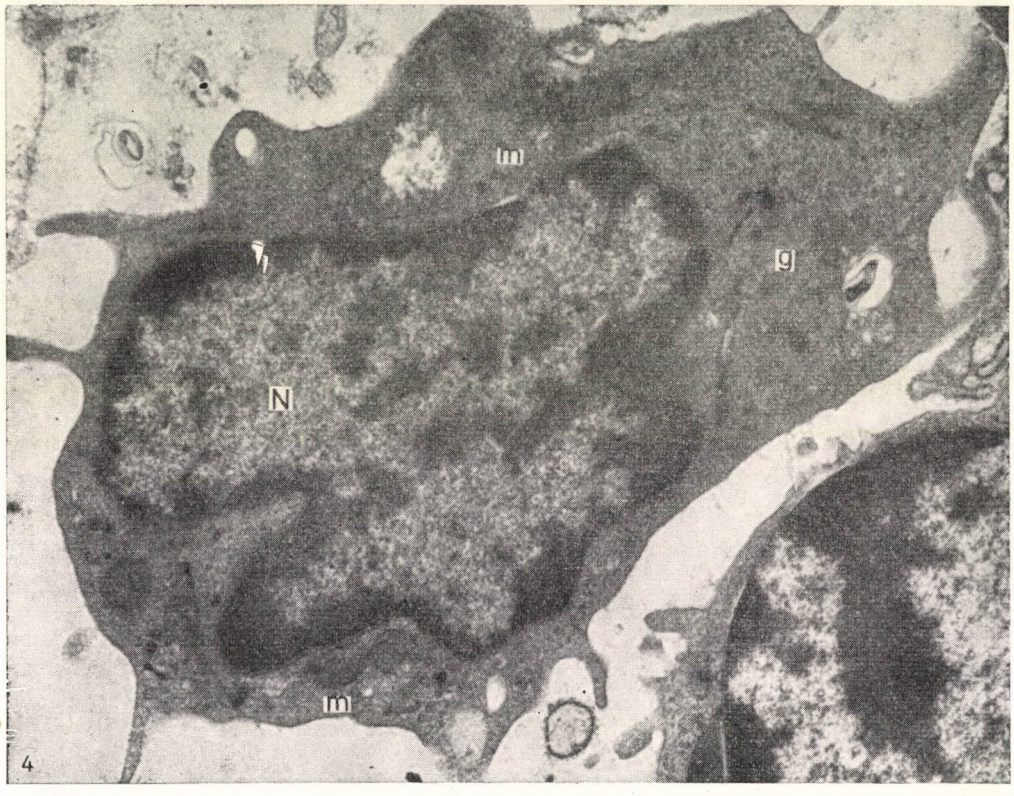


Fig. 4. Intermediate myeloid precursor from 21-day-old rat embryo. Beside the moderately mature nucleus and many mitochondria the low number and small size of granules are conspicuous (ø 230 mμ). ×17 500

Discussion

The 21st day of embryonal development in the rat is a point of time when the bone marrow undergoes rapid and profound morphogenetic changes. It contains beside many immature elements (stem cells, haemocytoblasts) myeloid and lymphoid cells. By that time erythropoiesis, previously concentrated in the liver and spleen [5, 7, 14], is transferred gradually to the bone marrow and progresses presumably very fast, because in the 1 to 2-week newborn the myeloid : erythroid quotient is already 0.6. Later, with the advance of growth, granulopoiesis gains preponderance again (Q = 1.9) [1, 10, 11].

In our previous study [3] we found 0.99% of basophilic granulocytes in the myelograms of adult animals (other authors estimated it at 0 to 1.10%), while the share of eosinophils was 9.98% (other authors, 3.80 to 20.89%). Though no quantitative analysis was made, the light and electron microscopic

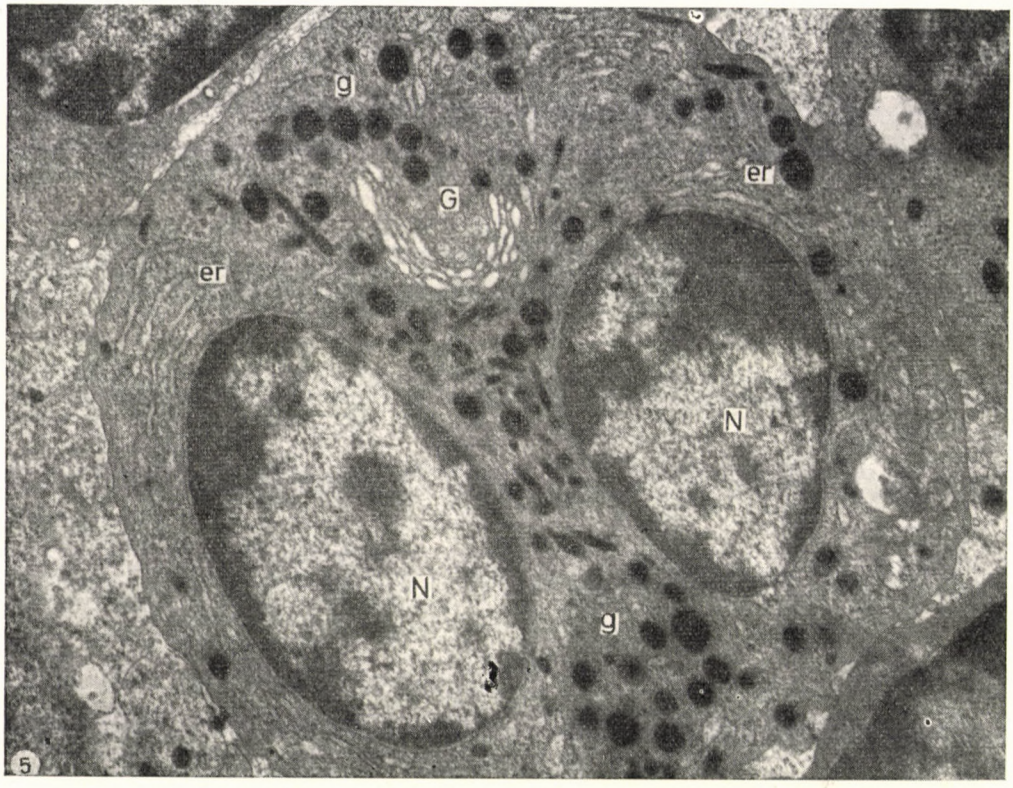


Fig. 5. Neutrophilic myelocyte from bone marrow of adult rat. ×16 100

patterns doubtlessly indicated a higher basophilic and mast cell and a lower eosinophilic granulocyte count. The late appearance of eosinophils in ontogenesis is presumably a phenomenon of more general validity, because it has been described also in a phylogenetically distant group of animals (e.g. Selachii). According to ANDREW [1] in embryonic development the cells appear in the following order in the lymphogranulopoietic organs: 1. haemocytoblast, 2. histiocyte, 3. neutrophilic granulocyte, lymphocyte, 4. eosinophilic granulocyte.

In our specimens, beside basophilic cells and haemocytoblasts, megakaryocytoblasts occurred in large numbers. They showed the structure observed by JONES [8] in those in the liver of 13 to 16-day-old embryos. They differed from the cells of similar type of adult rats or from those of rabbit embryos [14] mainly in the large number of mitochondria, but an increase of the chromoplasm : hyaloplasm ratio, as well as the scarcity of certain (mainly α) granules and ergastoplasmic elements were also observed.

As to the myeloid elements, the high number of mature neutrophils (readily identifiable by electron microscopy) on the 21st day supports the

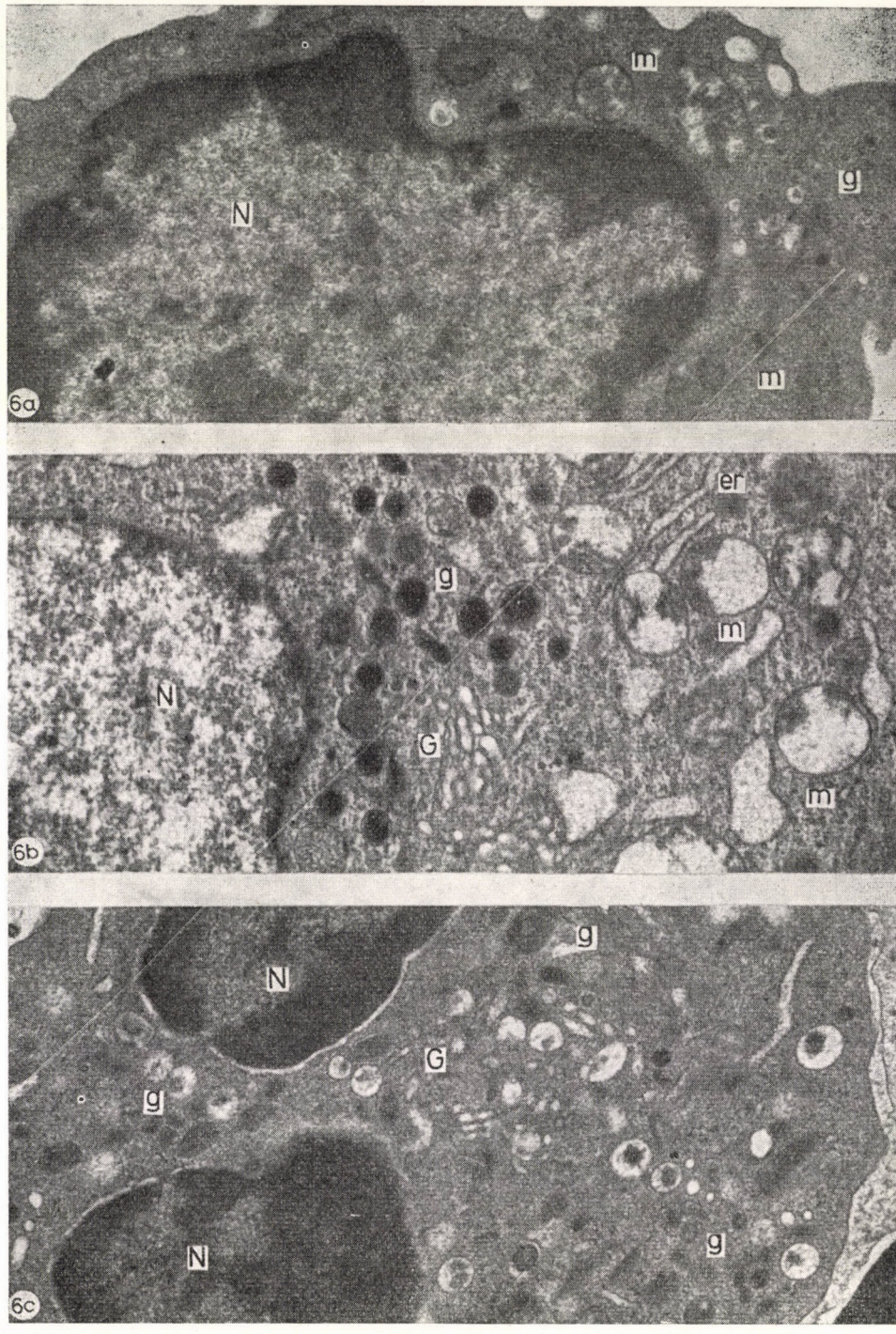
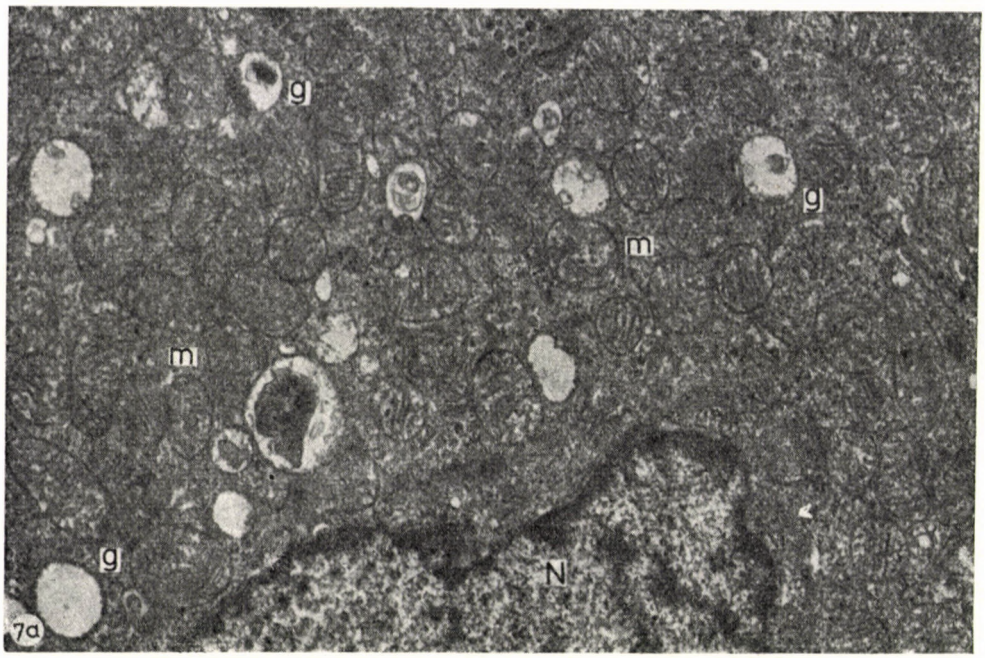


Fig. 6. Cytoplasmic organelles in myeloid cells of the rat. a) 21-day-old embryo, intermediate precursor; b) adult rat, promyelocyte, c) adult rat, granulocyte. ×20 000



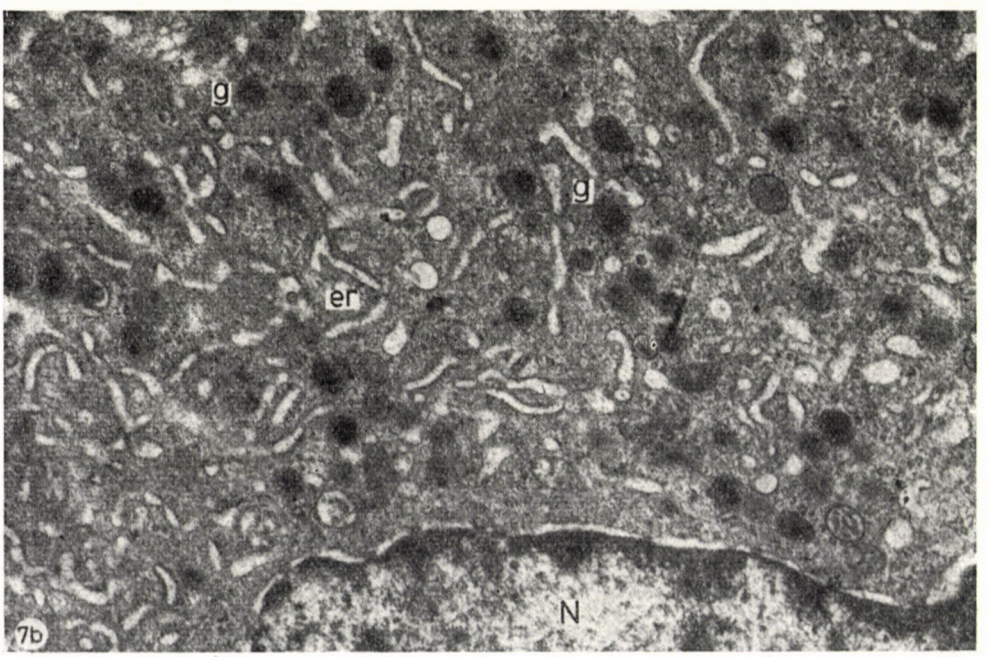


Fig. 7. a) Megakaryocytoblast from 21-day-old embryo. b) Megakaryocyte from bone marrow of adult rat. ×17 500

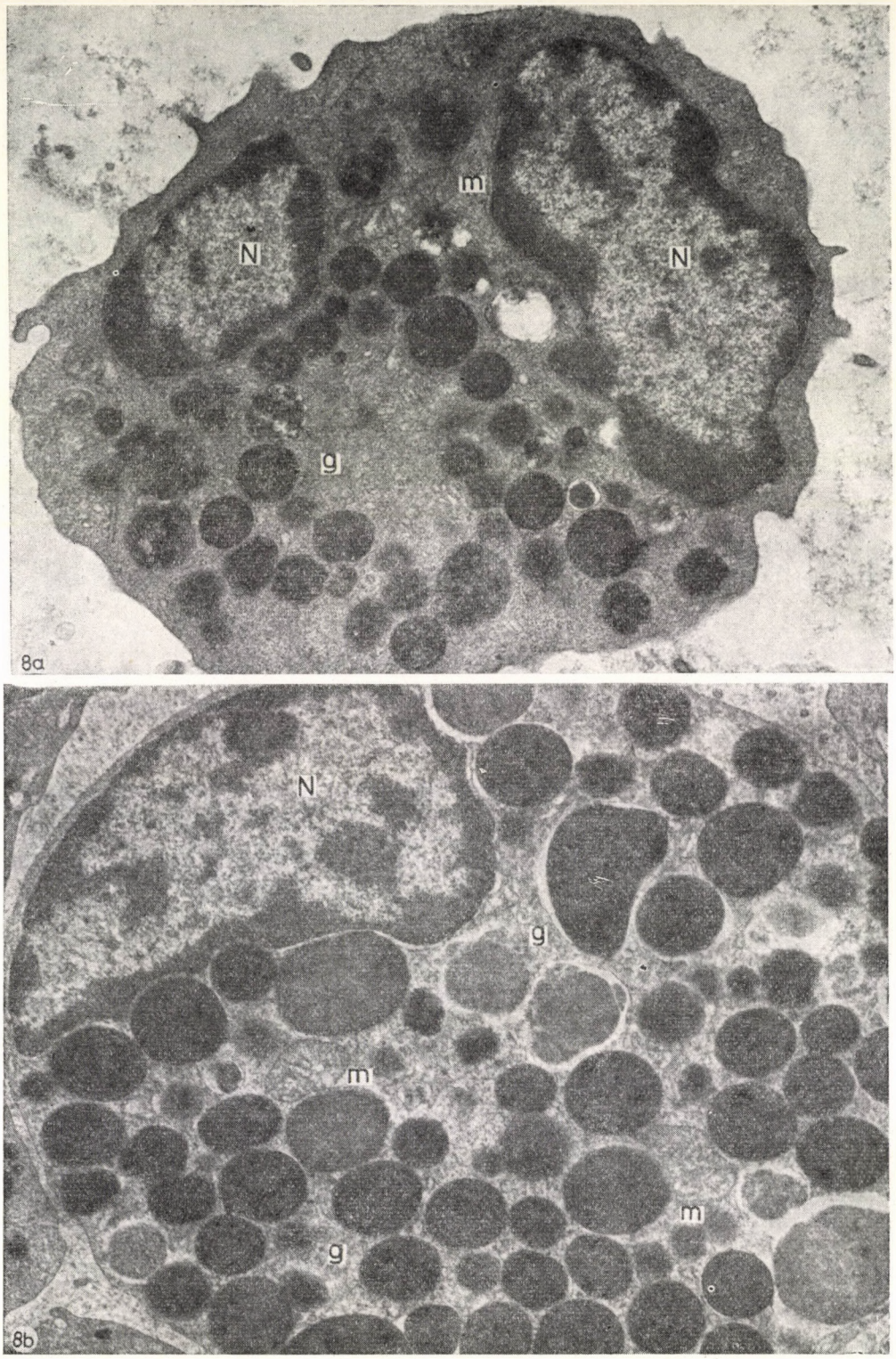


Fig. 8. Basophilic granulocytes of similar ultrastructure a) on the 21st day of intrauterine life, b) in bone marrow of adult rat. ×16 500

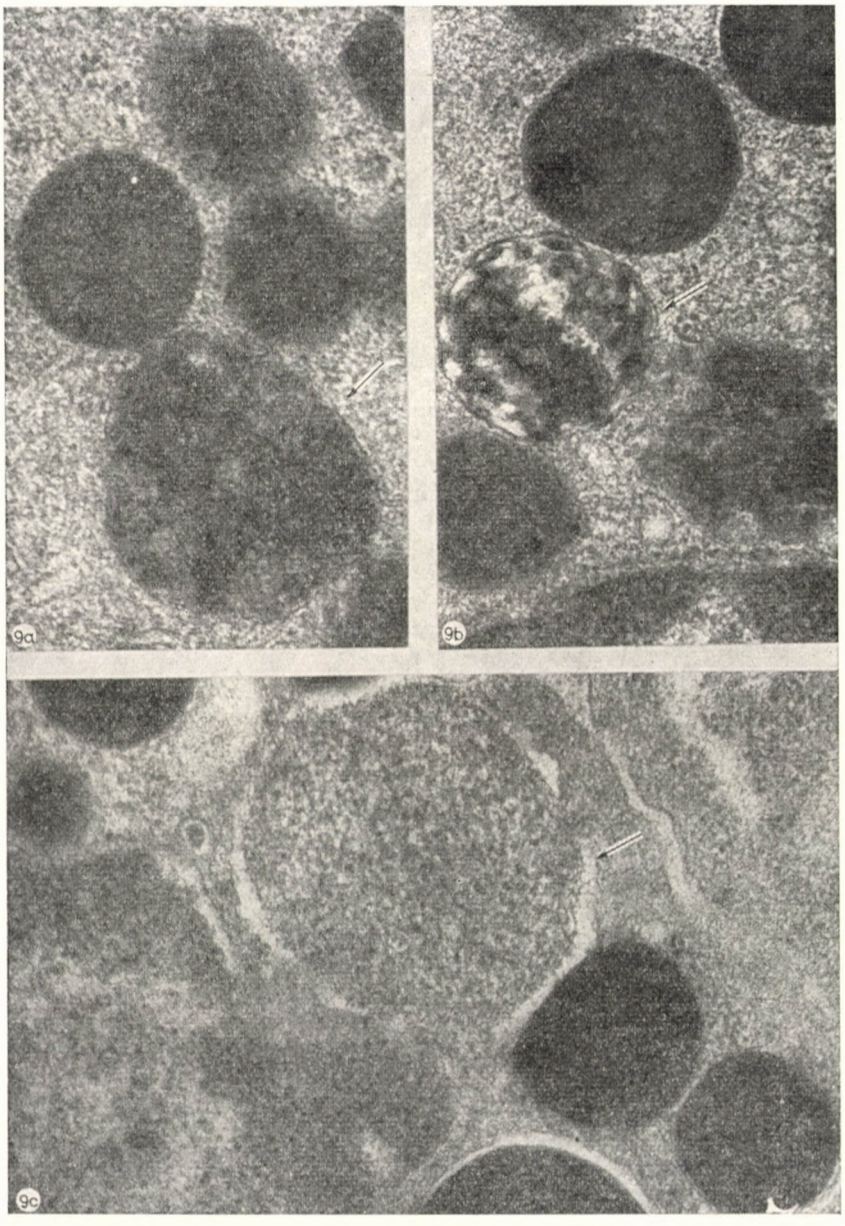


Fig. 9. Structure of immature granules (arrow) in the basophilic granulocytes of an embryo (a, b) and of an adult rat (c) ×68 000

light microscopic observation made by PLUM [11] notably that in the rat myelopoiesis begins on the 18th to 20th days of embryonal development. In the adults, the intermediate myeloid precursors of usual structure are lacking. The nuclear structure, the distribution of cytoplasmic organelles in promyelocytes, myelocytes and metamyelocytes have already been described [2] on the basis of electron microscopic studies. The immature neutrophils of the embryo resemble, however, rather the granuloid cells or histiocytes of lower vertebrates [1]. The differences in structure and biochemistry between foetal and adult blood cells are well known in the case of erythrocytes; the former have nuclei, are larger and contain less haemoglobin [6].

It should be borne in mind that certain elements of the prenatal rat bone marrow may come from other organs, through "embryonal repopulation". On the 11th–14th days of intrauterine life namely a proliferation of lymphoid, erythroid, megakaryocytoid, etc., elements may start in the centre of the thymus anlage [4, 12], in the lymph nodes [9, 13], in the liver [5, 8, 14], and in other animal species also in the kidney [1]. Blood, which in the embryo contains many immature elements [6, 10] such as myeloblasts, promyelocytes and myelocytes [9], may act as the "infusion" agent.

*

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REFERENCES

1. ANDREW, W.: Comparative hematology. Grune and Stratton, New York 1965.
2. BALÁZS, A., BUKULYA, B.: Biol. Közl. **16,** 13 (1968).
3. BALÁZS, A., RAPPAY, GY.: Acta biol. Acad. Sci. hung. **18,** 371 (1967).
4. BUKULYA, B.: Acta physiol. Acad. Sci. hung. **30,** 394 (1966).
5. GRASSO, J. A., SWIFT, H., ACKERMAN, G. A.: J. Cell Biol. **14,** 235 (1962).
6. HAGEMANN, E., SCHMIDT, G.: Ratte und Maus. Versuchstiere in der Forschung. De Gruyter, Berlin 1960.
7. HOLYOKE, E. A., LATTA, J. S., MCLEAN, J. V.: J. Ultrastruct. Res. **15,** 87 (1966).
8. JONES, P. O.: Anat. Rec. **138,** 105 (1960).
9. KNOLL, K.: Vergleichende Hämatologie. In: Heilmeyer, L. und Hittmair, A. (Eds): Handbuch der gesamten Hämatologie. Vol 1, Part 1, pp. 6—16. Urban und Schwarzenberg, München–Berlin–Wien 1957.
10. KÖHLER, H.: Knochenmark (einschliesslich Leukose). In: COHRS, P., JAFFÉ, R. und MEESSEN, H. (Eds): Pathologie der Laboratoriumstiere. Pp. 235–331. Springer, Berlin–Göttingen–Heidelberg 1958.
11. PLUM, C. M.: Folia haemat. **67,** 119 (1943).
12. SANEL, F. T.: Z. Zellforsch. **83,** 8 (1967).
13. YOFFEY, J. M.: Bone marrow reactions. Arnold, London 1966.
14. ZANBONI, L.: J. Ultrastruct. Res. **12,** 525 (1965).

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EFFECT OF A SINGLE NEONATAL GLYCOCORTICOID INJECTION ON THE THYMUS-LYMPHATIC AND ENDOCRINE SYSTEM AND ON THE GROWTH OF THE RAT AND THE DOG

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A single large dose of glycocorticoid administered 24 hours after birth to rats and dogs results in a fatal cachectic condition similar to the wasting syndrome which follows neonatal thymectomy. The survivors showed significantly lower body, thymic splenic and adrenal weight than the controls and the number of small lymphocytes was significantly decreased in the lymphatic organs and blood. Homologous skin grafts survived considerably longer and their reaction was much weaker than in the untreated controls. At 4 weeks following neonatal prednisolone treatment, serum total protein and all three globulin fractions were found to be less than normal. At 4 weeks the stress-induced rise in the blood corticosterone level was less than in the controls. In the development of fatal cachexia following neonatal glycocorticoid treatment, a primary role is attributed to disturbance of the thymic function but direct effects exerted by glycocorticoids on the peripheral lymphatic and endocrine organs and other tissues must also be taken into account.

In the past few years (1961 to 1967) it has become obvious that a normal thymic function is essential for the development of the lymphatic system, resistance to infection and immune defense of the body. It has been proved that the function of the thymus is particularly important in the neonatal period. In rodents thymectomy performed within 24 hours after birth led to a decrease in the lymphocyte count of blood and lymphatic organs, to an impairment of immune defense and in a variable percentage of cases to the development of the wasting syndrome [1–9, 16].

In young adult age, however, thymectomy has a slight influence on the activity of lymphatic organs [9–11], endocrine glands [12–15, 35] and body weight [10, 12, 16, 35] during the weeks following operation.

Development of the lymphatic system and the activity of the immune defense apparatus are controlled not only by the thymus, but also by other factors such as the adrenal cortical hormones, among others.

A single dose of glycocorticoid administered in adult age has no, or very slight influence on the immune response and induces only temporary lymphopenia and involution of the thymus-lymphatic system [17–20, 53, 54].

In view of the known consequences of neonatal thymectomy [1–9] it seemed interesting to study the influence on the development and function of the thymic-lymphatic system of a single dose of glycocorticoid administered

on the first day of life, when the lymphatic organs are not yet fully developed and the immune apparatus is not yet fully competent. The other effects of glycocorticoids have also been studied, thus the inhibition of growth and the impairment of pituitary-adrenocortical function.

The results obtained may be summarized as follows.

A. Effect of a single dose of glycocorticoid in the neonatal period on the thymus-lymphatic system and on body weight

It has been shown that in the rat a single high dose of glycocorticoid injected within 24 hours after birth causes fatal cachexia resembling the wasting syndrome arising after neonatal thymectomy ([20], Fig. 1). Lower doses caused the death of fewer animals at a later period. It was exclusively the glyco-

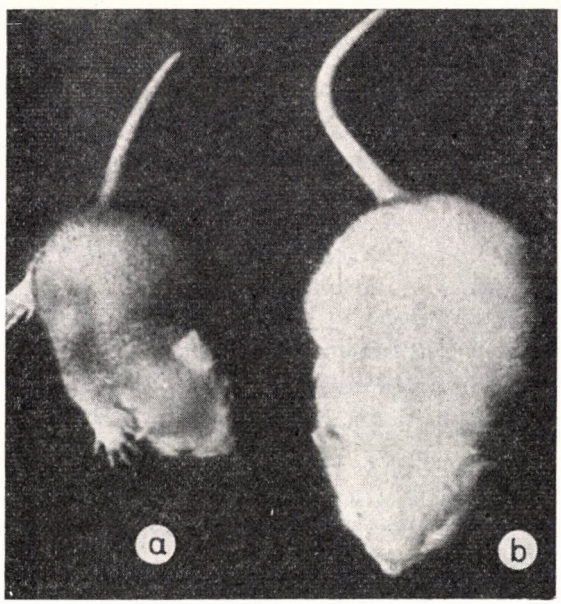

Fig. 1. Effect of a single neonatal cortisol dose in the rat, at 4 weeks. a : cortisol, 1 mg/animal, intraperitoneally; b: 0.9% NaCl solution, intraperitoneally

corticoids that caused fatal cachexia, aldosterone and DOCA did not. Nor did the glycocorticoid cause fatal cachexia when administered on the 5th day of life and in this case thymic involution was followed by rapid regeneration [20].

The neonatal injection of 0.5 mg of cortisol considerably reduced the rate of growth during the first week and only slightly during the second. Following the injection of 0.25 mg of prednisolone the rate of growth was even lower, but development increased rapidly during the 4th week. A 2.5 mg dose of cortisol completely blocked growth and the animals died in a few days.

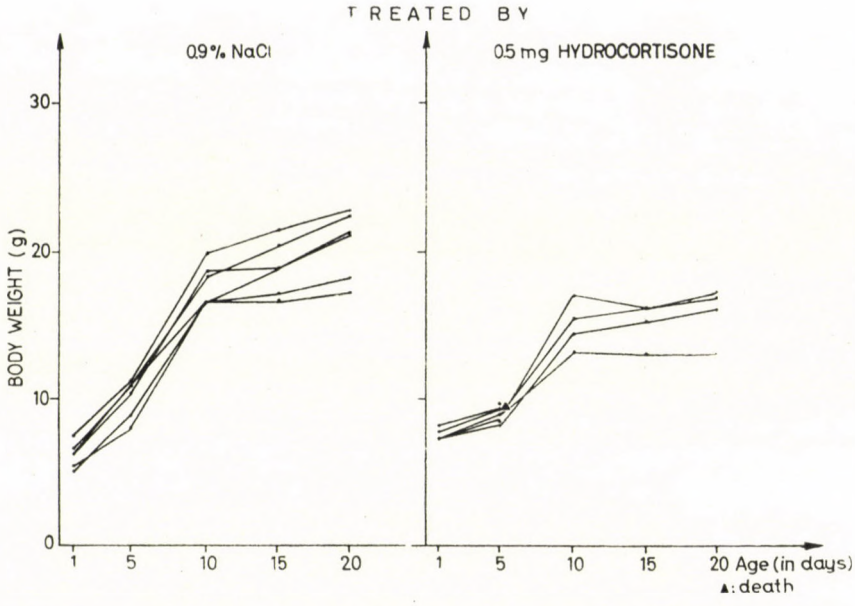

Fig. 2. Effect of a single glycocorticoid dose given 24 hours after birth on the weight curve of rats

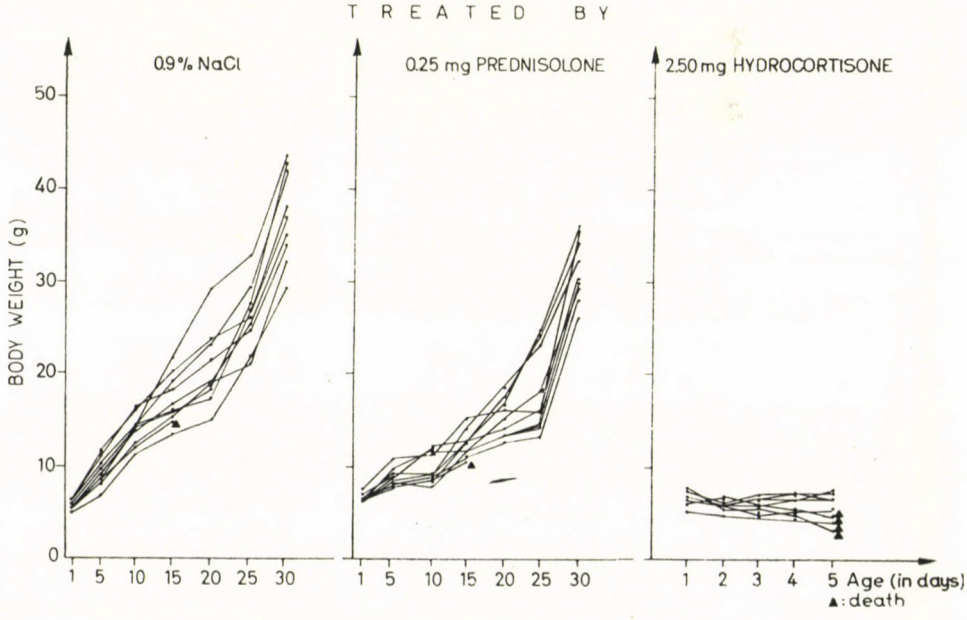

Fig. 3. Effect of a single glycocorticoid dose given 24 hours after birth on the weight curve of rats

Table I

Effect of a single dose of glycocorticoid administered to rats on the first day of life

Glycocorticoid treatment	Route of injections	Dose	Number of animals	Days after injection	Weight				WBC/mm³	Small Ly/mm³	Ly/My	Ratio Small Ly/ Large Ly
					body g	adrenal mg	thymus mg	spleen mg				
Cortisol	intraperitoneal	1.0 mg	9	8	4.38 ±0.20	1.55 ±0.20	5.20 ±1.75	7.80 ±1.13	4 875.0 ±149.3	562.0 ±234.5	0.93 ±0.28	0.32 ±0.11
0.9% NaCl	intraperitoneal	0.04 ml	9	8	14.40 ±0.44	4.05 ±0.50	45.50 ±3.34	73.25 ±4.70	5 550.0 ±150.0	2 885.0 ±107.3	3.57 ±0.24	2.01 ±0.10
Cortisol	intraperitoneal	1.0 mg	9	24	19.65 ±1.82	8.22 ±0.46	50.93 ±11.35	141.8 ±25.5	10 525.0 ±1 062.1	2 013.5 ±205.1	1.43 ±0.14	0.50 ±0.01
0.9% NaCl	intraperitoneal	0.04 ml	8	24	38.40 ±2.06	12.44 ±0.81	181.8 ±16.7	220.0 ±28.2	10 650.0 ±298.6	5 803.5 ±166.6	5.62 ±0.37	2.12 ±0.15
Prednisolone	intraperitoneal	0.25 mg	10	30*	75.00 ±5.80	—	—	—	7 460.0 ±1 099.1	2 803.8 ±421.3	4.75 ±0.24	0.83 ±0.03
0.9% NaCl	intraperitoneal	0.05 ml	9	30*	80.00 ±4.50	—	—	—	10 577.8 ±581.6	4 869.3 ±294.9	5.15 ±0.24	1.30 ±0.09
Dexamethasone	subcutaneous	0.25 mg	7	40	79.20 ±7.9	20.00 ±1.35	249.70 ±40.30	3 340.50 ±49.50	7 966.7 ±391.3	1 664.0 ±119.8	2.79 ±0.33	0.42 ±0.03
0.9% NaCl	subcutaneous	0.05 ml	8	40	113.1 ±6.7	24.63 ±1.54	345.50 ±33.30	486.90 ±75.30	12 475.0 ±86.1	5 832.0 ±247.0	4.70 ±0.32	1.85 ±0.48

(All values $\bar{x} \pm S\bar{x}$). * Organs not weighed.

In animals which survived the neonatal glycocorticoid treatment the following changes were observed. The weight of thymus, spleen and adrenals was significantly lower, but the index organ weight/100 g body weight was reduced in the case of the thymus only ([20, 24], Table I).

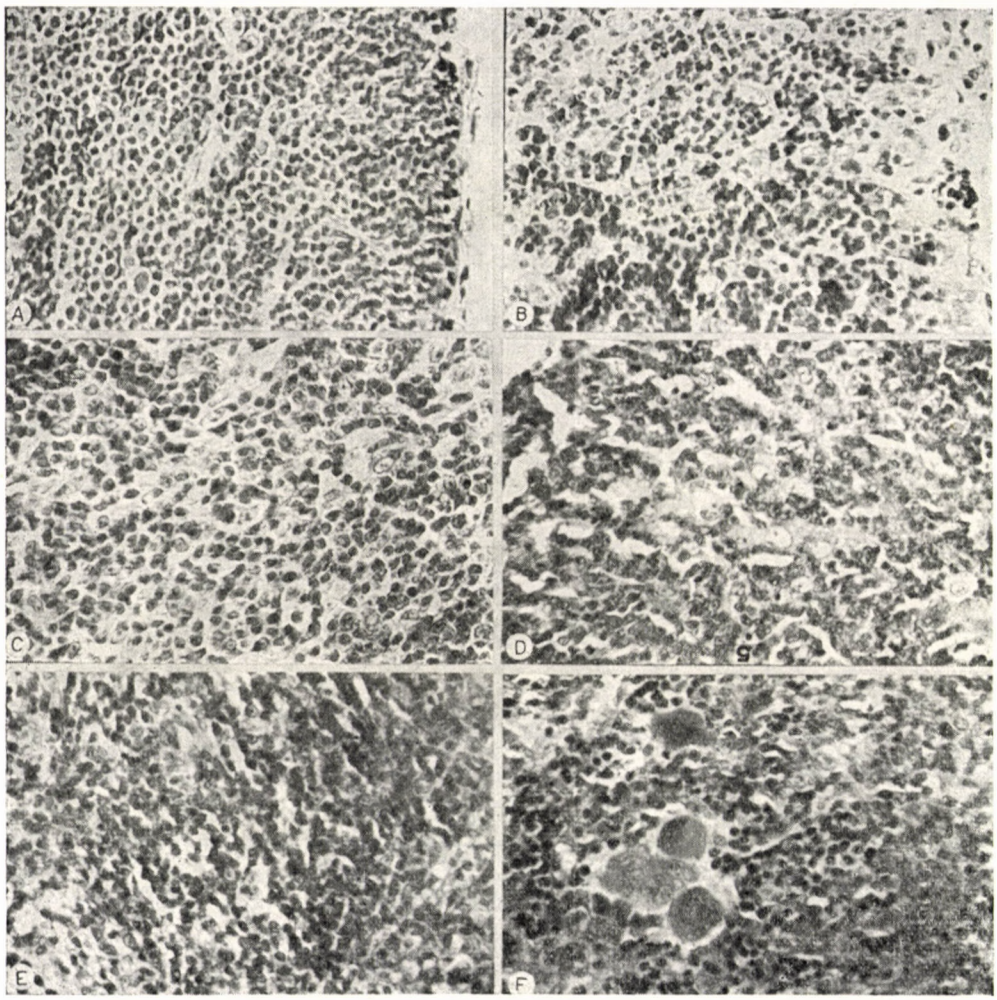

Fig. 4. Effect of a single neonatal cortisol dose on the cortex and medulla of the thymus gland and on the spleen. A and B: thymic cortex, C and D: thymic medulla, E and F: spleen, A, C and E: control B .D and F: treated with cortisol. Haematoxylin-eosin, ×375

The small lymphocyte count decreased significantly in every case ($p < 0.001$), while the leucocyte count was not reduced in every group. The small/large lymphocyte quotient, too, was significantly reduced ($p < 0.001$) (Table I).

Histological examination 8 days after the neonatal glycocorticoid treatment revealed that the medulla-cortex border was indistinct in the thymus; the structure of this organ had loosened up, the number of lymphoid elements decreased, while that of reticular elements increased (Fig. 4A, B, and Fig. 4C, D).

In the spleen the number of malpighian bodies and of the mature, small lymphocytes decreased, while that of the reticular elements and megakaryocytes conspicuously increased (Fig. 4E, F).

The lymph nodes displayed a dissociated structure, the small lymphocyte count was decreased and the number of reticular elements increased (Fig. 5A, B). These histological changes were still in evidence on the 40th day [20,22].

Table II

Effect on homologous and heterologous skin grafts of a single hydrocortisone injection administered to rats 24 hrs after birth

Group	Number of animals	Beginning of graft rejection on day							
		12th		15th		18th		20th	
		homol.	heter.	homol.	heter.	homol.	heter.	homol.	heter.
0.9% NaCl, intraperitoneally	6	*0/6	6/6	4/6	6/6	6/6	∅	∅	∅
1 mg hydrocortisone, intraperitoneally	6	0/6	6/6	0/6	6/6	0/6	∅	5/6	∅

* Number of animals showing first signs of rejection per total number of animals with skin graft

∅ = complete rejection.

Table III

Effect of single prednisolone dose administered on the first day of life on the absolute value of total serum protein and on fractions in rats

(All values are $\bar{x} \pm S\bar{x}$)

Groups	Treatment	Age in days	Number of animals	Total protein g/100 ml	Albumin mg/100 ml	α Globulin mg/100 ml	β Globulin mg/100 ml	γ Globulin mg/100 ml
Control	0.1 ml 0.9% NaCl intraperitoneally	28	8	4.94±0.05	2255±64	1156±40	1134±27	393±38
Prednisolone	1 mg intraperitoneally	28	11	4.19±0.06	2085±63	960±48	934±29	212±54
Adult control	—	90	4	5.50±0.09	1911±70	1139±96	1472±50	978±62

In view of the significant decrease in the number of lymphocytes in the blood and tissues it seemed justified to study the function of the thymus-lymphatic system following neonatal glycocorticoid treatment. For this purpose the observation of transplantation immunity, as an index of cellular immunity, seemed to be the most appropriate.

We have shown previously that after neonatal treatment with 1 mg of glycocorticoid the heterologous (CBA mouse) skin graft was rejected without while the homologous skin graft (R rat) was rejected with a certain delay ([22], Table II).

To study humoral immunity, we tested the serum gamma globulin level and found that at 28 days following neonatal prednisolone treatment serum total protein and all three globulin fractions were decreased ($p < 0.05$) ([23], Table III).

According to the results of our experiments now in progress antibody production is also diminished in the rat subjected to neonatal glycocorticoid treatment [36].

B. Effect of neonatal glycocorticoid treatment on the pituitary-adrenocortical system and on thyroid activity

Apart from its effect on the thymus-lymphatic system, neonatal glycocorticoid treatment may damage the pituitary-adrenocortical system and the thyroid gland; these organs may also have a role in the development of fatal cachexia.

It is well known [26–30] that in the adult rat a single large dose of glycocorticoid suppresses pituitary-adrenocortical activity and blocks its stress-

Table IV

Effect of a single 0.5 mg dose of cortisol administered on the first day of life on resting and post-formalin blood corticosterone level (0.25 ml 1% formalin/100 g b. wt.) Stress was followed by decapitation 30 min later

Neonatal treatment, intraperitoneally	Number of determinations	Weeks after neonatal treatment	Formalin subcutaneously 30 min before decapitation	Plasma corticosterone μg/100 ml
0.9% NaCl	5	4	0.9% NaCl	15.0±1.8
Cortisol	5	4	0.9% NaCl	17.1±0.9
0.9% NaCl	7	4	Formalin	50.1±2.2
Cortisol	6	4	Formalin	33.0±3.9
0.9% NaCl	6	8	0.9% NaCl	16.9±1.9
Cortisol	6	8	0.9% NaCl	19.2±3.7
0.9% NaCl	6	8	Formalin	51.1±2.7
Cortisol	6	8	Formalin	63.7±2.4

induced activation. This effect develops within hours and may last 24 to 48 hours. The duration of inhibition depends on the glycocorticoid dose and on the kind of non-specific stimulation employed [31]. We have therefore estimated the blood corticosterone level in both the resting and stress conditions

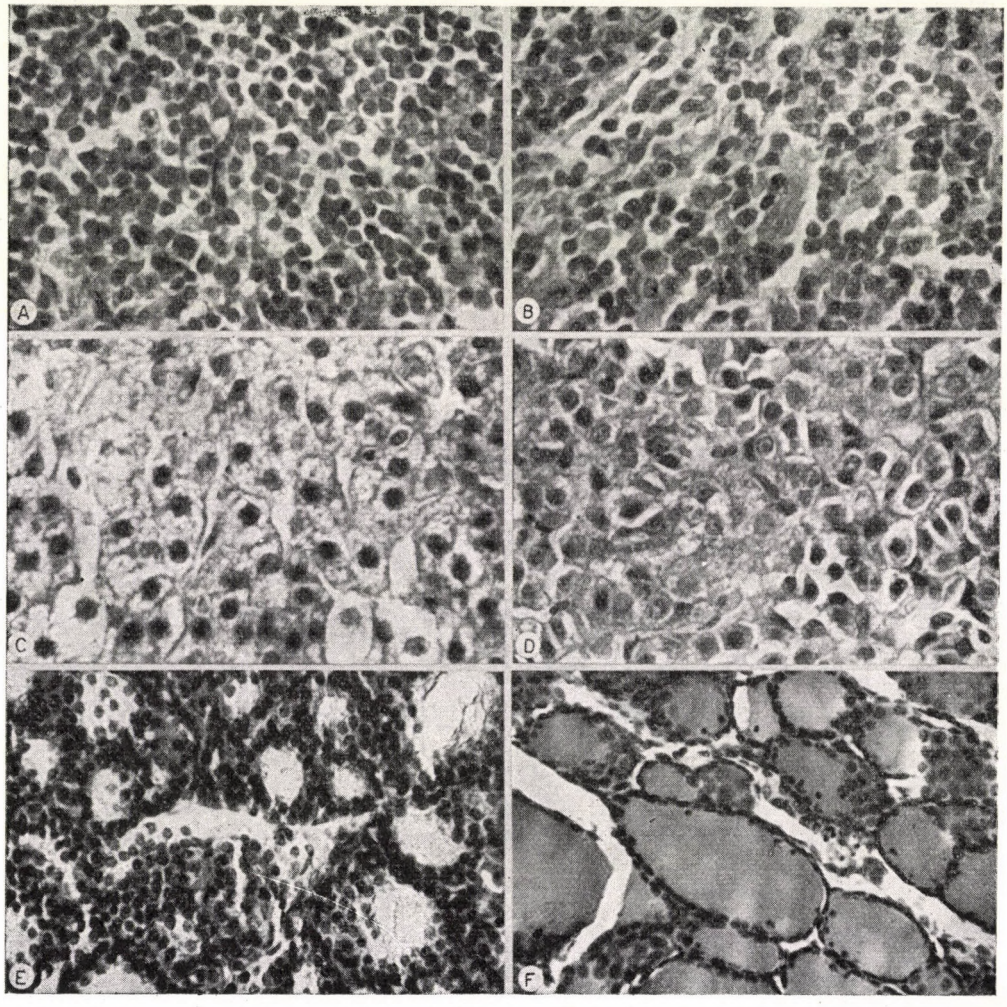

Fig. 5. Effect of a single neonatal cortisol dose on the lymph node (A and B), adrenal cortex (C and D) of the rat and thyroid gland (E and F) of the dog. A, C and E: control, B, D and F treated with cortisol. Haematoxylin-eosin, A, B, C and D: ×600, E and F: ×340

at various time intervals after neonatal glycocorticoid treatment. The corticosterone level in peripheral blood was determined by a fluorometric method [32].

Two weeks after neonatal glycocorticoid treatment formalin stress (0.25 ml per 100 g body weight of 3% formalin subcutaneously) killed 8 ani-

mals out of 10 within 30 minutes. At 4 and 8 weeks the concentration of formalin was reduced (0.25 ml per 100 g body weight of 1% formalin, subcutaneously) and the animals were decapitated 30 minutes later. At 4 and 8 weeks after neonatal glycocorticoid treatment the resting blood corticosterone level was similar to that found in the controls. At 4 weeks the stress-induced rise in the blood corticosterone level was less and at 8 weeks more than in the controls (Table IV), these changes were significant statistically ($p < 0.01$).

Pituitary-adrenocortical reactivity was in accord with the histologic appearance of the adrenal cortex. Eight days after neonatal glycocorticoid treatment the changes were indicative of hypofunction (Fig. 5C, D), while at 40 days rather of hyperfunction [22]).

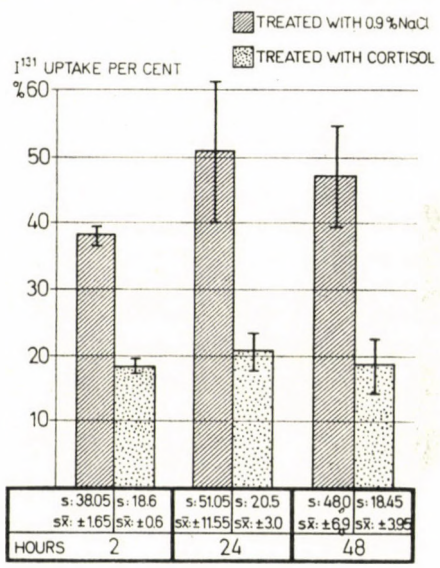

Fig. 6. Effect on thyroid ^{131}I uptake of 10 mg/100 g of cortisol administered intramuscularly on the first day of life, in the 6-week-old dog

It is known that in hyperthyroidism or following thyroxine treatment thymic hyperplasia occurs [19, 25], in spite of a significant rise in the blood corticosterone level [14]. We have, therefore investigated the effect of a single neonatal glycocorticoid injection on thyroid function.

It was found that 8 and 40 days after neonatal glycocorticoid treatment the thyroid gland of the rat shows histological changes suggestive of mild hyperfunction [22]. In the newborn dog a single 10 mg/100 g body weight dose of cortisol-induced thyroid hypofunction by 6 weeks; thyroid ^{131}I uptake decreased (Fig. 6) ($p < 0.001$), the acini were enlarged, filled with colloid and the epithelial layer was flattened (Fig. 5E, F, [33]).

Discussion and conclusions

According to our experiments conducted in recent years, fatal cachexia is induced in the rat by a single neonatal glycocorticoid injection. If the dose is high enough, the mortality rate is 100%. It has been shown that the same treatment is followed by fatal cachexia in the mouse [21, 40, 41], in the rat [39, 42 44] and in the dog [23], too. The fact that this fatal cachexia is closely similar to the wasting syndrome arising after neonatal thymectomy [1–8, 16, 45] has led us to investigate whether the thymic damage caused by a single neonatal glycocorticoid injection had any role to play in the fatal cachexia.

In the early phase of our experiments the above assumption seemed to be strengthened by the fact that a single glycocorticoid injection administered on the 5th day of extrauterine life did not cause fatal cachexia [20], and it was thought that the same treatment employed on the first day of life was effective because then the lymphatic apparatus is still immature and the glycocorticoid suspended thymic function, just when it would have been essential for the development of the lymphatic and immune apparatus [1–9, 16, 34, 45]. This hypothesis has been confirmed by our own results and also by the evidence reported in the literature.

We have observed a significant involution of the thymus, spleen and lymph nodes following neonatal glycocorticoid treatment. In these organs and the blood the lymphocyte count decreased significantly [20, 22, 24]. The tests for thymic function likewise showed a damage, as transplantation immunity and furthermore the gamma globulin level [22, 23, 24] as well as according to experiments now in progress, antibody production, were all reduced [36].

Our results are in accord with the published evidence that neonatal glycocorticoid treatment is followed by a decrease of the immunoglobulin level and antibody production [40, 42, 46]. The importance of the immunological damage has been stressed by REED and JUTILA [40, 41], as well as DUHIG [47], according to whom in both germ-free and antibiotic-treated non-germ-free mice, the incidence of fatal cachexia decreased significantly.

The neonatal glycocorticoid treatment cannot be considered to be merely a "hormonal thymectomy", because in the survivors the thymus-lymphatic system regenerates and the other symptoms subside in a few weeks. A further difference from wasting syndrome following neonatal thymectomy is the extra-thymic actions of the neonatal glycocorticoid treatment.

While following neonatal thymectomy, lymphopenia and the damage to cellular immunity are the most characteristic symptoms [1–8, 16], neonatal glycocorticoid treatment causes an additional impairment of humoral immunity manifesting itself with a decrease of the gamma globulin level and of antibody production.

In birds the bursa of Fabricius is responsible for the development and function of the antibody-producing apparatus, i.e. for humoral immunity

[48, 49]. It may be assumed that during the neonatal period an equivalent organ may exist and act in mammals [50, 51], but this has not yet been confirmed. Our results seem to suggest that glycocorticoid treatment in neonatal age is damaging not only the thymus, but also the tissue or organ responsible for the organization of humoral immunity in the neonatal period.

We have assumed that, in addition, an inhibition of pituitary-adrenocortical activity may be responsible for the high mortality, it being known that normal adrenocortical function is essential for the survival of acute stress [17, 18].

After a single injection of a high dose of glycocorticoid in adult rat, the pituitary-adrenocortical system is inhibited within a few hours then its activation by stress is not possible and this inhibition persists for 48 hours at most [26–31]. The mortality due to stress at 2 weeks and the inhibition of the rise in the blood corticosterone level at 4 weeks suggest that neonatal glycocorticoid treatment caused a long-lasting inhibition of the pituitary-adrenocortical system. During the 4th week the corticosterone binding capacity was 26.2 ± 2.5 μg/100 ml, as compared with the control value of 19.5 ± 2.9 μg/100 ml, thus the pituitary-adrenocortical inhibition may hardly be explained by a change in the free/bound corticoid ratio.

At 8 weeks, however, formalin stress caused a somewhat higher increase of the corticosterone level than in the controls. We assume that by that time the pituitary-adrenocortical system must have regenerated. This is supported by the observation of SZEBERÉNYI [39] that 30 days after neonatal dexamethasone treatment corticosteroid production and pituitary ACTH content were higher than in the controls, and SHAPIRO [43] found that at 40 days following neonatal glycocorticoid treatment the rise in the corticosteroid level in response to stress was practically the same as in the controls.

We believe that the inhibition of pituitary-adrenocortical activity depends on the dose of glycocorticoid and on the time elapsed after the injection, and the reactivity to stress is different in the different phases of recovery.

Neonatal glycocorticoid treatment might damage, in addition, some other metabolic processes. This is suggested by the subsequent inhibition of growth, wasting of the skin, the delay in hair growth, the decrease in the serum hexosamine and glycoprotein levels and the neutral mucopolysaccharide contents in liver, skin and cartilage [37, 38] and of the DNA, RNA and protein contents in certain organs [52].

Our results tend to indicate that adequate thymic, pituitary and adrenocortical functions are of particular importance in the neonatal period.

In the development of fatal cachexia following neonatal glycocorticoid treatment, a primary role is attributed to disturbance of the thymic function, because neonatal thymectomy in itself may give rise to the wasting syndrome.

*

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REFERENCES

1. Miller, J. F. A. P.: Lancet **2,** 748 (1961).
2. Jankovic, B. D., Waksman, B. W., Arnason, B. G.: J. exp. Med. **116,** 159 (1962).
3. Parrott, D. M. V., East, J.: Nature (Lond.) **195,** 347 (1962).
4. Good, R. A., Dalmasso, A. P., Archer, C. D. K., Pierce, J. C., Papermaster, B. W.: J. exp. Med. **116,** 773 (1962).
5. Sherman, J. D., Damashek, W.: Nature (Lond.) **197,** 469 (1963).
6. Fachet, J., Palkovits, M., Vallent, K.: Acta med. Acad. Sci. hung. **21,** 305 (1965).
7. Földes, P., Szeri, I., Bános, Zs., Anderlik, P., Balázs, M.: Acta microbiol. Acad. Sci. hung. **11,** 77 (1964–65).
8. Parrott, D. M. V.: Transplant. Bull. **29,** 102 (1962).
9. Fachet, J., Stark, E., Palkovits, M., Mihály, K.: Acta med. Acad. Sci. hung. **21,** 297 (1965).
10. Manning, M. J.: J. Endocr. **19,** 143 (1959).
11. Bierring, F.: Quantitative investigations on the lymphomyeloid system in thymectomized rats, In: Ciba Found. Symp. on Haemopoiesis, Cell Production and its Regulation. Eds: G. E. W. Wolstenholme and M. O'Connor; Churchill, London 1960. Pp. 185–203.
12. Fachet, J., Stark, E., Vallent, K., Palkovits, M.: Acta med. Acad. Sci. hung. **18,** 461 (1962).
13. Fachet, J., Vallent, K., Palkovits, M., Földes, J.: Endokrinologie **45,** 247 (1963)
14. Fachet, J., Vallent, K., Palkovits, M., Ács, Zs.: Acta med. Acad. Sci. hung. **20,** 281 (1964).
15. Fachet, J., McIntosh, B., Labrie, F., Fortier, C.: Acta physiol. Acad. Sci. hung. **32,** 43 (1967). Suppl.
16. Miller, J. F. A. P., Osoba, D.: Physiol. Rev. **47,** 437 (1967).
17. Selye, H.: Brit. J. exp. Path. **17,** 234 (1936).
18. Selye, H.: Stress. Acta Inc. Montreal 1950.
19. Dougherty, T. F.: Physiol. Rev. **32,** 379 (1952).
20. Fachet, J., Palkovits, M., Vallent, K., Stark, E.: Acta endocr. (Kbh.) **51,** 71 (1966).
21. Schlesinger, M., Mark, R.: Science **143,** 965 (1964).
22. Fachet, J., Palkovits, M., Petrányi, G.: Acta morph. Acad. Sci. hung. **14,** 269 (1966).
23. Fachet, J., Stark, E., Palkovits, M., Vallent, K.: Gen. comp. Endocr. **9,** 449 (1967).
24. Fachet, J., Stark, E., Vallent, K., Palkovits, M.: Acta physiol. Acad. Sci. hung. **29,** 374 (1966).
25. Ernström, U.: Studies on growth and cytomorphosis in the thymolymphatic system. Acta path. microbiol. scand. Suppl. **178** (1965).
26. Schapiro, S., Geller, E., Eiduson, S.: Proc. Soc. exp. Biol. (N. Y.) **109,** 935 (1962).
27. Hodges, J. R., Jones, M. T.: J. Physiol. (Lond.) **167,** 30 (1963).
28. Smelik, P. G.: Proc. Soc. exp. Biol. (N. Y.) **113,** 616 (1963).
29. Stark, E., Fachet, J.: Acta med. Acad. Sci. hung. **19,** 367 (1963).
30. Stark, E., Fachet, J., Mihály, K.: Arzneimitt.-Forsch. **16,** 1574 (1966).
31. Stark, E., Fachet, J.: Unpublished results.
32. Guillemin, G. R., Clayton, W. G., Lipscomb, H. S.: J. Lab. clin. Med. **53,** 830 (1959).
33. Vallent, K., Hernády, T.: In the press.
34. Chatten, J.: Amer. J. med. Sci. **248,** 715 (1964).
35. Jaanus, S. D., Martin, C. R.: Gen. comp. Endocr. **10,** 147 (1968).
36. Fachet, J., Petrányi, G.: Unpublished data.
37. Vallent, K., Fachet, J., Stark, E.: Acta med. Acad. Sci. hung. **22,** 215 (1966).
38. Vallent, K., Palkovits, M., Mándi, B.: In the press.
39. Szeberényi, Sz.: J. Endocr. **35,** 323 (1966).
40. Reed, N. D., Jutila, J. W.: J. Immunol. **99,** 238 (1967).
41. Reed, N. D., Jutila, J. W.: Science **150,** 356 (1965).
42. Branceni, D., Arnason, B. G.: Immunology **10,** 35 (1966).
43. Schapiro, S.: Proc. Soc. exp. Biol. (N. Y.) **120,** 771 (1965).
44. Csaba, G., Fischer, J.: Acta biol. Acad. Sci. hung. **17,** 75 (1966).
45. Azar, H. A.: Proc. Soc. exp. Biol. (N. Y.) **116,** 817 (1964).
46. Schapiro, S., Huppert, M.: Proc. Soc. exp. Biol. (N. Y.) **124,** 744 (1967).

47. DUHIG, J. T.: Nature (Lond.) **207,** 651 (1965).
48. GLICK, B., CHANG, T. S., JAAP, R. G.: Poultry Sci. **35,** 224 (1956).
49. WARNER, N. L., SZENBERG, A.: Ann. Rev. Microbiol. **18,** 253 (1964).
50. GOOD, R. A., GABRIELSEN, A. E., COOPER, M. D., PETERSON, R. D. A.: Ann. N. Y. Acad. Sci. **129,** 130 (1966).
51. FICHTELIUS, K. E.: Exp. Cell Res. **46,** 231 (1967).
52. WINICK, M., COSCIA, A.: In the press.
53. ISHIDATE, M., METCALF, D.: Australian J. of exp. Biol. and Med. Sci. **41,** 637 (1963).
54. DOUGHERTY, T. F., WHITE, A.: Endocrinology **39,** 370 (1946).

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ADRENERGIC NEURONE BLOCKING AGENTS: EFFECTS AND SITE OF ACTION

By

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The pharmacological properties of the selective adrenergic neurone blocking agent N-cyclohexyl-benzyl-3 alpha-granatanium bromide (N-1024) and of its derivatives are discussed. Adrenergic neurone blocking activity does not seem to be necessarily associated with tyramine and cocaine-like, noradrenaline depleting effects and with anticholinergic action at various sites. Returning to the original theory of Hey and WILLEY, as well as BOURA and GREEN, the adrenergic neurone blocking agents are described as local anaesthetic-like compounds acting on special membranes.

The pharmacology of the first adrenergic neurone blocking agent was described in 1954 by HEY and WILLEY [46]. This compound, xylocholine (choline 2,6-xylyl ether bromide, TM-10), inhibits the responses of the effector organs to sympathetic postganglionic stimulation without diminishing the effect of physiological transmitters. According to the first description, xylocholine would be a "local anaesthetic" active on a special site, on the sympathetic postganglionic fibre. This assumption has been extensively debated in recent years.

In 1959 the pharmacology of two new adrenergic neurone blocking agents, bretylium and guanethidine, was described and in subsequent years their effects and mode of action were dealt with in many papers. In spite of the extensive studies their mode of action is not fully clarified. There are still many controversial problems awaiting elucidation.

Clarification of the mode of action has been rendered difficult by the fact that guanethidine, the compound most extensively studied, has a wide variety of multi-directional actions in addition to the inhibition of the response to sympathetic postganglionic stimulation and of the release of noradrenaline. Guanethidine possesses namely noradrenaline depleting, parasympatholytic, ganglioplegic, neuromuscular blocking, tyramine-like (indirect sympathomimetic), anti-tyramine (cocaine-like), local anaesthetic and other effects. Likewise, the adrenergic neurone blocking agents of tropine structure described by us in 1962 [24] had many side effects.

We have been interested in the first place how and to what extent the various activities are in connection with the adrenergic neuronoplegic effect.

The question had emerged long ago as to whether the noradrenaline depletion caused by guanethidine was responsible for the blocking of adrenergic neurones. Back in 1961, CASS and SPRIGGS [13] demonstrated that bretylium did not give rise to noradrenaline depletion and the adrenergic blocking effect was not parallel with depletion in the case of guanethidine, either. It was claimed that the latter compound had a primary bretylium-like and a secondary reserpine-like activity. Similar results were obtained by GAFFNEY et al. [36] who found that the tachycardia elicited in the dog by stimulation of the accelerant nerve was blocked by guanethidine long before noradrenaline depletion. FIELDEN and GREEN [33] compared the pharmacological actions of guanethidine with those of one of its derivatives. They found that beta-hydroxy-phenylethylguanidine depleted the noradrenaline depots in a low dose already, but even much higher doses failed to cause a rapid adrenergic inhibition similar to that induced by guanethidine, and claimed that the two kinds of activity were not correlated.

Recently, CHANG et al. [16] have arrived at an opposite conclusion. They found namely that guanethidine reduced by about 30% the noradrenaline level of the particulate fraction of the rat's vas deferens in 30 minutes, when there was no demonstrable change yet in the supernatant. According to them this depletion was responsible for the adrenergic neurone blocking effect of guanethidine. The following facts, however, are at variance with this view. Their own experiments showed that the rat's vas deferens was insensitive to guanethidine, a dose as high as 25 mg/kg having been required to block the responses to nervous stimulation. Furthermore, it is questionable whether the particulate noradrenaline would be responsible for the response to nervous stimulation, and the rat's vas deferens might represent a special organ, since on exhaustive stimulation the noradrenaline content decreases in the particulate fraction only, and not in the supernatant [15]. However, in the organs the situation is different. For example, according to STITZEL et al. [59] in the isolated perfused rabbit heart noradrenaline decreases in the soluble fraction and remains unchanged in the particulate one following stimulation of the accelerator nerve, and BHAGAT [1] stated that in the rat heart guanethidine decreased the noradrenaline content mainly in the soluble fraction. Thus, it is not proved that guanethidine would act faster and more intensely on the particulate than on the soluble fraction, nor is it proved that nervous stimulation would mobilize the noradrenaline located in the particles. Thus, the hypothesis suggested by CHANG et al. cannot be considered of a general value, and there is no proof of the essential role of the noradrenaline-depleting activity in the rapid adrenergic neurone blocking action of guanethidine.

Guanethidine elevates blood pressure, causes contraction of the nictitating membrane, tachycardia, effects of sympathomimetic nature. These are inhibited not only by the alpha or beta blocking agents, but also by reserpine,

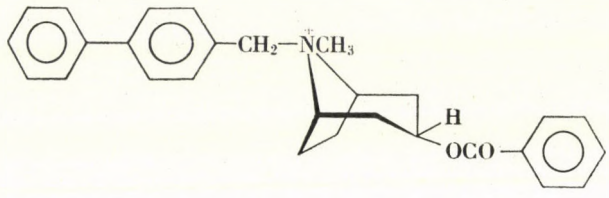
cocaine, imipramine and desipramine, i.e. it may be considered an indirect sympathomimetic, tyramine-like activity. According to the scheme of FURCHGOTT et al. [35] this means that guanethidine enters the neurone at a transfer site, through a transport mechanism, releases noradrenaline there, and that, coming out of the neurone, exerts its characteristic effect on the adrenergic receptor. According to CARLSSON and WALDECK [12] if a compound utilizes this transport mechanism, it will more or less inhibit it. Blocking of this mechanism leads to an inactivation of tyramine, to an inhibition of noradrenaline uptake, and to an increase of its effect. In fact, guanethidine has such cocaine-like activity; it inhibits the pressor, noradrenaline-liberating and other effects of tyramine and other sympathomimetic amines of indirect action. In addition, guanethidine behaves as a pseudo-transmitter; if it is accumulated in noradrenaline depots, it may be liberated therefrom by nervous stimulation [2].

The question emerges whether these effects of guanethidine are capable of inhibiting adrenergic neurones. Neither the indirect sympathomimetics such as amphetamine [19, 26], nor the compounds blocking noradrenaline uptake such as cocaine, nor the false transmitters and their precursors are adrenergic neurone blocking agents and no such effect is displayed by alpha-methyl-m-tyramine, a compound possessing all three properties [44, 50], either. The adrenergic neurone blocking activity of guanethidine is independent of these properties.

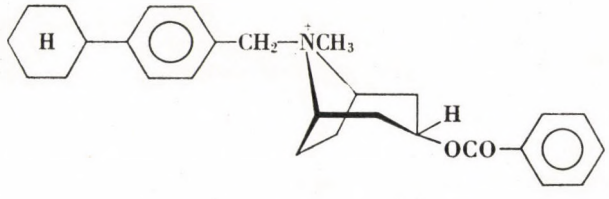
Bretylium is similar to guginethidine in many respects, and differs from it in others. It blocks adrenerac neurones, has a sympathomimetic activity inhibited by cocaine [38, 47], but has neither noradrenaline depleting [13], nor cocaine-like activities [1, 9, 14, 47, 56, 57]. Thus, it exhibits somewhat more clearly the adrenergic neurone blocking action, but still has many "side effects", more than the granatanium derivatives synthetized and studied by us.

How did we begin to study the adrenergic neurone blocking agents of tropine and granatane structure? Following the administration of high doses of N-phenyl-benzyl-3-alpha-benzoyl-tropinium bromide (N-361), described by GYERMEK and NÁDOR (Fig. 1a) [42], that had a strong nicotine-like ganglionic stimulating effect, we observed in the cat a characteristic, protracted hypotension and such a lasting inhibition of the nictitating membrane contractions evoked by cervical sympathetic stimulation which could not be explained by a mere ganglioplegic effect. The response to postganglionic sympathetic stimulation was also inhibited, but here the adrenergic neurone inhibition was only a "side effect", beside the intensive nicotine-like activity. Transformation to cyclohexyl of the terminal aromatic ring markedly diminished the ganglionic stimulating effect of the compound [21, 24] and thus we obtained a molecule, N-cyclohexyl-benzyl-3-alpha-benzoyl-tropinium bromide, whose adrenergic neurone blocking activity was a main effect. This derivative, N-718 (Fig. 1a),

is an adrenergic neurone blocking agent in the first place, but it still has the original molecule's special, ganglionic or chemoreceptor stimulating action marked especially in the dog [51]. This effect was completely abolished on the addition of another CH_2 group to the pyrrolidine ring, preventing thereby, presumably by further space filling, the molecule's link with the cholinergic receptor. The new compound, N-cyclohexyl-benzyl-3-alpha-benzoyl-granatanium bromide (N-1024), as well as N-cyclohexyl-oxybenzyl-3-alpha-benzoyl-



N – 361



N – 718

Fig. 1a

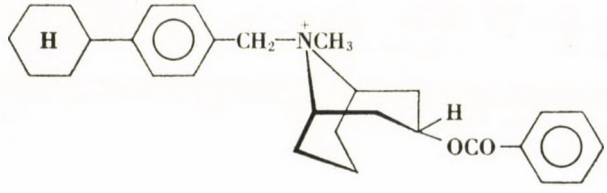
Table I

Compound	Ganglionic stimulating activity	Ganglionic blocking activity	Tyramine-like activity	Antityramine activity	Noradrenaline depleting dose (Rat heart)
Guanethidine	—	+	+	+	5 mg/kg
N-361	+	(+)	—	—	0
N-718	+	+	—	—	20 mg/kg inactive
N-1024	—	—	—	—	20 mg/kg inactive
N-1054	—	—	—	—	0

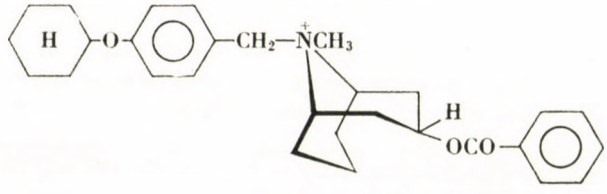
granatanium bromide (N-1054) (Fig. 1b) and their derivatives had no ganglionic or chemoreceptor stimulating, vasopressor, tachycardizing or other effects of similar nature, not even in the dog [27].

The most important pharmacological properties of N-1024 are as follows (Table I).

(1) Blocking of adrenergic neurones. The compound inhibits the nictitating membrane contractions induced by postganglionic cervical sympathetic



N-1024



N-1054

Fig. 1b

Blocking concentration g/ml, guinea pig vas deferens (Transmural stimulation)	Antiouabain dose (Dog)	Local anaesthetic concentration (Rat tail) per cent	Effect on heart rate (Cat and dog)	Neuromuscular blocking dose, cat's tibialis anterior muscle (d-Tubocurarine = = 0.18 mg/kg)	Carotid occlusion, blocking dose (Cat)
$2-3\times10^{-6}$	—	0.25—0.5 *	tachycardia/ bradycardia	12 mg/kg	5 mg/kg
0	0	0	tachycardia	>2 mg/kg	—
$2-5\times10^{-7}$	0	0.2	tachycardia/ bradycardia	>5 mg/kg	0.5—1 mg/kg
$2-5\times10^{-7}$	1.5—2 mg/kg	0.1	bradycardia	>6 mg/kg	0.5—1 mg/kg
$0.5-1.0\times10^{-6}$	0	0.25—0.5	bradycardia	>6 mg/kg	0.5—1 mg/kg

+ = active, — = inactive, 0 = not tested
* RAND and WILSON [54] (Mouse-tail)

stimulation, the contractions of the spleen in response to postganglionic stimulation of the splenic nerve, the tachycardia induced by stimulation of the stellate ganglion, the contractions of the vas deferens in response to transmural stimulation, without blocking the effects of adrenaline and noradrenaline [27, 28]. These inhibitory actions are abolished by dexamphetamine [26].

(2) Lasting reduction of blood pressure and pulse rate, inhibition of the reflex rise in blood pressure in response to clamping the common carotids in the cat and dog [27], of the pressor effect of physostigmine in the rat [29].

(3) It has no parasympathomimetic, parasympatholytic, ganglion stimulating effects [27], its ganglion blocking activity is insignificant, and it has no neuromuscular blocking action in the whole animal. In the isolated frog rectus it acts as a carbachol antagonizer, but in this antagonism the competitive factor is insignificant [29].

(4) N-1024 has no cocaine-like (antityramine) activity and it does not cause noradrenaline depletion in the rat heart [27, 29].

(5) In the dog it suspends for a long time the arrhythmia evoked by cardiac glycosides and, though less intensely, it inhibits the disturbances of rhythm caused by aconitine in the rat and those induced by electrical stimulation in the cat [23].

Recently, we have subjected to investigation the local anaesthetic action of N-1024, N-1054 and of several other tropine and granatane derivatives. A difficulty of such tests is that the maximum solubility in water of the compounds varies from 0.1 to 0.5%, but it could be confirmed beyond doubt that both N-1024 and its derivatives possess the slowly developing, protracted regional and mucosal anaesthetic effect characteristic of the quaternary local anaesthetics [45]. The former was studied in the rat's tail by the heat irradiation method in which compounds soluble up to 0.1% are also active. Mucosal anaesthesia was estimated on the guinea pig cornea after 60 sec of contact; in this way the compounds soluble to 0.2% or more could only be tested (Table I).

Thus, the granatanium compounds represent such adrenergic neurone blocking agents which show this activity more clearly and selectively than any other such compound. They have no depleting, tyramine and cocaine-like, anti-acetylcholine effects. Beside the adrenergic neurone blocking effect in the strict sense of the word we have observed only two important activities: the antiarrhythmic and local anaesthetic ones. Thus, these compounds are suitable for illuminating the mechanism of the adrenergic neurone blocking effect.

Before discussing the mode of action, a few words should be said about the relative inactivity of the compounds on the cholinergic receptors. As mentioned beforehand, the original molecule was a strong ganglionic stimulant. Within a series of tropine esters the ganglionic effect increases when the space-filling of the quaternarizing group is increased [25, 41, 43], maximum activity

being usually reached with a phenylbenzyl quaternary group. In our series of compounds this activity could be abolished in two steps, by hydrogenation of the terminal aromatic ring and by expanding the pyrrolidine ring. Ultimately, both increased the size of the molecule, making it more difficult to fit to the ganglionic receptors. In this way had been reached N-1024, inactive on the cholinergic receptors, but possessing a strong and selective adrenergic neurone blocking activity.

As to the ester groups of the molecules in the present paper only the benzoic acid esters are mentioned, but in recent years we have tested a number of tropine and granatane compounds esterified with aromatic hydroxy acids. These compounds proved to be potent parasympatholytics, but did not block adrenergic neurones [53]. Thus, the parasympatholytic and adrenergic neurone blocking effects are separate properties. How should this phenomenon at variance with the hypotheses adopted by many authors be interpreted?

According to BURN [7] bretylium over its adrenergic neurone blocking action to the acetylcholine antagonist property, in the sympathetic postganglionic neurone noradrenaline would be liberated as a result of acetylcholine mobilization and not in direct response to nervous excitation. Just like acetylcholine has its specific antagonists (atropine, hexamethonium, tubocurarine) at its other sites of action, in the sympathetic postganglionic fibres this specific antagonist would be bretylium. But just like the other specific antagonists show a certain overlapping of actions, it could be expected that bretylium and the other adrenergic neurone blocking agents would have anticholinergic activity in other areas as well.

Where and how does the cholinolytic activity of the adrenergic neurone blocking agents manifest itself? GOKHALE et al. [39] showed on the rabbit and rat intestine and the rabbit trachea that guanethidine and bretylium antagonized acetylcholine. RAND and WILSON [55] observed the same in the guinea pig intestine and together with others stated that guanethidine had also ganglionic blocking activity. KOSTERLITZ and LEES [48] reported that in the guinea pig intestine bretylium shifted to the right the dose-response curve of acetylcholine, but the compound was a more potent antihistamine agent. GARRETT and SOUSA [37] as well as DŽOLJIČ [30] stated that in the isolated guinea pig intestine the antiacetylcholine effect of guanethidine was not specific, but was due to the spasmolytic, direct muscle depressing activity.

As to the action of guanethidine on the neuromuscular junction, BURN and SELTZER [10] showed in phrenic nerve-diaphragm preparations that bretylium and guanethidine had a neuromuscular blocking activity and at the same time found, that, although at high concentrations, tubocurarine and decamethonium exerted an adrenergic neurone blocking effect on the isolated rabbit intestine. From this they concluded to a close correlation between the adrenergic neurone blocking and the neuromuscular blocking activities. RAND and

WILSON [55] claimed on the basis of studies on frog and bird muscles that guanethidine is a competitive acetylcholine antagonist, but they tried to influence the contraction by just one dose of acetylcholine and neglected to study the dose-response curves before and after guanethidine administration. Another indirect evidence against the competitive antagonistic action of guanethidine on the neuromuscular synapse is the fact that it blocks not only the contractions in response to direct nervous stimulation, but also those evoked by muscle stimulation [20, 40, 49]; and CHANG et al. [17] state firmly that "in the case of guanethidine the main effect appeared to be on muscle fibres".

Our adrenergic neurone blocking agents of tropine structure diminish only at high dosage and in a hardly reproducible fashion the contractions of the cat's anterior tibial muscle evoked by sciatic nerve stimulation. This and the similar effect of guanethidine is not antagonized by neostigmine [29]. In the isolated rectus abdominis muscle of the frog 2×10^{-6} g/ml concentration of N-1024 causes only an insignificant shift to the right in the dose-response curve of carbachol ($pA_2 = 5.07$; the pA_2 value of tubocurarine was in our experiments 6.72–6.79). At higher concentrations, N-1024 considerably reduced the maximum effect of carbachol, thus the antagonism was non-competitive. In the frog muscle guanethidine caused no shift to the right in the dose-response curve of carbachol, but only a depression of the peak; this also pointed to the antagonism not being competitive [22].

Accordingly the following may be stated as regards the anti-acetylcholine effect of the adrenergic neurone blocking agents. Certain compounds are totally inactive on various kinds of cholinergic receptors (N-1024 has no parasympatholytic effect, its ganglionic blocking action is insignificant). The anti-acetylcholine activities observed are non-competitive in nature and are presumably not specific.

The guanethidine-carbachol antagonism observed by us on the frog rectus muscle is apparently similar to the tetracaine-carbachol antagonism demonstrated by FEINSTEIN and PAIMRE [32]. They have proved that the local anaesthetics inhibit non-specifically the effects of various spasmogenic compounds in different organs. It was plausible therefore to think that the anti-acetylcholine effect of guanethidine may be traced back to its local anaesthetic effect and at the same time also the question emerged as to whether the adrenergic neurone blocking effect was a consequence of such an activity.

Among the classical local anaesthetics there was no compound containing quaternary nitrogen. The first such quaternary local anaesthetic was produced by NÁDOR et al. [52], and HERR et al. [45] stated that they differed from their non-quaternary analogues mainly in that their effect developed slowly but lasted fora long time. They also reported that the benzyl-quaternaries were more active than the methyl-quaternaries. When HEY and WILLEY [46] described the pharmacology of xylocholine, the first adrenergic neurone blocking agent,

they quoted the above mentioned paper by HERR et al. and explained the adrenergic neurone blocking action of xylocholine so that this, as a local anaesthetic 2 to 10 times more potent than cocaine, would act primarily on the sympathetic postganglionic neurones. EXLEY [31] rejected this hypothesis by stating that xylocholine does not block conduction in the adrenergic fibre. According to him the compound would be active on the nerve ending or near to that. BOURA and GREEN [3] observed that bretylium had a protracted local anaesthetic effect and reported that the compound inhibited the responses to sympathetic nerve stimulation also when this had been applied to the nerve trunk. They showed that bretylium acted in the way as HEY and WILLEY supposed xylocholine to act. Even in 1965, BOURA and GREEN [5] claimed that "the possibility remains that the depressant action on the adrenergic fibre terminals is analogous to the impairment of conduction in a nerve trunk caused by local anaesthetics".

In general, the adrenergic neurone blocking agents have a local anaesthetic property [4, 54]. In this respect the effect of the guanidine compounds develops just as slowly and lasts just as long as that of the quaternary ones. In spite of this, a number of authors are rejecting the view that the adrenergic neurone blocking and the local anaesthetic effects were causally correlated. According to BOYD et al. [6], although procaine inhibits the vas deferens contractions elicited by hypogastric stimulation, it acts at the same concentration when applied to the nerve trunk as when added to the bath, in contrast to bretylium which is about 200 times less active on the nerve trunk than in the bath. However, this fact does not justify to reject the above theory, because it is not certain that every segment of the fibres is equally sensitive to bretylium. CLARK and HUGHES [18] found no parallelism between the adrenergic neurone blocking and local anaesthetic activities of different xylocholine derivatives. In our view the only conclusion derivable from this is that not every quaternary local anaesthetic is an adrenergic neurone blocking agent, but it does not discredit the contention that in general the adrenergic neurone blocking agents have local anaesthetic properties. RAND and WILSON [54] argued against the correlation between the two activities after they had shown that the adrenergic neurone blocking effect of procaine develops fast and can be eluted rapidly, whereas that of guanethidine develops slowly and is difficult to elute. These differences, however, follow from the fact that the lipophilic procaine and the lipophobic guanethidine presumably link up differently or to different membrane elements. In support of the local anaesthetic hypothesis is the observation by CABRERA et al. [11] that guanethidine and bretylium at low concentrations inhibit for a long time the antidromic impulse-eliciting effect of acetylcholine. The authors think that this effect is due to the circumstance that the compounds, like the local anaesthetics, keep the membrane polarized.

N-1024 and the other granatane-type adrenergic neurone blocking compounds show this activity more purely and are free of the side effects interfering with the study of the mode of action. It has already been discussed why these compounds have no anti-acetylcholine activity, but why are they potent adrenergic neurone blocking agents? In this relation we can put forward merely speculations. If the OH radical of the ester group and the phenylbenzyl group of the N atom on two receptor types yielded the most specific acetylcholine antagonists with a 28-fold atropine activity and a 100-fold TEA activity [43, 25], then this means that such structural elements are forming strong physical bonds. If the molecule is lacking such elements and by the hydrogenation of the quaternary aromatic group and by expanding the structure we strongly impaired the fitting to such sites, it is conceivable that the affinity of the compound to other, silent sites and eventually to inactivating enzyme systems deteriorates in the same way. In such a case, however, larger quantities of the substance reach presumably other (at present not adequately known) receptor groups of special membranes. In support of this may be mentioned that many compounds are blocking adrenergic neurones in isolated organs, but few less do so in the whole animal. For example, in the isolated organ dimethylphenylpiperazinium ion blocks the adrenergic neurone [8], but nobody has observed such an effect in the whole animal.

Finally, we have shown that the noradrenaline-depleting, cocaine and tyramine-like, anti-acetylcholine effects cannot play any role in the adrenergic neurone blocking property. The effects which remain in the case of N-1024 and the other granatane derivatives are their adrenergic neurone blocking, local anaesthetic and antiarrhythmic activities. In our opinion these three are closely interrelated. The correlation between the antiarrhythmic and local anaesthetic effects is well known [34, 58] and it is also known that with the decrease of lipid-solubility (e.g. procaine—procaine amide) the duration of the antiarrhythmic effect increases, in the same way as the duration of action of quaternary local anaesthetics is longer than that of the tertiary analogues.

Each of the adrenergic neurone blocking agents contains a strongly basic group (quaternary ammonium, amidoxime, guanidine, aminoguanidine, or amidine) and the site and duration of the bonds formed by these lipophobic molecules obviously differ from those of the lipophilic compounds. There are great differences in chemical structure and physicochemical properties between the membranes of various organs [60] and therefore it is possible that certain areas (e.g. the terminal parts of adrenergic neurones, the heart) are more affine to these molecules and they act on them. Ultrastructural investigations are required to supply the final solution to this problem. In our own studies we started from a compound with a strong cholinergic activity having also a marked adrenergic neurone blocking effect. By producing new derivatives we have succeeded in eliminating the cholinergic action; the anticholinergic

effect could thus be separated from the adrenergic neurone blocking activity. By an up-to-date interpretation of the correlation between effect and structure we could synthetize an adrenergic neurone blocking agent of a new type, free of side effects, which acts more selectively than any other known compound of this kind. Its study has allowed a deeper insight into the mode and site of action of adrenergic neurone blocking agents.

REFERENCES

1. Bhagat, B.: Brit. J. Pharmacol. **22,** 238 (1964).
2. Boullin, D. J.: J. Pharm. Pharmacol. **18,** 709 (1966).
3. Boura, A. L. A., Green, A. F.: Brit. J. Pharmacol. **14,** 536 (1959).
4. Boura, A. L. A., Green, A. F.: Brit. J. Pharmacol. **20,** 36 (1963).
5. Boura, A. L. A., Green, A. F.: Ann. Rev. Pharmacol. **5,** 183 (1965).
6. Boyd, H., Chang, V., Rand, M. J.: Arch. int. Pharmacodyn. **131,** 10 (1961).
7. Burn, J. H.: Brit. Med. J. **I,** 1623 (1961).
8. Burn, J. H., Froede, H.: Brit. J. Pharmacol. **20,** 378 (1963).
9. Burn, J. H., Rand, M. J.: Brit. J. Pharmacol. **15,** 56 (1960).
10. Burn, J. H., Seltzer, J.: J. Physiol. (Lond.) **179,** 569 (1965).
11. Cabrera, R., Torrance, R. W., Viveros, H.: Brit. J. Pharmacol. **27,** 51 (1966).
12. Carlsson, A., Waldeck, B.: Acta pharmacol. (Kbh.) **22,** 293 (1965).
13. Cass, R., Spriggs, T. L. B.: Brit. J. Pharmacol. **17,** 442 (1961).
14. Cession-Fossion, A.: Arch. int. Pharmacodyn. **158,** 45 (1965).
15. Chang, C. C., Chang, J. C.: Brit. J. Pharmacol. **25,** 758 (1965).
16. Chang, C. C., Chang, J. C., Su, C. Y.: Brit. J. Pharmacol. **30,** 213 (1967).
17. Chang, C. C., Chen, T. F., Cheng, H. C.: J. Pharmacol. exp. Ther. **158,** 89 (1967).
18. Clark, E. R., Hughes, I. E.: Brit. J. Pharmacol. **28,** 105 (1966).
19. Cowan, F. F., Cannon, C., Koppanyi, T., Maengwyn-Davies, G. D.: Science **134,** 1069 (1961).
20. Dixit, B. N., Gulati, O. D., Gokhale, S. D.: Brit. J. Pharmacol. **17,** 372 (1961).
21. Dóda, M., Fehér, O., György, L., Nádor, K.: Brit. J. Pharmacol. **21,** 10 (1963).
22. Dóda, M., György, L., Bite, Á.: J. Pharm. Pharmacol. **20,** 575 (1968).
23. Dóda, M., György, L., Kenyeres, J., Nádor, K.: Acta physiol. Acad. Sci. hung. **34,** 107 (1968).
24. Dóda, M., György, L., Nádor, K.: Nature (Lond.) **195,** 1108 (1962).
25. Dóda, M., György, L., Nádor, K.: Arch. int. Pharmacodyn. **145,** 264 (1963).
26. Dóda, M., György, L., Nádor, K.: Arch. int. Pharmacodyn. **164,** 247 (1966).
27. Dóda, M., Molnár, J., György, L., Nádor, K.: Acta physiol. Acad. Sci. hung. **31,** 257 (1967).
28. Dóda, M., Nádor, K.: Acta physiol. Acad. Sci. hung. **31,** 177 (1967).
29. Dóda, M.: To be published.
30. Džoljič, M.: Arch. int. Pharmacodyn. **151,** 334 (1964).
31. Exley, K. A.: Brit. J. Pharmacol. **12,** 297 (1957).
32. Feinstein, M. B., Paimre, M.: Nature (Lond). **214,** 151 (1967).
33. Fielden, R., Green, A. L.: Brit. J. Pharmacol. **30,** 155 (1967).
34. Frey, H. H., Nielsen, C. K.: Arch. int. Pharmacodyn. **162,** 93 (1966).
35. Furchgott, R. F., Kirpekar, S. M., Rieker, M., Schwab, A.: J. Pharmacol. exp. Ther. **142,** 56 (1963).
36. Gaffney, T. E., Chidsey, C. A., Braunwald, E.: Circular. Res. **12,** 264 (1963).
37. Garrett, J., Sousa, C.: Arzneimittel-Forsch. **13,** 125 (1963).
38. Gokhale, S. D., Gulati, O. D., Kelkar, V. V.: Brit. J. Pharmacol. **20,** 362 (1963).
39. Gokhale, S. D., Gulati, O. D., Kelkar, V. V., Joshi, N. Y.: Arch. int. Pharmacodyn. **145,** 243 (1963).
40. Green, A. F., Hughes, R.: Brit. J. Pharmacol. **27,** 164 (1966).
41. Gyermek, L., Nádor, K.: Acta physiol. Acad. Sci. hung. **4,** 341 (1953).
42. Gyermek, L., Nádor, K.: Pharmazie **10,** 485 (1955).
43. György, L., Dóda, M., Nádor, K.: Arzneimittel-Forsch. **11,** 444 (1961).
44. György, L., Molnár, J., Dóda, M.: Arzneimittel-Forsch. **15,** 219 (1965).

45. HERR, F., NÁDOR, K., PATAKY, GY., BORSI, J.: Naunyn-Schmiedebergs Arch. exp. Path. Pharmakol. **217,** 447 (1953).
46. HEY, P., WILLEY, G. L.: Brit. J. Pharmacol. **9,** 471 (1954).
47. JOHNSON, G. L., KAHN, J. B., JR.: J. pharmacol. exp. Ther. **152,** 458 (1966).
48. KOSTERLITZ, H. W., LEES, G. M.: Brit. J. Pharmacol. **17,** 82 (1961).
49. KRONEBERG, G., STOEPEL, K.: Naunyn-Schmiedebergs Arch. exp. Path. Pharmakol. **243,** 36 (1962).
50. MOLNÁR, J., GYÖRGY, L., DÓDA, M.: Acta physiol. Acad. Sci. hung. **28,** 185 (1965).
51. MOLNÁR, J., GYÖRGY, L., DÓDA, M., NÁDOR, K.: Arch. int. Pharmacodyn. **151,** 22 (1964).
52. NÁDOR, K., HERR, F., PATAKY, GY., BORSY, J.: Nature (Lond.) **171,** 788 (1953).
53. NÁDOR, K., DÓDA, M., GYÖRGY, L.: To be published.
54. RAND, M. J., WILSON, J.: Europ. J. Pharmacol. **1,** 200 (1967).
55. RAND, M. J., WILSON, J.: Europ. J. Pharmacol. **1,** 210 (1967).
56. ROSS, C. A., WENGER, H. C., LUDDEN, C. T., STONE, C. A.: Arch. int. Pharmacodyn. **142,** 141 (1963).
57. SCHMITT, H., SCHMITT, H., DEPOILLY, F.: Nature (Lond.) **196,** 73 (1965)
58. SOMANI, P., LUM, B. K. B.: J. Pharmacol. exp. Ther. **147,** 194 (1965).
59. STITZEL, R. E., CAMPOS, H. A., SHIDEMAN, F. E.: Int. J. Neuropharm. **4,** 327 (1965).
60. ZIPF, H. F.: Dtsch. med. Wschr. **9,** 405 (1968).

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ON RED CELL LIPIDS

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Changes in the lipid composition of erythrocytes has been studied 1. during ontogenesis; 2. under the effect of a fat-free diet; and 3. in some metabolic diseases.

Cholesterol, TEFA, lipid P, phospholipid classes and total phospholipid fatty acid pattern of erythrocytes were studied with respect to changes during ontogenesis, those induced by a fat-free diet, in essential and secondary hyperlipidaemia, and in hypolipidaemia due to acanthocytosis.

The constancy of red cell lipids in essential hyperlipidaemia and the limits of the type and extent of the changes that can be induced in the red cells by dietary means were suggestive of regulating factors other than the relative change of any component in diet, plasma or adipose tissue.

Attention is drawn to the striking similarities in the phospholipid fatty acid pattern of foetal cells, red cells in essential fatty acid deficiency, in acanthocytosis, and in some haemolytic diseases. It is suggested that the resemblance in lipid structure, non-electrolyte permeability and viability of these erythrocytes may originate from the essential fatty acid deficiency as an effect of the fat-free diet, secondary to malabsorption in acanthocytosis, and as a secondary local fatty acid deficiency in conditions with highly increased demand for red cells. In these conditions there is a "foetal type" erythropoiesis with accelerated type of maturation possibly skipping some cell divisions.

During the last decade much information has accumulated about the biophysical and biochemical properties of cell membranes. The fine structure of these important cell constituents is a very complex one. In spite of the striking similarities in the overall structure of cellular membranes, the classical "unit membrane" concept of DAWSON and DANIELLI [1] and ROBERTSON [2] cannot be applied to biological membranes in general owing to differences in membrane protein-lipid ratios, metabolism, and enzymatic reactions depending on their special functions [3].

The essential components of the red cell membrane are lipids, structural proteins, ions and water. Any chemical agent before entering the cell will first come into contact with the membrane and its penetration into the cell will depend on its molecular dimensions and charge, and its lipid/water partition coefficient. Human erythrocytes contain approximately 7.5×10^{-16} M of lipid per cell. Phospholipids, non-esterified cholesterol and glycolipids in a molar ratio of about 4 : 3 : 0.5 are the main lipid constituents of erythrocytes [4]. Current reports [5–8] agree in that neutral lipids other than free cholesterol, i.e. cholesterol esters, triglycerides and free fatty acids are not integral parts of the red cell membrane. Phosphatidyl choline, phosphatidyl serine, phospha-

tidyl ethanolamine and sphingomyelin are the major phospholipid components, representing about 90% of the lipid phosphorus of human erythrocytes, while phosphatidic acid, phosphatidyl inositol and lysolecithin are found in minute amounts [9, 10]. The red cell membrane is a metabolically active system with active transport and well demonstrable turnover of its lipid constituents. Most of the cholesterol is situated in the circumference of the erythrocyte. The high interfacial tension due to this high concentration of cholesterol with its numerous hydrophobic bounds is believed to be an important factor in determining the biconcave shape of normal intact erythrocytes [11]. Decreasing metabolic activity of aging erythrocytes or during incubation elicits membrane changes resulting in decreased plasticity and in increased permeability to sodium and potassium, membrane condensation and lipid loss. The consequent decrease of critical haemolytic volume explains the decrease of cell viability [12].

Erythrocyte lipids display remarkable qualitative differences in the different animal species, correlated with differences in permeability of the erythrocyte membrane in the given species [9, 13–15]. Erythrocyte lipids are affected by dietary factors and undergo pathological changes in some diseases.

All the lipid present in the intact erythrocyte can readily be recovered from the haemoglobin-free ghosts, hence the red cell membrane is the most easily accessible model for studying the paramount role in cell functions of even minute changes of the lipid structure of biointerfaces.

The aim of this paper is to report on investigations into the changes in erythrocyte lipids 1) during ontogenesis; 2) as an effect of changes in dietary fats; and 3) in some metabolic diseases.

Materials and methods

Thirteen human cord blood samples were obtained at the end of term pregnancies. Delivery was uncomplicated and the infant appeared normal at birth in each case. Blood was drawn from the umbilical vein immediately after the delivery of the placenta using sodium citrate as anticoagulant (1 vol to 4 vol blood). Adult blood samples were taken from 30 healthy blood donors, from 5 patients with essential hyperlipidaemia, from 2 with secondary hyperlipidaemia and from 1 with acanthocytosis.

The influence of dietary lipids was investigated in animal experiments. Two groups each of 30 male weanling rats of the inbred R Amsterdam strain were used. One group was fed the following "fat-free" diet for 120 days:

30% skimmed milk powder
2% "Foszkál" (calcium phosphate, Phylaxia)
1% salt premix (Phylaxia)*
1% vitamin premix (Phylaxia)**
66% flour

In the control group the above diet was complemented with 6% sunflower oil. Diets were fed as desired. Tap water was accessible at all times. Body weights, packed cell volume (microhaematocrit) and haemoglobin values were recorded weekly.

* Components of Phylaxia salt premix: $MnSO_4$ 35 g/kg, $MgSO_4$ 50 g/kg, $FeSO_4$ 5 g/kg, $CuSO_4$ 2.5 g/kg, $ZnSO_4$ 0.4 g/kg, KJ 0.2 g/kg, NaCl 500 g/kg, bran 1000 g.

** Components of Phylaxia vitamin premix: Vitamin A 600,000 IU, vitamin B_1 300 mg, vitamin B_2 600 mg, niacin 3000 mg, Ca-dl-pantothenic acid 1200 mg, vitamin B_{12} 2 mg, vitamin D_2 1000 IU.

Red blood cell lipids were extracted by the III method of WAYS and HANAHAN [16]. The only modification was the addition of BHT as antioxidant to the solvents at a final concentration of 0.005%. The extracts were stored in sealed glass vials under N_2 at −20°C.

Cholesterol was determined by Lieberman and Burchard's method, total esterified fatty acids (TEFA) as described by HACK [17], and lipid phosphorus as described by ALLEN [18].

Thin layer chromatography of the main lipid classes was performed on 0.25 mm Kieselgel G layers, activated at 110°C for 25 minutes. Petroleum ether–diethyl ether–acetic acid (90 : 10 : 1, v : v : v) mixture was used as solvent. The spots were revealed by spraying with phosphomolybdenic acid solution and heating at 110°C for 20—25 minutes. Phospholipids were separated on Kieselgel H (Merck) layers. The plates were activated at 110°C for one hour. Before activation a mixture of chloroform, methanol, acetic acid and water, 75 : 45 : 12 : 6 (v : v : v : v) was run through the layer for cleaning. The same solvent was used for the development of the chromatogram [45]. The spots were revealed by exposure to iodine vapour or by spraying with 50% H_2SO_4 and heating at 180°C for 30 minutes.

Fatty acid distribution of red cell total phospholipids was studied by gas-liquid chromatography of the corresponding fatty acid methyl esters. Phospholipids were converted into methyl esters using methanolic sulphuric acid. Gas-liquid chromatography was performed on a 5 feet column using an Aerograph A-600-C type gas chromatograph (H_2 flame detector) with diethylene glycol succinate as the stationary phase. The column temperature was 188°C.

Results

1. *Changes in erythrocyte lipids during ontogenesis* were studied by comparing the lipid composition of foetal and adult human red blood cells.

Quantitative determination of cholesterol, total esterified fatty acids and lipid phosphorus revealed considerable individual differences (Table I). Most of the values were, however, within or near the normal adult range.

Table I

Main lipid class levels of foetal erythrocytes

Cord blood sample No.	Cholesterol	TEFA	Lipid P
	10^{-12} mg/cell		
135	88	128	11.3
187	83	250	16.8
225	170	237	15.1
245	236	208	17.2
252	328	332	8.8
253	182	172	12.3
17	306	415	18.8
42	170	178	5.8
49	129	113	3.6
62	126	133	4.2
67	124	165	10.9
83	83	125	5.6
90	180	192	13.5
Adult RBC normal range (30 blood donors)	100—145	146—214	9.5—13.5

Thin layer chromatography of the main lipid classes using the petroleum ether–diethyl ether–acetic acid solvent system did not reveal any difference between cord blood and normal adult erythrocyte extracts. Both contained free cholesterol and free fatty acids, with traces of triglyceride and cholesterol esters. Phospholipids were resolved into sphingomyelin, phosphatidyl choline, phosphatidyl serine, phosphatidyl ethanolamine, and phosphatidyl inositol fractions. Sphingomyelin, phosphatidyl choline and phosphatidyl ethanolamine

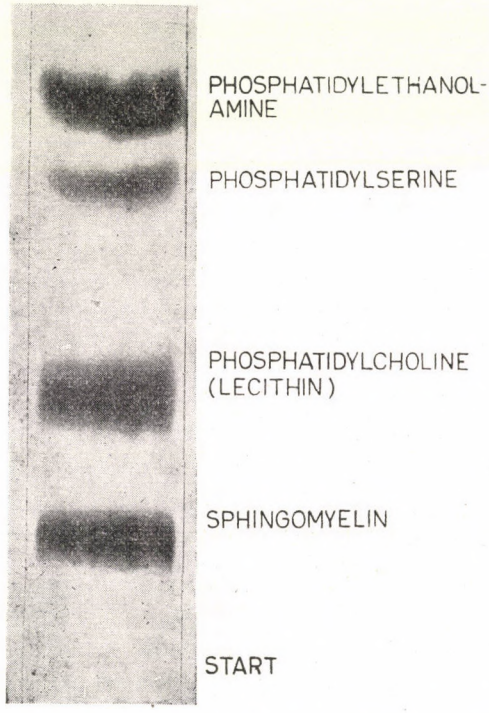

Fig. 1. Phospholipid fractions of adult erythrocytes separated by TLC

were identified by comparison with reference materials. Comparing the phospholipids of cord blood erythrocytes with those of normal adult blood we found in some of the cord blood samples a markedly stronger spot of phosphatidyl inositol (Figs 1, 2).

Fatty acid distribution of the total phospholipids of cord blood erythrocytes was found to differ from that of adult red blood cells in its significantly lower level of linoleic acid ($C_{18:2}$), in the higher concentration of palmitic acid ($C_{16:0}$) and a lower oleic acid ($C_{16:1}$) content. Eicosotrienoic acid ($C_{20:3}$) could be detected in 4 of the 9 cord blood samples (Table II).

2. *Influence of changes in dietary fats* was studied in rats kept on fat-free diet. Body weights, packed cell volume and haemoglobin levels revealed no significant changes as compared to the control group. No definite changes could be found in the main lipid and phospholipid classes of the rats kept on fat-free diet. Striking changes could be detected in the fatty acid distribution of erythrocyte total phospholipids (Table III). The relative amount of linoleic acid ($C_{18:2}$) decreased significantly, there was a slight, but definite decrease

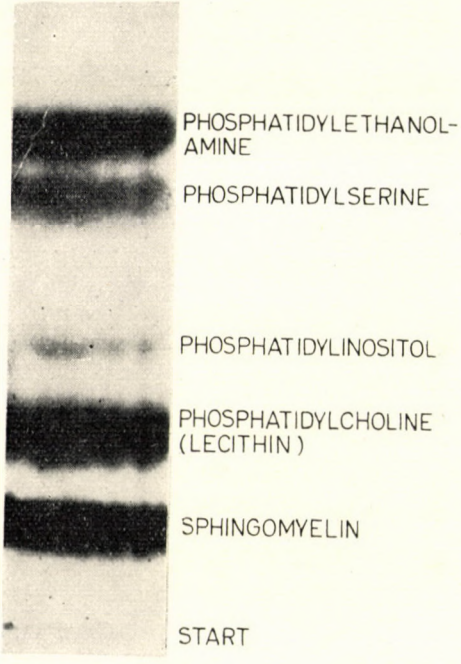

Fig. 2. Phospholipid fractions of foetal erythrocytes separated by TLC

in stearic acid ($C_{18:0}$) and a marked elevation in oleic acid ($C_{18:1}$). Eicosotrienoic acid ($C_{20:3}$) could be detected in 17 of the 30 blood samples.

3. *Serum and erythrocyte lipids in patients with hyper- and hypolipidaemia.* Lipid composition of serum and red cells of 5 subjects with essential hyperlipidaemia and of 2 patients with secondary hyperlipidaemia was studied. One of the patients with secondary hyperlipidaemia suffered from a severe recurrent intrahepatic cholestasis of pregnancy while the other had a malignant reticulosis. The hypolipidaemic patient revealed typical signs and symptoms of acanthocytosis, i.e. *a)* erythrocytes of thorny appearance, the so-called acanthocytes which in contrast to normal erythrocytes do not exhibit rouleaux formation in a wet blood film (Fig. 3); *b)* bilateral pigmentary degeneration of the

retina; and *c)* ataxic neuropathy. The patient had damaged liver function but no steatorrhoea.

Our results summarized in Fig. 4 indicate that in essential hyperlipidaemia, in spite of the extreme changes in plasma lipids, the erythrocyte lipids did not differ from those of the normal red cells. The patients with secondary hyperlipidaemia had low red cell cholesterol and TEFA levels, and the patient with cholestasis displayed in addition a low lipid P value. In both patients

Table II

Fatty acid distribution of the total phospholipid of adult and foetal erythrocytes
(major components in relative percentage)

Fatty acids as methyl esters	Adult*	Foetal**
$C_{16:0}$	23.3 (19.9–25.9)	28.9 (25.8–31.0)
$C_{16:1}$	2.0 (0.7– 3.6)	2.2 (1.6– 3.4)
$C_{18:0}$	20.8 (18.7–23.4)	20.7 (18.2–22.7)
$C_{18:1}$	20.2 (17.5–23.6)	17.1 (16.6–17.6)
$C_{18:2}$	12.6 (10.9–14.5)	5.5 (4.3– 6.6)
$C_{20:3}$***		2.7 (2.3– 3.9)
$C_{20:4}$	21.0 (17.0–24.7)	22.5 (18.9–27.5)

* Average of 30 experiments
** Average of 9 experiments
*** $C_{20:3}$ acid was found in 4 out of 9 cases

Table III

Influence of a fat-free diet on the fatty acid distribution of erythrocyte total phospholipids in the rat

Fatty acid	Erythrocytes	
	Control*	Fat-free diet**
$C_{16:0}$	29.2 (26.4–30.3)	31.6 (28.8–35.7)
$C_{16:1}$	4.1 (3.3– 5.9)	4.4 (2.6– 7.7)
$C_{18:0}$	17.9 (13.3–20.1)	13.7 (10.9–17.1)
$C_{18:1}$	11.7 (9.8–13.6)	17.4 (15.0–20.2)
$C_{18:2}$	10.8 (9.4–12.3)	3.8 (2.7– 6.3)
$C_{18:3}$		1.4 (0.8– 4.2)***
$C_{20:4}$	26.4 (20.5–30.5)	27.8 (25.6–37.6)

* Average of 30 controls
** Average of 30 experiments
*** $C_{20:3}$ acid was found in 17 of the 30 blood samples

there were changes in the fatty acid profile of red cell phospholipids; the level of palmitic acid ($C_{16:0}$) was increased and that of arachidonic acid ($C_{20:4}$) decreased. In addition, in the patient with cholestasis there was a slight decrease in the relative amount of stearic acid ($C_{18:0}$) and the percentage of linoleic acid was less in the patient with malignant reticulosis.

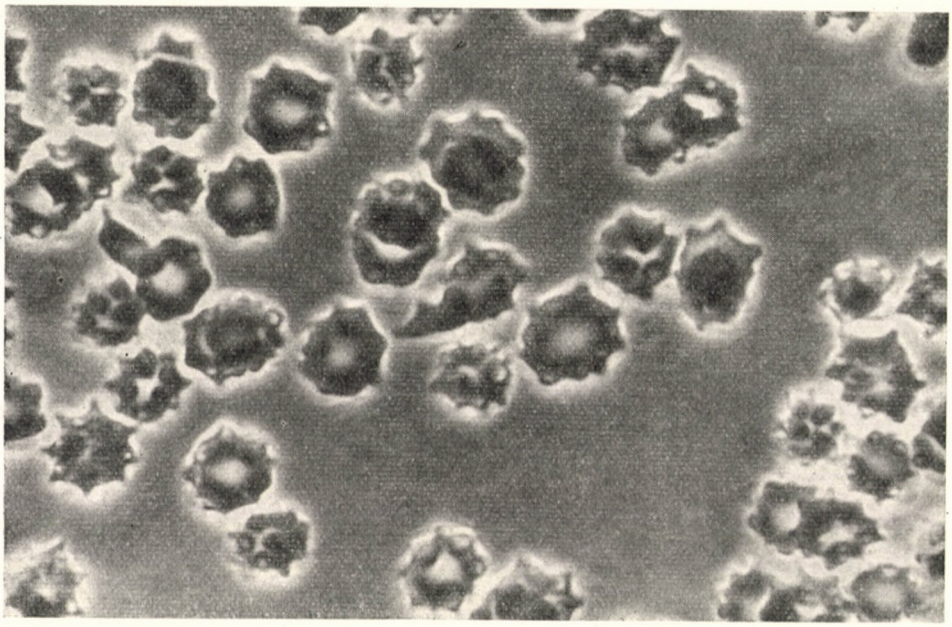

Fig. 3. Acanthocytes. Blood film in physiological saline. Phase contrast micrograph

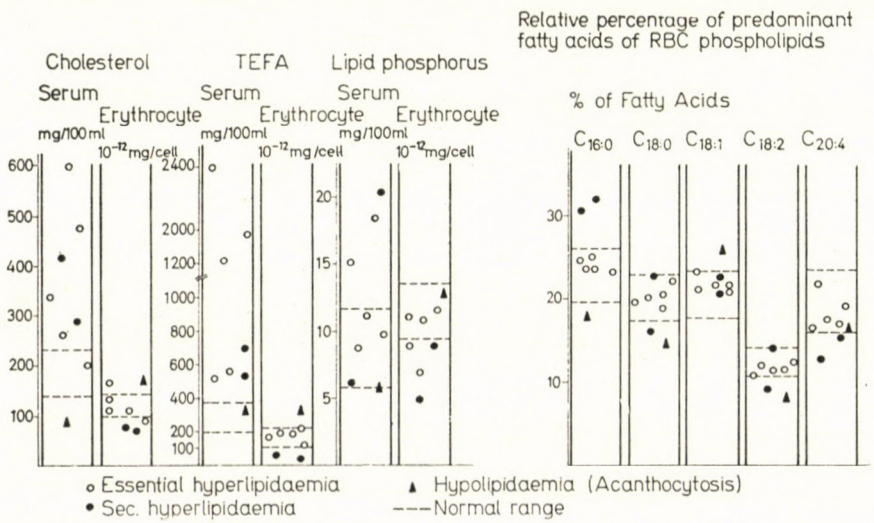

Fig. 4. Serum and erythrocyte lipids in hyper- and hypolipidaemia

The patient suffering from acanthocytosis had marked hypolipidaemia, and a considerable decrease of the β-lipoprotein level. The serum cholesterol level was low, the erythrocyte TEFA level was slightly increased. Changes occurred in the relative amount of many individual fatty acids. There was a definite decrease in palmitic acid ($C_{16:0}$) and stearic acid ($C_{18:0}$), an increase in oleic acid ($C_{18:1}$) and a marked decrease in linoleic acid ($C_{18:2}$). Arachidonic acid($C_{20:4}$) corresponded to the lowest level of the normal range.

Discussion

It is well known that the major haemoglobin component in foetal mammalian red blood cells is Hb F, while in the adult erythrocyte it is Hb A. Still, the basic functional characteristic of foetal erythrocytes, their great affinity to oxygen cannot be explained by the properties of Hb F. Hb F solutions do not show an increased oxygen affinity, nor do so the erythrocytes of adults with hereditary persistence of Hb F. Between foetal and adult erythrocytes, there are definite differences also in permeability [19–21], ultrastructure, metabolism and enzyme activities. They are easy to differentiate cytologically [22, 23]. All these reflect differences in cellular organization between foetal and adult red cells.

We found great individual differences in the cholesterol, TEFA and lipid P levels. They may have originated from the varying relative amount (60–80%) of foetal cells in different cord blood samples. There were, however, no significant differences between foetal and adult erythrocytes in these lipid characteristics.

The analyses of the fatty acid profile of foetal red cell phospholipids yielded more relevant results. In good agreement with the data of CROWLEY et al. [24] the linoleic acid ($C_{18:2}$) content of foetal cells was found to be significantly lower, and their oleic acid ($C_{18:1}$) content slightly lower, than that of adult erythrocytes. Besides, we have found higher concentration of palmitic acid ($C_{16:0}$) in foetal red cells. Eicosotrienoic acid ($C_{20:3}$) could be detected in nearly 50% of the foetal erythrocyte extracts, while in none of the adult samples.

The lower linoleic acid ($C_{18:2}$) content and the appearance of eicosotrienoic acid ($C_{20:3}$) were found to be characteristic of red blood cells produced during a fat-free diet [25].

In a number of investigations changes in dietary fats have shown to affect the composition of the red cell lipids. Alterations in the fatty acid content of the red cell were observed to follow changes in food intake. In good agreement with literary data [26, 27] our experiments on rats kept on a fat-free diet revealed no changes in the proportions of the various lipid classes.

There were striking shifts in linoleic and oleic acid; the former decreased significantly, while there was a marked elevation of the latter accompanied by a slight but definite decrease in stearic acid. Eicosotrienoic acid could be detected in more than 50% of the blood samples. Volunteers consuming a fat-free diet or patients with malabsorption syndrome also show a greatly reduced linoleic acid content in their erythrocytes. Linoleic acid content of red cells increases if the diet is rich in this fatty acid [26, 30]. The same is true for rats kept on a diet rich in polyunsaturated fatty acids [28, 29, 31]. Red blood cell fatty acids change rapidly after changing the diet [7]. Fatty acids are incorporated into the phospholipids, principally into the lecithin fraction of the circulating erythrocytes. It is likely that in dietary experiments the change in the phospholipid fatty acids of circulating red cells is a result of a combination of acylation processes and an exchange of phospholipid molecules between plasma and cells [29]. Nevertheless, the fatty acid composition of red cell phospholipids is not a simple reflection of the composition of the dietary lipids. In contrast to the rapidity with which the appropriate diet leads to the increase of red cell linoleic acid, it is difficult or even impossible to increase the red cell content of certain other fatty acids [32]. The limits of the type and extent of the changes that can be induced in the red cells by dietary means suggest the effect of regulating factors other than the relative change of any component in diet, plasma or adipose tissue.

Most of the erythrocyte lipids are in a dynamic exchange with the corresponding plasma component. The exchange of cholesterol is much more rapid than that of the phospholipids [4]. In spite of this a number of investigations have shown that although dietary fats affect the cholesterol content of plasma, the amount of this lipid in erythrocytes remains constant. Similarly, normal values for red cell cholesterol were found in several cases of hypercholesterolaemia [26, 27, 29]. However, the extreme increase in free plasma cholesterol elicited by cholesterol feeding of guinea pigs is reflected in their erythrocyte lipids [33]. Abnormally low red cell cholesterol levels have been reported in rats following treatment with triparanol [34].

The results of our studies in patients with essential hyperlipidaemia speak unequivocally for regulating factors maintaining the constancy of red cell lipids in spite of the extreme changes in plasma lipids. The main lipid and phospholipid class levels and the fatty acid composition of red cell phospholipids were all within the normal range. This normal structure of membrane lipids is in good agreement with the harmless nature of this genetically determined defect. On the contrary, our severely ill patients with secondary hyperlipidaemia had significant changes in the composition of their red cell lipids.

Definite changes in red cell lipids could be detected also in our hypolipidaemic patient suffering from acanthocytosis. Our results correspond well with the recent results of PHILLIPS and DODGE [35] and with the most con-

sistent change, the decrease in linoleic acid content, reported previously by others [26, 36, 37].

On the basis of the similarities between the fatty acid changes in acanthocytosis and in essential fatty acid deficiency in various animal species, it is suggested [35] that the fatty acid changes in acanthocytosis may be explained largely, if not entirely, by a deficiency of essential fatty acids secondary to malabsorption and perhaps by an additional low fat intake. The deficiency in these patients is induced apparently genetically. As a result of the deficiency, the relative amount of red cell linoleic acid is decreased and that of oleic acid increased. The decrease of the linoleic acid level was observed also in the adipose tissue [26], in the liver and intestinal mucosa [38] of patients with acanthocytosis. These changes in membrane lipids are well reflected in the clinical symptoms of the syndrome, manifesting themselves with acanthocytes, malabsorption, liver dysfunction and multiple neurologic signs.

From the present results two main conclusions may be drawn.

1. The lipid composition of the red cells is specific and constant. Plasma lipids are much more responsive to dietary changes than are the red cell lipids. The total amount of none of the cell lipids undergoes a change by alteration of dietary lipids. Deficiency in essential fatty acids is reflected in the membrane lipid pattern. Normal red cell lipid values in essential hyperlipidaemia point to regulating factors maintaining the constancy of membrane lipid structure.

2. There is a conspicuous resemblance in the results of the reported experiments, i.e. the decrease in polyunsaturated fatty acids, in the first place the decrease of linoleic acid in the phospholipids of foetal cells, in the cells of animals kept on a fat-free diet, in the cells of patients with secondary hyperlipidaemia, and in genetically determined hypolipidaemia.

In previous papers [39, 40] we have reported relatively low levels of polyunsaturated fatty acids of red cell phospholipids in various haemolytic diseases and in hereditary and secondary sideroachrestic anaemia, and suggested that the changes in the lipid structure and permeability of cellular and mitochondrial membranes may play an important role in the development of the intracellular iron overload. Relatively low levels of linoleic acid were found previously by MUNN [41], MUNN and CROSBY [42] and DE GIER et al. [43] in various red cell abnormalities. This abnormality was considered primarily as a consequnce of an immature red cell population. It is suggested that an increased demand for red cells elicits a local deficiency of the essential fatty acid, linoleic acid, in the production of erythrocytes.

The ow level of linoleic acid in foetal erythrocytes may also be explained by the hgh proportion of immature red cells in cord blood. During the last one hundred days of pregnancy the foetus trebles its weight and thus approximately tebles its blood volume. In previous papers [21, 15, 44] we have drawn

attention to the fact that cord blood cells resemble in many characteristics the red cells in certain types of haemolytic anaemia. In both conditions blood volume expands rapidly with accelerated maturation by possibly skipping some divisions [46, 47]. This "foetal type" of erythropoiesis is reflected in the iron incorporation *in vitro* and in the non-haemoglobin iron binding protein content of these cells [44].

A long-term or permanent change in the concentration of one fatty acid, even though compensated by the replacement by another fatty acid, may have an important physiological significance. The red cell is a highly integrated system. Modification of one of its elements may affect the structure and function of the entire membrane. Cell membranes are sites of complex metabolic functions and are of key importance in cell permeability and viability. This is well reflected in the fact that foetal cells, red cells in essential fatty acid deficiency, in acanthocytosis and in certain haemolytic diseases, resemble not only in their low linoleic acid levels but in their permeability to non-electrolytes and in their decreased viability as well.

*

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REFERENCES

1. Davson, H., Danielli, J. F.: The permeability of natural membranes. Cambridge University Press, 1943.
2. Robertson, J. D. in: Cellular membranes and development, M. Locke (ed.), Academic Press, New York 1964, p. 1.
3. Korn, E. D.: Science **153,** 1491 (1966).
4. Wead, R. I., Reed, C. F.: Amer. J. Med. **41,** 681 (1966).
5. de Gier, J., van Deenen, L. L. M.: Biochim. biophys. Acta (Amst.) **49,** 286 (1961).
6. Hanahan, D. J., Watts, R. M., Pappajohn, D. J.: Lipid Res. **1,** 421 (1960).
7. Hill, J. G., Kuksis, A., Beveridge, J. M. R.: J. Amer. Oil Chemists Soc. **41,** 393 (1964).
8. Nelson, G. J.: Lipid Res. **8,** 374 (1967).
9. van Deenen, L. L. M., de Gier, J.: The Red Blood Cell, Eds Bishop, Ch. and Surgenor, D. M. Academic Press, New York 1964.
10. Dodge, J. T., Phillips, G. B.: J. Lipid Res. **8,** 667 (1967).
11. Murphy, J. R.: J. Lab. clin. Med. **65,** 757 (1965).
12. Jacob, H. S.: Amer. J. Med. **41,** 734 (1966).
13. Jacobs, M. H., Glassman, H. N., Parpart, A. K.: J. cell. comp. Physiol. **7,** 197 (1935).
14. de Gier, J., van Deenen, L. L. M., van Senden, K. G.: Experientia (Basel) **22,** 20 (1966).
15. Holián, S. R., Szelényi, J. G.: XVth Coll. Protides of the Biol. Fluids, Brugge **15,** 377 (1967).
16. Ways, P., Hanahan, D. J.: J. Lipid Res. **5,** 318 (1964).
17. Hack, M. H.: Arch. Biochem. **58,** 19 (1955).
18. Allen, R. J. L.: cit. Ivor Smith (ed.). Chromatographic and Electrophoretic Techniques. William Heinemann Ltd., London 1960. Vol. I, p. 370.
19. Barton, T. C., Brown, D. A. J.: J. gen. Physiol. **47,** 839 (1964).
20. Reimann, F., Yenen, E.: Acta haemat. (Basel) **27,** 359 (1962).
21. Holián, S. R., Szelényi, J. G., Breuer, J. H., Medgyesi, G., Sőtér, V. N.: Haematologia (Budapest) **1,** 416 (1968).
22. Kleihauer, E., Braun, H., Betke, K.: Klin. Wschr. **35,** 637 (1957).

23. SZELÉNYI, J. G., HOLLÁN, S. R.: Vox Sang. (Basel) **12,** 234 (1967).
24. CROWLEY, J., WAYS, P., JONES, J. W.: J. clin. Invest. **44,** 989 (1965).
25. FARNSWORTH, P., DANON, D., GELLHORN, A.: Brit. J. Haemat. **11,** 200 (1965).
26. FARQUHAR, J. W., AHRENS, E. H.: J. clin. Invest. **42,** 675 (1963).
27. HILL, J. G., KUKSIS, A., BEVERIDGE, J. M. R.: J. Amer. Oil Chemists Soc. **42,** 137 (1965).
28. VAN DEENEN, L. L. M., DE GIER, J., HOUTSMULLER, U. M. T., MONFOORT, A., MULDER, E.: Dietary effects on the lipid composition of biomembranes in biochemical problems of lipids. Frazer, BBA Library Vol. 1, **40,** 4 (1963).
29. DE GIER, J.: Proc. XIth Congr. Int. Soc. Blood Transfusion, Sydney 1966, p. 135.
30. HORWITT, M. K., HARVEY, C. C., CENTURY, B.: Science **130,** 917 (1959).
31. WALKER, B. L., KUMMEROW, F. A.: J. Nutr. **81,** 75 (1963).
32. MULDER, E., DE GIER, J., VAN DEENEN, L. L. M.: Biochim. biophys. Acta (Amst.) **70,** 94 (1963).
33. OSTWALD, R., SHANNON, A.: Biochem. J. **91,** 146 (1964).
34. BLOHM, T. R., KARIYA, T., LAUGHLIN, M. W.: Arch. Biochem. **82,** 250 (1959).
35. PHILLIPS, G. B., DODGE, J. T.: J. Lab. clin. Med. **71,** 629 (1968).
36. WAYS, P., REED, C. F., HANAHAN, D. J.: J. clin. Invest. **40,** 1088 (1961).
37. WAYS, P., REED, C. F., HANAHAN, D. J.: J. clin. Invest. **42,** 1248 (1963).
38. ISSELBACHER, K. J., SCHEIG, R., PLOTKIN, G. R., CAULFIELD, J. B.: Medicine (Baltimore) **43,** 347 (1964).
39. HOLLÁN, S. R., BREUER, J. H., MEDGYESI, G. A.: Haemat. hung. **5,** 17 (1965).
40. HOLLÁN, S. R., GÁBOR, I., LELKES, G., CEGLÉDI, Á., MEDGYESI, G. A., BREUER, J.: Haemat. hung. **5,** 23 (1965).
41. MUNN, J. I.: Brit. J. Haemat. **4,** 344 (1958).
42. MUNN, J. I., CROSBY, W. H.: Brit. J. Haemat. **7,** 523 (1961).
43. DE GIER, J., VAN DEENEN, L. L. M., VAN VERLOOP, M. C., VAN GASTEL, C.: Brit. J. Haemat. **10,** 246 (1964).
44. HOLLÁN, S. R., SZELÉNYI, J. G., ÁRKY, I.: Proc. 1st Int. Symposium on Metabolism and Membrane Permeability of Erythrocytes and Thrombocytes, Vienna 1968.
45. SKIPSKI, V. P., PETERSON, R. F., BARCLAY, M.: Biochem. J. **90,** 374 (1964).
46. BORSOOK, H., RATNER, K., TATTRIE, B., TEIGLER, D.: Nature (Lond.) **217,** 1024 (1968).
47. LORD, B. I.: Nature (Lond.) **217,** 1026 (1968).

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THE ROLE OF LYMPH AND BLOOD CIRCULATION IN PANCREATIC SELF-DIGESTION AND THE DEVELOPMENT OF EXTRAPANCREATIC NECROSES

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After infusion of ^{131}I-albumin and injection of in vivo protein-bound ^{125}I rose bengal into the pancreas of the dog, their levels were found to be significantly higher in thoracic duct lymph than in pancreatic and portal venous plasma. In the dog with bile-induced pancreatitis, lipase and amylase levels were also higher in thoracic duct lymph than in portal venous serum.

By its transport function, lymph circulation plays a protective role in the process of pancreatic self-digestion. A certain danger may be inherent in the fact that the lymph carries into the blood enzymes, proteins, and protein-bound substances at high concentrations which have not passed through the liver.

In acute pancreatitis, digestive enzymes find their way into the pancreatic interstices through acini and the walls of the efferent ducts [1–8], and protein [9], protein-bound substances, even red blood cells [10], reach them across the capillary walls. From the interstices they are carried away by blood and lymph. Data in the literature are indicative of the significance of the transport by lymph: pancreatic lipase and amylase levels are higher in lymph than in plasma [6, 7, 11, 12, 13, 14]. In the dog, combined pancreatic ductal and lymphatic obstruction aggravates enzymatic self-digestion of pancreatic tissue and fat necrosis [15]; in rats with trypsin-induced pancreatitis, ligation of the thoracic duct substantially increases mortality [16] and in dogs with bile-induced pancreatitis, venous congestion is manifest in the gland [17]. Further proofs are that pancreatic blood flow, as measured by the ^{86}Rb method, is significantly reduced [18], due to a narrowing of the pancreatic arteries [17], distention of arteriovenous anastomoses [17], and failure of the blood capillaries to fill up [17, 20, 21]. These changes can readily be studied after filling the pancreatic vascular system with 5% polyvinyl chloride [19].

In pancreatitis, aetiological significance appears to attach to the transport from the pancreatic interstices in two respects. First, the removal of enzymes, proteins, and breakdown products of protein nature, decreases pancreatic self-digestion and impedes the development of fibrosis after acute pancreatitis. Second, it furthers the formation of organ necroses induced by pancreatic digestive enzymes, and the development of shock and toxaemia.

When transported by the lymph, enzymes, protein, protein-bound substances, and protein breakdown products reach the blood circulation without passing through the liver, thus without being inactivated or detoxicated. The enzymes and substances released from the pancreas into the abdominal cavity are taken up by blood capillaries across the peritoneum; in this way they also bypass the liver, on their way into the superior vena cava.

Two questions remain to be answered, viz.

(i) how much protein is carried away from the pancreas by lymph and how much by blood, and is the total amount transported away sufficient to reduce pancreatic self-digestion?

(ii) is the site of the enzyme-induced extrapancreatic necroses dependent on the route of transport?

(i) The results of experiments carried out in the dog, using the methods and techniques described in earlier work [22], showed that after infusion into the pancreas of ^{131}I-tagged albumin the protein-bound iodine level was 94% to 97% in 1 ml of lymph and 73% to 84% in 1 ml of plasma from the femoral artery (Table I). The concentration of ^{131}I-albumin was in the lymph 12 to 24 times that in the plasma of the pancreatic vein (Table II). Mean activity (cpm) leaving the pancreas during the first three hours after infusion was 7.5% of the total input both in thoracic duct lymph and in pancreatic venous plasma (Table III).

Table I

Protein-bound iodine in per cents of total iodine
(mean ± S. E.)

Protein-bound iodine in 1 ml of	30 min	60 min	90 min	120 min	150 min	180 min
lymph	97.2±0.6	96.8±0.6	95.6±0.9	95.5±0.9	95.0±0.9	94.7±0.8
plasma of femoral artery	73.0±6.4	74.3±4.3	78.1±3.7	82.2±3.6	83.8±3.5	84.1±2.9
Number of animals	6	7	7	7	6	6

Table II

Quotients of ^{131}I-albumin concentration in thoracic duct and pancreatic venous plasma
(mean ± S. E.)

	30 min	60 min	90 min	120 min	150 min	180 min
Quotient	24.3±12.8	23.4±4.5	20.2±4.6	14.6±2.6	13.2±3.5	11.9±3.2
Number of animals	5	6	5	7	6	6

Table III

Total amount of ^{131}I-albumin (cpm) leaving the pancreas expressed in percentage of activity input

Hour	Lymph		Blood		Number of animals	
	mean	extreme values	mean	extreme values	lymph	blood
First	1.7	0.3–3.8	3.5	2.0–5.4	7	5
Second	4.5	0.9–12.2	5.2	3.3–9.4	7	6
Third	7.4	1.4–19.1	7.6	2.6–11.8	6	5

Infusion of ^{51}Cr-tagged [23] red blood cells produced no appreciable activity either in blood or in lymph [10].

Our finding that in 3 hours nearly as much protein leaves the pancreas in the lymph as in the pancreatic vein, both in intact dogs and in dogs with pancreatitis, is evidence in favour of a protective role being played by the lymph and blood circulation during acute pancreatitic self-digestion [22].

(ii) In acute pancreatitis, large amounts of digestive enzymes leave the gland with the blood [2, 3, 4, 7, 10–15, 24–25] and may give rise to necrosis in liver, kidney, lungs and pleura [15, 26–37], although a proteinase-inhibitory system is at work not only in the pancreas [27], the liver [38], and the blood [35, 39], but also in the lung [38] where lipase is also inactivated [40]. It seems that the necroses in the lungs and the pleura, but possibly also those in other organs, are induced by the enzymes transported into the superior vena cava at first only by the lymph and in the later phase also after having been absorbed from the peritoneum and the pleura [31, 45]. To test this assumption, two series of experiments have been performed.

Materials and methods

In a model experiment imitating the transport of enzymes from the pancreas into the blood and their excretion by the liver, seven dogs of either sex, weighing 8 to 17 kg, were anaesthetized with chloralose, laparotomized, and each was given through the duct of Santorini into the pancreas a single 10 ml dose of ^{125}I rose bengal (27.5μCi/pancreas) [41] known to be bound by protein *in vivo* [42]. Bile was diverted through a cannula inserted into the common duct. The duct of Wirsung was ligated near its duodenal orifice. The efferent lymphatics of the liver were also ligated. Lymph was collected from the thoracic duct for one hour, at four consecutive 5-minute, two 10-minute, and one 20-minute intervals. With each lymph collection, blood was withdrawn from the portal vein, the femoral artery, and a hepatic vein. Into the latter, a cardiac catheter was introduced under radiographic control. Excretion of ^{125}I rose bengal by the liver was calculated from the difference between its concentration in the portal and the hepatic vein. The collected lymph was heparinized, and the protein was precipitated from it and from the blood plasma with trichloro-acetic acid. Activity was estimated by means of a well-type scintillation counter. No experiment was evaluated in which protein-bound activity was less than 80% of the activity of 1 ml lymph. Plasma volume was determined by the Evans blue dilution method. The radioactivity in the plasma volume is the product of the volume and the radiochemical concentration in the blood plasma. Activity in pancreas and

liver homogenates, and also in the bile collected during one hour, was then estimated and expressed in per cents of the total input.

In another experiment, involving seven dogs, weighing between 7 and 16 kg, anaesthetized with chloralose and laparotomized, the lipase [43] and amylase [44] activities in the blood serum of the femoral artery, and the femoral, the portal, and the hepatic veins, as well as in the thoracic duct lymph were measured at 15-minute intervals during two and a half hours. After the first interval, 10 ml of bile from the gall bladder were injected through Santorini's duct into the pancreas, to elicit pancreatitis. Enzyme extraction by the liver and by one hind-leg was calculated from the activity difference between inflowing and outflowing plasma. Statistical analysis was performed using Student's t test and the sign test.

Results and discussion

As early as 15 minutes after the administration of ^{125}I rose bengal its concentration was found to be significantly higher ($p < 5\%$) and by the end of the first hour about six times as high ($p < 1\%$) in lymph than in portal venous blood plasma (Fig. 1). An average 30% of the dye by the extracted liver At one hour, the pancreas contained about 73%, the bile and the liver together about 15%, the whole volume of plasma roughly 7%, and the lymph collected during one hour 1%, of the activity input (Table IV).

At 60 minutes after the injection of bile into the pancreas, amylase activity, and at 75 minutes lipase activity, was significantly higher in lymph than in portal venous serum ($p < 5\%$) (Figs 2 and 3).

About 20% lipase was extracted by the liver and none by the hind-legs used for control purposes. Amylase extraction by the liver could not be demonstrated.

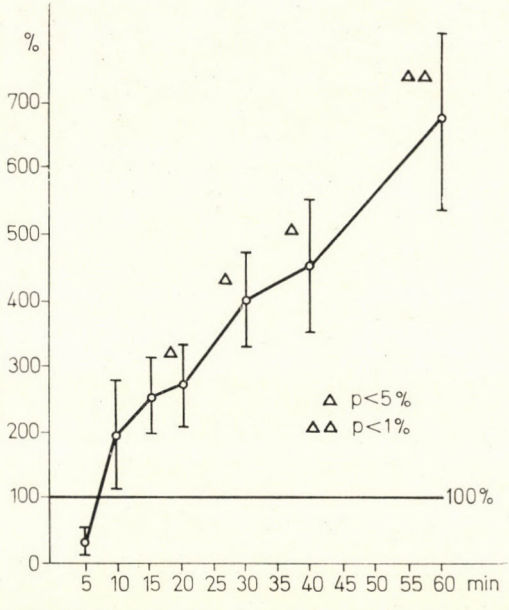

Fig. 1. Activity (cpm/ml) of thoracic duct lymph in per cent of activity in portal venous plasma. Vertical bar: S.E. (Means of 7 dogs)

Table IV

Distribution of activity (cpm) one hour after injection of ^{125}I rose bengal into the pancreas

Measured in	Number of animals	Activity in percentage of input
Pancreas	4	72.66±1.828
Liver and bile	6	15.13±1.55
Blood circulation	6	6.76±0.42
Lymph (collected in 1 hour)	6	1.24±0.33

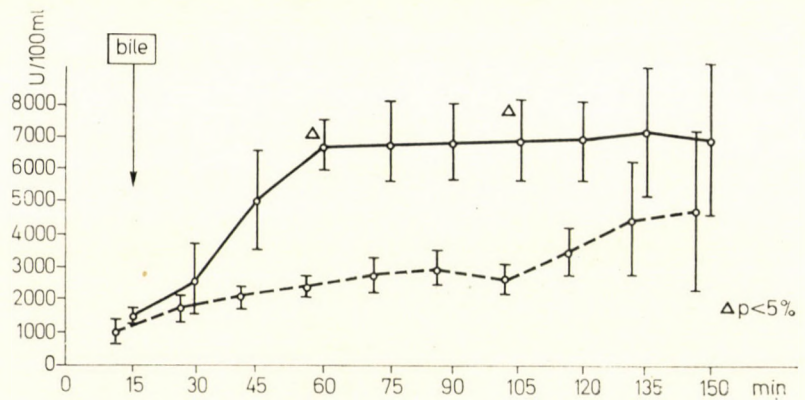

Fig. 2. Amylase activity in thoracic duct lymph and portal venous serum in bile-induced pancreatitis. Vertical bar: S.E. ———— = thoracic duct lymph. — — — — — = portal venous serum. (Means of 5 dogs)

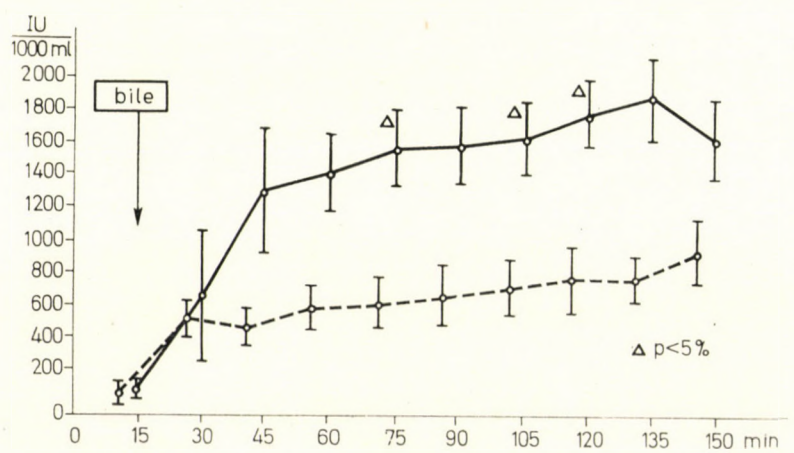

Fig. 3. Lipase activity in thoracic duct lymph and portal venous serum in bile-induced pancreatitis. Vertical bar: S.E. ———— = thoracic duct lymph. — — — — — = portal venous serum. (Means of 7 dogs)

Although our experimental results have in several respects failed to verify our assumption, they do suggest two conclusions.

First, pancreatic lipase and amylase transported by the portal venous route produce necroses primarily, but not exclusively, in the liver. When transported by the lymph, these enzymes give rise to perilymphatic necroses and necroses in the lungs and the pleura.

Secondly, in pancreatitis we must establish, separately for each and every pancreatic digestive enzyme, the site and the mechanism of the special inhibitory system.

Such investigations are necessary for a clear understanding of the pathogenesis of the extrapancreatic necroses induced by pancreatic enzymes.

*

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REFERENCES

1. Bainbridge, F. A.: J. Physiol. (Lond.) **32,** 1 (1905).
2. Dumont, A. E., Doubilet, H., Mulholland, J. H.: Ann. Surg. **152,** 403 (1960).
3. Dumont, A. E., Mulholland, J. H.: Surg. Forum. **10,** 243 (1959).
4. Duprez, A., Godart, S., Plattenborze, R., Dupont, M.: Interstitial and Lymphatic Pathway of the External Secretion of the Pancreas. In: Heinkel, K., Schön, K. (eds): Pathogenese, Diagnostik, Klinik und Therapie der Erkrankungen des exokrinen Pankreas. Schattauer, Stuttgart 1964. Pp. 207–213.
5. Litvine, J.: Acta chir. belg. **63,** 698 (1964).
6. Osato, S.: Tohoku J. exp. Med. **2,** 514 (1921).
7. Papp, M., P. Németh, É., Koltay, E.: Acta physiol. Acad. Sci. hung. **14,** 149 (1958).
8. Rasin, E., Feldman, M. G., Dreiling, A.: J. surg. Res. **2,** 320 (1962).
9. Egdahl, R. H.: Ann. Surg. **148,** 389 (1958).
10. Sim, D. N., Duprez, A., Anderson, M. C.: Acta gastro-ent. belg. **29,** 235 (1966).
11. Bartos, V., Brzek, V., Groh, J.: Amer. J. med. Sci. **252,** 660 (1966).
12. Dumont, A. E., Doubilet, H., Mulholland, J. H.: Ann. Surg. **152,** 403 (1960).
13. Hatano, M., Grenier, J. F., Adloff, M., Weiss, A. G.: C. R. Soc. Biol. (Paris) **157,** 1505 (1963).
14. Popper, H. L., Necheles, H.: Proc. Soc. exp. Biol. (N. Y.) **43,** 220 (1940).
15. Papp, M., P. Németh, É., Feuer, I., Fodor, I.: Acta med. Acad. Sci. hung. **11,** 203 (1958).
16. Papp, M.: Personal communication.
17. Papp, M., Ungvári, Gy., P. Németh, É., Munkácsi, I., Zubek, L.: Orv. Hetil. **109.** 2610 (1968).
18. Papp, M., Makara, G. B., Hajtman, B., Csáki, L.: Gastroenterology **51,** 524 (1966).
19. Munkácsi, I.: Z. wiss. Mikr. **63,** 352 (1957).
20. Galletti, G., Cavallari, A., Battisti, C., Martinelli, G., Berardi, P.: Boll. Soc. ital. Biol. sper. **43,** 82 (1966).
21. Cavallari, A., Galletti, G., Battisti, C., Martinelli, G., Berardi, P.: Boll. Soc. ital. Biol. sper. **43,** 83 (1966).
22. Papp, M., Ormai, S., Csáki, L.: Z. exp. Med. **147,** 285 (1968).
23. Gray, I. I., Sterling, K.: J. clin. Invest. **29,** 1604 (1950).
24. Howard, J. M., Smith, A. K., Peters, J. J.: Surgery **26,** 161 (1949).
25. Litvine, I.: Acta chir. belg. **63,** 698 (1964).
26. Wanke, M., Grözinger, K. H.: Langenbeck's Arch. klin. Chir. **310,** 36 (1965).
27. Wanke, M.: Experimentelle Pankreatitis. Thieme, Stuttgart 1968.
28. Achord, J. L., Gerle, R. D.: Amer. J. dig. Dis. **11,** 453 (1966).
29. Dos Reis, L.: Arch. Surg. **87,** 604 (1963).
30. Fisher, E. R., McCloy, D.: Surgery **37,** 213 (1955).
31. Kalser, M. H., Roth, J. L. A., Bockus, L.: Gastroenterology **28,** 842 (1955).
32. Leger, L., Morin, M., Fabiani, P., Wagnier, M., Patel, J. C.: Presse méd. **74,** 1403 (1966).

33. MAYORAL, J. W., GOMPARTZ, M. L., YOUNG, J. M.: Gastroenterology **36,** 884 (1959).
34. PANABOKKÉ, R. G.: J. Path. Bact. **75,** 319 (1958).
35. SARLES, H., CAMATTE, R.: Pancréatites aigues. Conceptions et thérapeutics récentes. Masson, Paris 1963.
36. VOGEL, S. F.: Arch. Path. **52,** 355 (1951).
37. WARTER, J., KEMPF, F., TEMPE, J. D.: J. Radiol. Électrol. **46,** 55 (1965).
38. WERLE, E., MARX, R., TRAUTSCHOLD, I., REICHENBACH-KLINKE, K. E.: Blut **14,** 206 (1967).
39. SCHULMAN, M. R.: J. biol. Chem. **213,** 655 (1955).
40. PROTSENKO, V. C.: Fed. Proc. (Transl. Suppl.) **24,** 203 (1965).
41. HALLABA, E., RAIEH, M.: Int. J. appl. Radiat. **18,** 533 (1967).
42. ROSENTHAL, S. M., WHITE, E. C.: J. Pharmacol. exp. Ther. **24,** 265 (1925).
43. WEBER, H.: Dtsch. med. Wschr. **90,** 1170 (1965).
44. SMITH, B. W., ROE, J. H.: J. biol. Chem. **179,** 53 (1949).
45. WARTER, J., MÉTAIS, P., WEIL, J. P., STORCK, D.: Rev. int. Hépat. **8,** 1033 (1962).

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ROLE OF CATECHOLAMINES IN THE CENTRAL EFFECTS OF AMPHETAMINE

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Reserpine and alpha-methyl-m-tyrosine do not influence amphetamine hypermotility, but reduce the elevation of metabolic rate caused by amphetamine.

Prenylamine and guanethidine inhibit both central effects of amphetamine.

There is no direct correlation between the decrease of the brain catecholamine level and the influence on the different amphetamine effects.

Reserpine inhibits the hypermotility caused by p-chloroamphetamine.

Independently of their ability to cause hypermotility, the amphetamine derivatives diminish the facilitation of convulsions caused by reserpine and thyroxine.

Amphetamine is a sympathomimetic amine possessing peripheral and central actions. While its peripheral effect is exerted through an indirect mechanism, i.e. the liberation of noradrenaline from tissues, its role played in the central nervous system (CNS) is unclarified. Though in high doses amphetamine lowers the noradrenaline level in the brain [14, 16, 26], until recently most authors have claimed that the central effect of amphetamine was a direct one, exerted on the catecholamine receptors [2, 28, 33, 34]. When DAHLSTRÖM and FUXE [4] had demonstrated specific noradrenergic and dopaminergic neurones in the CNS, having the same properties as the peripheral neurones, the question arose as to how the same compound may exert its central and peripheral effects by different mechanisms. The claim that the central effect of amphetamine was a direct one was based in the first place on the fact that reserpine, which causes a complete monoamine depletion in the brain, did not inhibit the hypermotility [33]. Earlier we had suggested the possibility that the central action of amphetamine might also be an indirect one, because prenylamine [27] and guanethidine, which cause noradrenaline depletion in the brain [21], as well as diethyldithiocarbamate, which inhibits the formation of noradrenaline from dopamine reducing thereby the brain noradrenaline level [3] diminish the central locomotor effect of amphetamine [9, 22]. Alpha-methyltyrosine blocks noradrenaline synthesis by the inhibition of tyrosine hydroxylase [18] and lowers the cerebral noradrenaline and dopamine levels without influencing the serotonin contents [29]. The compound inhibits certain central effects of amphetamine and therefore more and more authors have claimed that amphetamine would act also centrally in an indirect way [5, 10, 25, 30] and its activity depends not on the actual noradrenaline level, but on intact noradrenaline synthesis [5, 31].

Methods

White mice of either sex, weighing 18 to 22 g each and white rats of the Wistar strain, weighing 120 to 150 g each, were used. The animals were grouped at random. Statistical analysis was done following variance analysis by DUNNET's multiple comparison method [8], or by Student's *t* test.

Cerebral noradrenaline and dopamine levels were estimated by spectrophotofluorometry according to DRUJAN et al. [7]. The mice were killed by decapitation, the brain was dropped in liquid N_2 and stored at $-30°C$ until tested. Four mouse brains were pooled for each test.

The motility of mice was estimated by KNOLL's method [13]. The mice were placed into the motimeter 30 minutes after the administration of physiological NaCl solution or of the test substance and motility was observed for 30 minutes.

O_2 consumption was determined according to ISSEKUTZ [11] at 30°C.

The susceptibility to seizures was tested in mice according to ORLOFF et al. [19]. A 0.5% solution of pentetrazole was injected into a tail vein at a rate of 0.05 ml/10 sec until tonic extensor convulsions had developed. Convulsive threshold is understood to mean the dose of pentetrazole required to evoke tonic extensor convulsions.

Electroshock was elicited by means of bitemporal electrodes and a Disa stimulator, with 125/sec frequency, 6 msec delay, 3 msec duration and 8.5 to 10.5 mA intensity for mice and 15 to 16 mA for rats. The duration of stimulus was 0.5 sec in the case of mice and 1.0 sec in that of rats. A tonic extensor convulsion was accepted as being a convulsion.

The amphetamine level of the brain was estimated according to AXELROD [1].

The amine depletors (reserpine, prenylamine, guanethidine, alpha-methyl-m-tyrosine) were injected 2 hours prior to the experiments.

Results

The data in Table I show the influence of reserpine, prenylamine and guanethidine on amphetamine hypermotility and on the cerebral noradrenaline and dopamine levels in mice. Prenylamine and guanethidine significantly diminished the hypermotility, while reserpine had no influence on it. At the same time, prenylamine reduced the brain noradrenaline and dopamine levels in the same measure as reserpine, and guanethidine lowered the noradrenaline level to a lesser extent than reserpine and had no influence at all on the dopamine level. DINGELL et al. [5] and SULSER et al. [31] showed that reserpine and the specific noradrenaline depletor alpha-methyl-m-tyrosine lowered the brain noradrenaline level more markedly than did the enzyme inhibitor alpha-methyltyrosine, but only the latter inhibited the excitation caused by amphetamine. To explain these results, it was assumed that amphetamine activity requires intactness of synthesis and that the actual noradrenaline level plays no significant role. Our results did not support this view, because neither prenylamine, nor guanethidine influenced catecholamine synthesis and yet both inhibited the locomotor effect of amphetamine.

SULSER et al. [32] stated that desmethylimipramine potentiates amphetamine hypermotility and increases at the same time the brain amphetamine level. Thus, the opposite influence on amphetamine hypermotility exerted by reserpine, prenylamine and guanethidine, might be independent of the catecholamine depletion and these compounds may influence differently the entry/accumulation of amphetamine. However, the experiments indicated that 30 or 60 minutes after administration, at the time of maximum hypermotility,

Table I

Effects of reserpine (2.5 mg/kg, intraperitoneally), prenylamine (50 mg/kg, subcutaneously) and guanethidine (5 mg/kg, intraperitoneally) on the noradrenaline and dopamine levels of the mouse brain, as well as on the hypermotility caused by 50 μM/kg (±) amphetamine sulphate

In brackets: number of experiments

	Noradrenaline	Dopamine	
Control	0.436 ± 0.017 μg/g (12)	0.624 ± 0.064 μg/g (12)	Increase of motility, per cent of controls
	control per cent		Amphetamine =100±8
Reserpine	23±5 (10)	47±2 (10)	120±10
Prenylamine	24±7 (9)	48±8 (9)	30±7
Guanethidine	58±13 (9)	100±14 (9)	36±9

Table II

Effect of reserpine (2.5 mg/kg, intraperitoneally) and guanethidine (5 mg/kg, intraperitoneally) on the amphetamine level of the mouse brain following the intraperitoneal injection of 75 μM/kg (±) amphetamine

In brackets: number of experiments

μg/g ± S. E.

	Following amphetamine injection, at	
	30 minutes	60 minutes
Control	10.64±1.66 (5)	10.30±1.79 (5)
Reserpine	9.48±1.74 (4)	9.35±0.16 (4)
Control	9.37±0.83 (7)	
Guanethidine	10.33±1.22 (7)	

the brain amphetamine level is the same in the control mice and in those pretreated with reserpine or guanethidine (Table II). Accordingly, the different actions of the two noradrenaline depletors cannot be explained by a change in the accumulation of amphetamine in the brain. The same cannot be claimed in the case of prenylamine pretreatment: amphetamine being produced from prenylamine in the brain [20]. Thus the determination of amphetamine is difficult in the presence of prenylamine. Beside hypermotility the other characteristic central effect of amphetamine consists in its increasing the metabolic

rate. In our previous experiments, pretreatment with 1 mg/kg of reserpine completely inhibited in 4 hours the increase of O_2 consumption caused by amphetamine. In the present experiments, reserpine, prenylamine and guanethidine significantly diminished the increase of metabolic rate caused by amphetamine in mice (Table III). Alpha-methyl-m-tyrosine, which reduces the brain catecholamine level, has no influence on the locomotor effect of amphetamine [32], but in our experiments intensely inhibited the rise in metabolic rate caused by amphetamine (Table III). (In the doses employed

Table III

Effect of pretreatment with reserpine (2.5 mg/kg, intraperitoneally), prenylamine (50 mg/kg, subcutaneously), guanethidine (5 mg/kg, intraperitoneally) and (±) alpha-methyl-m-tyrosine (50 mg/kg, intraperitoneally) on the increase of O_2 consumption caused by 25 μM/kg (±) amphetamine in mice

Groups of 6 mice each. Basal O_2 consumption, 14.1±0.7 ml/dm²/10 minutes

Pretreatment	Increase of O_2 consumption at 30 minutes following amphetamine administration ml/dm²/10 min mean + S. E.
0.9% NaCl	14.8 ± 0.4
Reserpine	6.3 ± 0.4*
Prenylamine	8.2 ± 0.7*
Guanethidine	9.4 ± 0.9*
Alpha-methyl-m-tyrosine	3.2 ± 0.4*

* $p < 0.01$

the amine depletors do not influence basal O_2 consumption.) Thus, on the basis of the experimental evidence obtained the catecholamine depletors may be classified into two groups. To the first belong reserpine and alpha-methyl-m-tyrosine, which inhibit the central hypermetabolic effect of amphetamine, but do not influence hypermotility. In the other group belong prenylamine and guanethidine, which inhibit both the locomotor and the metabolic rate-increasing effects.

Earlier we had shown that the increased tendency to convulsions caused by reserpine was correlated with a decrease of the brain noradrenaline level and when the decrease of the level was inhibited by MAO blocking pretreatment, the tendency to convulsions decreased [23]. As it is known, following MAO-inhibitor pretreatment reserpine causes excitation similar to that induced by amphetamine. We have therefore investigated the influence of amphetamine on the convulsant action of reserpine and its correlation with hypermotility.

As pointed out above, reserpine did not influence amphetamine hypermotility, but diminished hypermotility induced by (±)-p-chloroamphetamine and methamphetamin considerably (Table IV). A further derivative, trifluoromethyl ampheetamine had no influence on the spontaneous activity of mice.

Table IV

Effect of reserpine pretreatment (2.5 mg/kg, intraperitoneally) on the hypermotility induced in mice by (±) amphetamine (50 μM/kg), methamphetamine and (±) p-chloroamphetamine

Groups of 15 mice each

Treatment	Motimeter value in 30 minutes, mean ± S. E. (pretreatment)	
	0.9% NaCl	reserpine
0.9% NaCl	228± 26	8.1± 1.5
Amphetamine	750±134	800 ±137
Methamphetamine	584± 79	168 ±41*
p-chloroamphetamine	543± 43	145 ±36*

* $p < 0.01$

Of the amphetamine derivatives tested, the lowering of convulsant threshold caused by reserpine was most intensely inhibited by p-chloroamphetamine. When the tendency to convulsions of mice was tested by means of an intravenous pentetrazole infusion, the threshold lowered by reserpine by about 50% was completely normalized by p-chloroamphetamine. Under the effect of electroshock treatment 100% of the reserpine-treated but only 50% of the p-chloroamphetamine treated animals developed tonic extensor convulsions. Methamphetamine and trifluoromethyl amphetamine were about equipotent in the case of pentetrazole convulsion, while methamphetamine was more potent in action of the response to electroshock. As examined by both tests amphetamine had the weakest effect. In the presence of reserpine amphetamine continued to cause excitation, while methamphetamine and chloroamphetamine did not, in spite of their inhibiting the convulsion facilitating effect of reserpine which is certainly correlated with catecholamine depletion. Trifluoromethyl amphetamine, which caused no excitation, was also effective (Table V.).

The susceptibility to convulsions is increased by thyroxine in the guinea pig [12] and the rat [24]. While facilitating convulsions, reserpine sedates the animals, whereas thyroxine enhances their excitability and their sensitivity to amphetamine [6]. We treated rats with 1 mg/kg of thyroxine subcutaneously daily for 3 days and tested them for sensitivity to electroshock on the fourth day. All four amphetamine derivatives significantly diminished the convulsion facilitating effect of thyroxine (Table VI). In thyroxine-pretreated animals the amphetamine derivatives had a full excitatory effect and 50 μM/kg of trifluoromethyl amphetamine, which causes no toxic symptoms in untreated animals, proved to be lethal. If we compare these results with those obtained for reserpine, we have to conclude that the influence of amphetamine derivatives on the tendency to convulsions is independent of their excitatory action.

Table V

Effect of 50 μM/kg (±) amphetamine, methamphetamine, (±) p-chloroamphetamine and 75 μM/kg trifluoromethyl amphetamine subcutaneously on the facilitation of convulsions by 2.5 mg/kg reserpine injected intraperitoneally. The experiments were carried out 30 minutes after the administration of amphetamine derivatives

The control mice were shocked with 10.5 mA, the reserpine-treated ones with 8.5 mA current. In brackets: number of experiments

Treatment	ml/10 g pentetrazole mean ± S. E.	Number of mice with convulsions/number of experimental mice
0.9% NaCl	0.183±0.012 (8)	15/16
Reserpine	0.092±0.003 (8)	16/16
Reserpine + amphetamine	0.126±0.012 (8)	14/16
Reserpine + methamphetamine	0.161±0.012* (8)	10/16
Reserpine + p-chloroamphetamine	0.183±0.015* (8)	8/16
Reserpine + trifluoromethyl amphetamine	0.160±0.024* (8)	12/16

* $p < 0.01$

Table VI

Effect of 50 μM/kg (±) amphetamine, methamphetamine, (±) p-chloroamphetamine and 12.5 μM/kg of trifluoromethyl amphetamine on the facilitation of convulsions by thyroxine in the rat

Groups of 10 rats each. The animals pretreated with 0.9% NaCl solution were shocked with 16 mA, the thyroxine-pretreated ones with 15 mA current

Treatment	Number of animals with tonic extensor convulsion (pretreatment)	
	0.9% NaCl	Thyroxine
0.9% NaCl	5	7
Amphetamine	0	2
Methamphetamine	1	0
p-chloroamphetamine	1	0
Trifluorometyl amphetamine	1	0

Discussion

The central action of amphetamine has been amply discussed in the past 15 years, mainly because the characteristic excitation caused by amphetamine resembles the excitation induced by an elevation of the cerebral catecholamine level observed for instance after the administration of the noradrenaline precursor DOPA, or of reserpine following MAO-inhibitor pretreatment. There is

ample evidence supporting the view that amphetamine acts directly on the central noradrenaline or dopamine receptors, mimicking the effect of physiologic mediator substances. At the same time, many authors think that amphetamine acts indirectly, through releasing noradrenaline or dopamine. VAN ROSSUM [33, 34] believes the action of amphetamine to be a direct one, because its locomotor effect is not inhibited by reserpine, as opposed to that of cocaine which he thinks to be an indirect one, since reserpine inhibits hypermotility which is restored then by DOPA. SMITH [28], too, thinks that amphetamine acts directly. He claims that the liberation of noradrenaline by high amphetamine doses does not prove the indirect nature of the effect, because noradrenaline and adrenaline are also capable of liberating tissue catecholamines. HANSON [10] supports the view that the amphetamine effect is indirect: the blocking of conditioned reflexes in the cat by reserpine is abolished by amphetamine, but when the animals are treated also with the tyrosine hydroxylase inhibitor alpha-methyltyrosine, amphetamine is ineffective, but if DOPA, too, is given, amphetamine becomes active again. In contrast to this, MOORE and RECH [17] demonstrated a diminution of the defensive reflex in the rat in response to alpha-methyltyrosine, while the reflex was restored by amphetamine in the same way as by DOPA. In support of the indirect effect is the fact that alpha-methyltyrosine inhibits the hyperthermia caused by amphetamine in the mouse [15]. WEISSMAN and KOE [36] showed that alpha-methyltyrosine blocked the stimulation by amphetamine of the defensive reflex of the rat. The blocking effect was not abolished by DOPA, while it was abolished by MAO inhibitors. Comparing the effects of alpha-methyl-m-tyrosine and alpha-methyltyrosine on the brain catecholamine level, DINGELL et al. [5] as well as SULSER et al. [31] found that although alpha-methyl-m-tyrosine lowered the brain catecholamine level more markedly than alpha-methyltyrosine, the latter inhibited amphetamine hypermotility, while alpha-methyl-m-tyrosine did not inhibit it. They claimed that the amphetamine action was an indirect one and its activity is dependent not on the actual brain catecholamine level, but on the newly synthesized catecholamine. This would explain why central amphetamine stimulation is inhibited by alpha-methyl-tyrosine inhibiting synthesis and not by alpha-methyl-m-tyrosine, which latter compound causes a greater depletion, but does not influence synthesis.

As the above data indicate, the view that the central effect of amphetamine is exerted directly on the catecholamine receptors is based in the first place on the fact that the effect is not blocked by reserpine. On the other hand, the indirect nature of the effect is supported by the fact that it is inhibited by alpha-methyl-tyrosine, which inhibits noradrenaline synthesis. Our finding, however, of a central amphetamine effect (increase of O_2 consumption) which is inhibited by reserpine and of catecholamine depletors (prenylamine, guanethi-

dine) which are acting not by inhibiting synthesis and are inhibiting the locomotor and hypermetabolic effects of amphetamine, place the whole problem in an entirely different light. The results for reserpine and alpha-methyl-m-tyrosine, notably that they inhibit the increase of metabolism caused by amphetamine, but not the hypermotility evoked by it, substantiate in the first place the view that these two effects are exerted on two different levels of the central nervous system and, also, that reserpine and alpha-methyl-m-tyrosine act on one level only. Furthermore, the finding which is interpreted to prove that amphetamine acts directly, notably that reserpine does not inhibit its effect, is untenable because reserpine fails to inhibit only one of the central effects. It is possible that those structures in which amphetamine exerts its locomotor effect reserpine depletes catecholamines to a lesser extent than those in which the metabolic rate is increased. This would mean that at one site there is catecholamine that amphetamine can mobilize or "utilize", while at the other there is none. Also against this view is the fact that prenylamine and guanethidine equally inhibit the two central effects of amphetamine, although guanethidine lowers the brain noradrenaline level by 40% only and does not influence at all the dopamine level. It does not seem likely that guanethidine which reduces the NA level of the whole brain by 40% only would cause such a depletion in the structures in which amphetamine exerts its locomotor and metabolic effects that no noradrenaline would be left for being utilized by amphetamine. Furthermore, this would also mean that in the amphetamine effect exclusively noradrenaline had a role, while dopamine would have none, as guanethidine leaves the dopamine level unchanged. The results for prenylamine and guanethidine also prove that it is not an inhibition of synthesis that is responsible for the anti-amphetamine effect of alpha-methyltyrosine, because the synthesis of catecholamines is influenced neither by guanethidine nor by prenylamine. The findings concerning the convulsive action show that the inhibition by amphetamine and its derivatives of the facilitation of convulsions by reserpine is not correlated with the hypermotility effect. Amphetamine, which continues to cause hypermotility following reserpine treatment, reduces in the same way the electroshock sensitivity enhanced by reserpine as it is reduced by p-chloroamphetamine, whose hypermotility-inducing effect is inhibited by reserpine, or by trifluoromethyl amphetamine which causes no hypermotility. Thus, amphetamine and its derivatives seem to have three such effects (hypermotility, elevation of metabolic rate, inhibition of the facilitation of convulsions caused by reserpine and thyroxine) that develop independently of one another and may be influenced differently. This applies not only to the depletor substances, as for example the alpha-adrenergic receptor blocking phenoxybenzamine abolishes the locomotor effect of amphetamine [9], while it does not influence its effect on metabolism [35]. Thus, the mode of action of amphetamine and of the substances inhibiting its different effects

cannot be determined exclusively by biochemical studies, which ignore the possibility that the agents acting on amine metabolism may act differently on different structures of the CNS. It is possible that there are amphetamine effects exerted on those structures in which catecholamines play an important role (hypothalamus) and that there are "non-specific" ones exerted on those structures in which the mediator role of catecholamines is not a primary one. For instance, our preliminary experiments indicate that the level of amphetamine is higher in the cerebral cortex than in the rest of the brain and the cortex is known to contain a very low amount of catecholamines.

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REFERENCES

1. Axelrod, J.: J. Pharmacol. exp. Ther. **110,** 315 (1954).
2. Brodie, B. B., Spector, S., Shore, P. A.: Pharmacol. Rev. **11,** 548 (1959).
3. Carlsson, A., Lindquist, M., Fuxe, K., Hökfelt, T.: J. Pharm. Pharmacol. **18,** 60 (1966).
4. Dahlström, A., Fuxe, K.: Acta physiol. scand. **62,** Suppl. 232, 1 (1964).
5. Dingell, J. V., Owens, M. L., Norvich, M. R., Sulser, F.: Life Sci. **6,** 1155 (1967).
6. Dolfini, E., Kobayashi, M.: European J. Pharmacol. **2,** 65 (1967).
7. Drujan, B. D., Sourkes, T. L., Layne, D. S., Murphy, G. F.: Canad. J. Biochem. Physiol. **37,** 1153 (1959).
8. Dunnett, C. W.: J. Amer stat. Assoc. **50,** 1096 (1955).
9. Galambos, É., Pfeifer, A. K., György, L., Molnár, J.: Psychopharmacologia (Berl.) **11,** 122 (1967).
10. Hanson, L. C. F.: Psychopharmacologia (Berl.) **10,** 289 (1967).
11. Issekutz, B., Issekutz, B. Jr.: Naunyn-Schmiedebergs Arch. exp. Path. Pharmakol. **199,** 306 (1942).
12. Issekutz, B., Dirner, Z.: Naunyn-Schmiedebergs Arch. exp. Path. Pharmakol. **185,** 45 (1937).
13. Knoll, J.: Arch. int. Pharmacodyn. **120,** 141 (1961).
14. McLean, J. R., McCartney, M.: Proc. Soc. exp. Biol. (N. Y.) **107,** 77 (1961).
15. Menon, M. K., Dandiya, P. C., Bapna, J. S.: Psychopharmacologia (Berl.) **10,** 437 (1967).
16. Moore, K. E.: J. Pharmacol. exp. Ther. **144,** 45 (1964).
17. Moore, K. E., Rech, R. H.: J. Pharm. Pharmacol. **19,** 407 (1967).
18. Nagatsu, T., Levitt, M., Udenfriend, S.: J. Biol. Chem. **239,** 2910 (1964).
19. Orloff, J. J., Williams, H. L., Pfeiffer, C. C.: Proc. Soc. exp. Biol. (N. Y.) **70,** 254 (1949).
20. Palm, D., Grobecker, H.: Experientia (Basel) **24,** 467 (1968).
21. Pfeifer, A. K., Vizi, E. Sz., Sátory, É.: Biochem. Pharmacol. **11,** 397 (1962).
22. Pfeifer, A. K., Galambos, É., György, L.: J. Pharmacol. **18,** 254 (1966).
23. Pfeifer, A. K., Galambos, É.: Arch. int. Pharmacodyn. **165,** 201 (1967).
24. Pfeifer, A. K., Pataky, I., Sátory, É., Vértes, P.: Arch. int. Pharmacodyn. **127,** 44 (1960).
25. Randrup, A., Munkvard, J.: Nature (Lond.) **211,** 540 (1966).
26. Sanan, S., Vogt, M.: Brit. J. Pharmacol. **18,** 109 (1962).
27. Schöne, H., Lindner, E.: Arzneimittel-Forsch. **10,** 583 (1960).
28. Smith, Ch. B.: J. Pharmacol. exp. Ther. **147,** 96 (1965).
29. Spector, S., Sjoerdsma, A., Udenfriend, S.: J. Pharmacol. exp. Ther. **147,** 86 (1965).
30. Stolk, J. M., Rech, R. H.: J. Pharmacol. exp. Ther. **158,** 140 (1967).
31. Sulser, F., Owens, M. L., Norwich, M. R., Dingell, J. V.: Psychopharmacologia (Berl.) **12,** 322 (1968).

32. Sulser, F. Owens, M. L., Dingell, J. V.: Life Sci. **5**, 2005 (1966).
33. Van Rossum, J. M., Van Der Schoot, J. B., Hurkmans, J. A. Th. M.: Experientia (Basel) **18**, 229 (1962).
34. Van Rossum, J. M., Hurkmans, J. A. Th. M.: Int. J. Neuropharmacol. **3**, 227 (1964).
35. Vizi, E. Sz.: Magyar Farmakológiai Társaság Symposium. 1965.
36. Weissman, A., Koe, B. K.: Life Sci. **4**, 1037 (1965).

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EFFECT OF HYDROCORTISONE ON GROWTH IN VITRO OF FIBROBLAST-LIKE (FL) CELLS DERIVED FROM RAT ORGANS

By

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Cultures of fibroblast-like (FL) cells derived from rat thymus, bone marrow and heart muscle have been studied for growth, cellular division and cellular density in the growth zones in response to (a) exposure to hydrocortisone for one week; (b) exposure to the combined effect of hydrocortisone and demecolcin for one week; (c) short exposure to demecolcin. (1) The number of mitoses diminished in response to hydrocortisone and significantly increased under the joint influence of hydrocortisone + demecolcin while the cellular population density per surface area unit diminished. (2) The cells derived from heart muscle exhibited a different response pattern from those of the two other organs, all of the treatments having failed to elicit any response in the number of dividing cells. (3) On the evidence of histochemical studies none of the cultures revealed any 11-β-hydroxysteroid dehydrogenase activity. Thus, hydrocortisone seemed to influence the duration of individual phases of cellular life rather than the number of dividing cells, furthermore, the increase in the number of dividing cells in the hormone + demecolcin-treated cultures is unrelated to synergism.

The influence of glycocorticoids on cell cultures is intimately linked with the controversial issue of their antimitotic effect. While certain authors [12, 16] confirm this possibility, others [5, 15] not only reject it but even ascribe a mitosis-stimulating effect to glycocorticoids. These contradictions have various sources, for instance, different steroid derivatives were used in the experiments or the cultured fibroblast-like (FL) cells had been derived from different organs. Other possible sources of contradiction may be related to the magnitude of the doses together with other, additional factors: while in the presence of colchicine the rate of mitosis of L-cells adhering to an inert surface was not affected by 0.2 to 2.0 μg/ml or less than 0.005 μg/ml glucocorticoid (prednisolone-21-hemisuccinate-Na), the rate definitely increased when 0.005 to 0.2 μg/ml had been applied [14]. Examining suspension cultures derived from similar cell strains SEIFERT and HILZ [16] found 0.36 μg/ml cortisol to be inhibitive to mitosis in the absence of colchicine. Thus, on these grounds, at least as far as L-cells are concerned, inhibition of mitosis by steroids can be suspended by the use of poisons affecting mitosis. LETTRÉ et al. [8, 9] even claim the steroid hormones to synergise the effect of colchicine.

At any rate, if there exists a synergism of this kind, it is not clear on which types of FL cell it takes effect, and we also ignore the causes of this stimulative mechanism.

The aim of the present study was to examine the response of FL cells derived from different rat organs to the joint effect of steroid hormones and mitosis poisons.

Material and method

Tissue samples of 1 cu. mm obtained from thymus and femoral bone marrow of male albino Wistar rats weighing between 80 and 90 g and from the heart of newborn rats were explanted under aseptic conditions on coverslips mounted with a coagulate prepared from chick plasma and embryo extract, five tissue portions being placed on each coverslip. The preparations were then placed in Leighton (Bellco) tubes. 40 tubes were prepared from each organ. No fluid was added to the cultures for 24 hours, then 2 ml nutrient was added and changed daily. The nutrient was composed of 80% TC medium 199 (Difco), 20% heat-inactivated calf serum and 200 IU/ml penicillin. After the fourth day of explantation, for one week 20 culture tubes of each organ were treated with hydrocortisone, adding approximately 0.5 μg to each ml of the nutrient. On the 11th day all cultures were processed. 3 hours prior to processing 1 μg per ml nutrient of demecolcin (Colcemid, Ciba) was added to 10 tubes of each cortison-treated group, and to 10 tubes of each untreated group. In this manner we had four culture-types of each individual organ, i.e. I. untreated controls (IOO); II. demecolcin-treated (IIOC); III. steroid-treated (IIISO); and IV. steroid + demecolcin-treated (IVSC) groups. The cultures were processed in the following manner. (a) The coverslips were removed from the Leighton-tubes, rinsed in physiological saline, fixed in methanol for 10 minutes and stained with Giemsa solution (6 tubes per group); (b) demonstration of 11-β-hydroxysteroid-dehydrogenase [1] (3 tubes per group); (c) demonstration of nonspecific esterase and acid phosphatase activities by combined reaction [2] (one tube per group). For the quantitative estimation of hydrocortisone, fluorometry was used [6]. The zones of growth were measured by means of a calibrated ocular micrometer.

Results

By the 2nd or 3rd day following explantation growth of FL cells was noticeable in practically every tube. The cells were found to grow radially from nearly all implants. Non-adhering, floating or degenerating cells were removed when changing the nutrient for the first time. Owing to the vigorous growth in the untreated tubes (IOO) the period of hydrocortisone treatment was limited to seven days when the zones of growth, even in the untreated cultures, could not yet have attained the margins of the slides. In the growth of the demecolcin-treated explants (IIOC) no divergence whatsoever from the controls was noted. As regards the hydrocortisone-treated groups (IIISO and IVSC) the zones of growth of thymus and bone marrow cultures were generally smaller, those of heart muscle cell cultures slightly larger, than those of the untreated controls. While, however, the decrease in the zones of growth of thymus and bone marrow cultures attained the level of mathematical significance, the increase in those of heart muscle cultures failed to do so (Table I).

No significant change in cellular size and shape was noted. However, in the cellular population density of hydrocortisone-treated cultures, definite changes were manifest. This was particularly so in bone marrow cultures, where the number of cells was nearly 33% less than in the controls. The other two organ cultures in groups IIISO and IVSC also revealed a loss of density of FL-cells, this was, however, just at the borderline of significance (Table II).

Table I

Effect of treatments on the zones of growth

Culture	Type of treatment	Number of cultures	Zone of growth in sq.mm	Significance per cent
Thymus	IOO	25	30.1±3.0	$p < 0.1$
	IIISO	30	16.3±1.6	
Bone marrow	IOO	29	20.9±1.2	$p < 0.1$
	IIISO	30	13.9±1.0	
Heart	IOO	29	18.9±1.7	$10 < p < 25$
	IIISO	29	22.1±1.7	

Table II

Cellular population density per surface area unit

Culture	Type of treatment	Number of 0.05 sq.mm areas	Mean number of cells of the areas	Mean number of cells per cent per 1 sq.mm	Significance per cent
Thymus	IOO	50	13.68±0.76	273.6	$2.5 < p < 5$
	IIISO	50	11.74±0.69	234.8	
Bone marrow	IOO	45	14.71±0.75	294.2	$p < 0.05$
	IIISO	50	10.54±0.61	210.8	
Heart	IOO	50	14.14±0.72	282.8	$1 < p < 2.5$
	IIISO	50	12.32±0.51	246.4	

The number of dividing cells revealed significant changes which were related, apart from the type of treatment, also to the origin of the cells. The mitotic index of untreated FL cells derived from thymus fell to 50% in response to hydrocortisone treatment. Demecolcin when applied alone, caused a threefold, when combined with steroid, a sixfold rise in the mitotic index (Table III). The response of bone marrow cultures to the individual types of treatment were similar, with the exception of combined treatment (IVSC) which had failed to elicit an increase in the number of mitoses comparable to that attained in thymus cell cultures. The FL cell cultures derived from heart tissue revealed practically no change in the number of mitoses.

The hydrocortisone content of the discarded nutrient was checked daily. It ranged from 0.41 to 0.45 μg/ml against the theoretical 0.5 μg/ml. Presuming that a prolonged presence of hydrocortisone would result in the activation or induction of 11-β-hydroxysteroid dehydrogenase responsible for cortisol-cortisone-conversion, it was attempted to demonstrate this enzyme histochemi-

Table III

Number of dividing cells in the zones of growth

Culture	Type of treatment	Number of cells	Total number of mitoses	Mitotic index
Thymus	IOO	10 000	83	8.3
	IIOC	10 000	230	23.0
	IIISO	10 000	38	3.8
	IVSC	7 000	346	49.4
Bone marrow	IOO	10 000	70	7.0
	IIOC	10 000	156	15.6
	IIISO	10 000	32	3.2
	IVSC	10 000	188	18.8
Heart	IOO	10 000	37	3.7
	IIOC	7 000	31	4.4
	IIISO	10 000	19	1.9
	IVSC	10 000	35	3.5

cally. The cytoplasm of the FL-cells revealed, however, no sign of enzyme activity in any of the cultures.

The lysosomal enzymes were studied by demonstrating acid phosphatase and nonspecific esterase activity, using the same slide for the two procedures. The hormone-treated cultures of a sparser cell population gave the impression of containing more esterase-active cells and of displaying a fainter acid-phosphatase activity.

Discussion

The mechanism of action of steroid hormones in vertebrates is still obscure. Recent biochemical evidence [17] indicates that certain steroid derivatives, including hydrocortisone, act as de-repressors, by coupling with repressing histones and thus enabling particular sections of DNA to transmit information, i.e. to direct RNA-synthesis. The assumption that this de-repressing activity affected the DNA portions responsible for the initial phase of DNA-synthesis, would lead directly to the possibility of reduplication being triggered off. It has in fact been demonstrated that hormone treatment of epithelial cells resulted in a 15% increase in their DNA content [3].

In the present experiments a considerable increase in the number of dividing cells was found in the cultures in the case of combined hydrocortisone and demecolcin treatment as compared to the cultures treated with demecolcin alone (particularly as regards the cells of thymic origin). This might mean

that under the influence of hormone treatment a greater number of cells had started to divide, a supposition seemingly inconsistent with the observation that the number of mitoses had actually diminished in the exclusively hormone-treated cultures. This could be explained only by assuming that hormone treatment had shortened the duration of mitosis, since it is known that the number of dividing cells per time unit had definitely increased. On the grounds of these considerations the cultures would have to present a greater number of cells per surface area unit in response to hormone demecolcin treatment. Yet, the contrary is true, namely the number of cells definitely diminished in the growth zones of the cultures of all organs. The only explanation of this finding is that cellular destruction had occurred. We still ignore, however, whether the cells had been destroyed during the interphase or during mitosis, or, else, whether destruction had affected the daughter cells emerging from cellular division. Analysis of the nutrient medium might help to clear this point.

The present results are in agreement with the findings of LETTRÉ et al. [8, 9]. In our view, however, the colchicine derivative used by these authors as well as by ourselves results theoretically as well as practically in an arrest of cell division in the metaphase. Consequently, the increase in the number of cell divisions subsequent to the combined use of steroid and demecolcin cannot be the result of a synergism. Though colchicine and its derivatives do have an influence on the phase of DNA-synthesis [7, 11], this could not have affected the number of mitoses under the present experimental conditions where the effect of demecolcin had been limited to 3 hours.

Prolonged administration of cortisol to mice considerably enhanced the activity of 1-β-hydroxysteroid dehydrogenase, the enzyme responsible for cortisol-cortisone conversion [4]. Our histochemical method failed to demonstrate an enzyme activity in any of the preparations and the hormone added to the cultures was recovered practically *in toto* on renewal of the nutrient medium. This leaves us with two alternatives. Either the time was too short for activation or induction of the said enzyme or else the histochemical method was unsuited for the demonstration of 1-β-hydroxysteroid dehydrogenase activity under the present experimental conditions. This negative finding was the more puzzling as there is evidence [10] that steroid hormones are apt to induce alkaline phosphatase activity. As regards the two lysosomal enzymes acid phosphatase and nonspecific esterase, our failure to demonstrate any inhibition in their activities is in agreement with the findings of RASCHE et al. [13].

A comparison of the responses of cells derived from different organs, namely thymus versus bone marrow, or thymus and bone marrow versus heart, also revealed some differences. Intensity of growth or of cellular division of FL cells derived from heart muscle was unaffected by hormone treatment. On the evidence of the findings of COX and MACLEOD [3] who had studied

the effect of prednisolone on human epithelial cell lines and on fibroblasts, inhibition of cellular growth and division was confined to the epithelial elements. Since it is hardly possible that our thymus and bone marrow cultures should have yielded a cellular growth of exclusively epithelial origin but assuming the shape of fibroblasts, we must accept another, more plausible explanation, namely that the relationships between hormone and target organ become manifest *in vitro* too, it being known that glucocorticoid prevalence results in thymic involution *in vivo*.

*

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REFERENCES

1. Barka, T., Anderson, P. J.: Histochemistry. Theory, practice, and bibliography. Harper Row, New York 1963.
2. Bácsy, E., Vadász, Gy., Fazekas, I., Rappay, Gy.: Morph. és Ig. Orv, Szemle **8**, 216 (1968).
3. Cox, R. P., MacLeod, C. M.: J. gen. Physiol. **45**, 439 (1962).
4. Dougherty, Th. F., Berliner, M. L., Berliner, D. L.: Endocrinology **66**, 550 (1960).
5. Farnes, P., Barker, B. E.: Metabolism **14**, 75 (1965).
6. Guillemin, R., Clayton, G. W., Lipscomb, H. S., Smith, J. D.: J. Lab. clin. Med. **53**, 830 (1959).
7. Hell, E., Cox, D. G.: Nature (Lond.) **197**, 287 (1963).
8. Lettré, H., Lettré, R., Pflanz, Ch.: Naturwissenschaften **38**, 70 (1951).
9. Lettré, H., Lettré, R., Pflanz, Ch.: Naturwissenschaften **38**, 214 (1951).
10. Melnykovych, G., Bishop, C. F.: Endocrinology **81**, 251 (1967).
11. Rappay, Gy., Csemniczky, F.: Biol. Közl. **16**, 3 (1968).
12. Rasche, B., Leder, L. D., Ulmer, W. T.: Z. ges. exp. Med. **144**, 322 (1967).
13. Rasche, B., May, G., Ulmer, W. T.: Z. ges. exp. Med. **144**, 335 (1967).
14. Rasche, B., Ulmer, W. T.: Z. Zellforsch. **84**, 506 (1968).
15. Rhode, B., Schreiner, H. E., Vogel, A.: Arzneimitt.-Forsch. **11**, 392 (1961).
16. Seifert, R., Hilz, H.: Acta endrocr. (Kbh.) **53**, 189 (1966).
17. Stackhouse, H. L., Chetsanga, C. J., Tan, C. H.: Biochim. biophys. Acta (Amst.) **155**, 159 (1968).

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РЕЗЮМЕ

АРТЕРИО-ВЕНОЗНЫЕ ШУНТЫ ПОЧЕК

П. ГЁМЁРИ

Дается обзор многолетних исследований автора, проводившихся в целях уяснения артерио-венозных шунтов почек. В первой серии опытов автор с сотрудниками успешно воспроизводил эксперименты *Трюэта* и выявил, что при возникновении шунта Трюэта функция почек ухудшается, а клубочковая фильтрация, диурез и *Tm*ПАГК уменьшаются. В последующих исследованиях, проводившихся коррозионным методом, удалось выявить, что в состоянии шока артериовенозные соединения почек морфологически хорошо выявляемы. В последней же серии экспериментов было предоставлено функциональное доказательство артерио-венозных шунтов почек.

НОВЫЕ РЕЗУЛЬТАТЫ ИССЛЕДОВАНИЯ АНДРОГЕННОГО СТЕРОИДОГЕНЕЗА В ЧЕЛОВЕЧЕСКОЙ КОЖЕ

М. ЮЛЕС

Исходя из гипотезы, что гирсутизм связан с ферментативными системами кожи или ее придаточных образований, автор с сотрудниками приступили к изучению соединений со стерановым остовом в коже и в различных волосах. В докладе, прочитанном в Академии Наук Венгрии (1965 г.), а также на ряде международных конгрессов автор уже сообщил о том, что в человеческой коже был выявлен хромоген Циммермана, и что 1/5-ая часть его оказалась кетоновым хромогеном Циммермана. Существенная разница была выявлена между вытяжками кожи, покрытой волосами и кожи без волос, в пользу волосатой кожи. При распространении исследований на волосы выяснилось, что в человеческом оволосении, в частности в подмышечных волосах, имеется ряд 17-кетостероидов, и что главным образом подмышечные волосы содержат значительное количество дегидроэпиандростерона. Автору впервые в мировой литературе удалось доказать гистологическими исследованиями, что в человеческой коже можно выявить активность $\Delta 5—3\beta$-гидрокси-стероид-дегидрогеназы. Исходя из этого факта, в вытяжках волос удалось доказать андрост-4-ен-3,17-дион и андростан-дион.

При продолжении экспериментов были получены следующие результаты:

1. Из волос с помощью ряда методов замочки удалось экстрагировать стероиды без их разрушения.

2. Преобладающая часть 17-кетостероидов присутствует в волосах в форме эфира сульфата.

3. При инкубации кусков кожи радиоактивными стероидами оказалось, что человеческая кожа интенсивно метаболизирует радиоактивный дегидроэпиандростерон.

4. При количественных исследованиях выяснилось, что при пересчете на 1 г кожи кожа мужчин способна синтезировать в два с половиной раза больше андростерона, чем кожа женщин. В то же время оба вида кожи синтезируют один из важных исходных веществ двух андрогенных стероидов (андрост-4-ен-3,17-дион) с одинаковой радиоактивностью. Эти исследования показывают, что из биологически менее активных стероидов не только внутрисекреторные органы, но и кожа способна синтезировать стероиды со значительной биологической активностью.

5. Кожа способна преобразовывать дегидроэпиандростерон в сульфат дегидроэпиандростерона.

6. При инкубации кусков кожи лобка мужчины с агонадизмом было установлено, что из дегидроэпиандростерона и эта кожа способна синтезировать андростерон со значительной радиоактивностью и тестостерон с небольшой радиоактивностью. В инкубате до сих пор удалось идентифицировать 11 радиоактивных метаболитов.

7. В ходе проведения экспериментов по инкубации с радиоактивным андростендионом было установлено, что при таких экспериментальных условиях также образовываются андростерон и тестостерон, и что соотношение двух стероидов, в случае инкубации нормальной кожи, почти одинаковое.

8. При инкубации кожи больных гирсутизмом это соотношение изменяется: количество андростерона в два раза превышает количество тестостерона. При гирсутизме количество двух стероидов больше, чем у здоровых женщин.

9. Человеческий подмышечный пот содержит значительное количество эфира 17-кетостероид-сульфата, преимущественно радиоактивный сульфат дегидроэпиандростерона и сульфат андростерона.

На основе экспериментальных данных предполагается, что у части больных с так наз. идиопатическим гирсутизмом речь идет об энзимопатии кожи.

ПАТОГЕНЕЗ, ВЫЗЫВАЕМЫЙ СУЛЕМОЙ ПОЧЕЧНОЙ НЕДОСТАТОЧНОСТИ

П. БАЛИНТ

После внутривенного введения 2 мг на кг сулемы были определены параметры функции почек.

1. Количество крови, протекающей через почки, уменьшается в такой незначительной мере, что ишемию почек нельзя считать ответственной за развитие недостаточности почек.

2. При развитии почечной недостаточности решающую роль играет значительное уменьшение клубочковой фильтрации. Добавочной роли тубулярной редиффузии нельзя исключить.

3. Гипостенурия или астенурия являются вторичными последствиями уменьшения фильтрации, и они способствуют обострению симптомов почечной недостаточности.

4. Резкое уменьшение секреции и экстракции ПАГК обуславливаются обширным некрозом эпителия канальцев.

ЛИМФОГЕННАЯ ЭНЦЕФАЛОПАТИЯ — ЛИМФОСТАТИЧЕСКАЯ ЦЕРЕБРАЛЬНАЯ ГЕМАНГИОПАТИЯ

М. ФЁЛЬДИ

1. В противоположность теории, встречаемой в учебниках, согласно которой лимфообращение не участвует в сокообращении мозга, автор выявляет, что механическая недостаточность шейного лимфообращения вызывает лимфостатическую энцефалопатию.

2. Было установлено также, что механическая недостаточность шейного лимфообращения вызывает лимфостатическую гемангиопатию.

3. Сообщается новая теория о предлимфатическом дренаже жидкостей, происходящем в стенках кровеносных сосудов мозга.

4. Выдвигается представление о патомеханизме возникновения лимфостатической энцефалопатии.

5. Обсуждается совместная встречаемость лимфостатической энцефалопатии и известных неврологических заболеваний.

ИССЛЕДОВАНИЕ СОКРАТИМОСТИ И ОБЩЕГО ОБМЕНА ВЕЩЕСТВ МИОКАРДА ПРИ ДЕКОМПЕНСАЦИИ СЕРДЦА

Дь. ГАБОР, Б. КЕНЕЗ и Я. ШИМОНЬИ

Авторы исследовали изменения сократительной силы и обмена веществ миокарда у больных с недостаточностью левой половины сердца при декомпенсации и при восстановлении компенсации. Изменения сократительной силы миокарда они определили на основе первой производной кривой пульса сонной артерии (dc/dt), а изменения обмена веществ — на основе показателя давления-времени.

У больных, у которых удалось восстановить компенсацию, dc/dt повысилась, а показатель давления-времени уменьшился, значит сократительная сила миокарда повысилась при одновременном снижении его потребности в обмене веществ. В отдельных случаях повысились и обмен веществ и сократительная сила, однако, у этих больных не удалось восстановить компенсации. В других случаях повышения сократительной силы не наблюдалось, эти больные умерли.

Из результатов исследований авторы делают вывод, что под влиянием лечения наперстянкой вначале уменьшается обмен веществ, а затем следуют увеличение сократительной силы и восстановление компенсации.

ДЕЙСТВИЕ ПРЕДНИЗОЛОНА НА РАЗВИТИЕ АТОФАНОВОЙ ЯЗВЫ У СОБАКИ

А. ХАМОРИ, Т. НЕМЕШ и Т. ХАЛ

Применение терапической дозы преднизолона на протяжении трех недель у собак вызывает лишь у 20% животных изъязвление. Поражение поверхностное и наибольший диаметр не превышает 1 см. Экспериментальные результаты позволяют надеяться, что человеческую язву от преднизолона можно будет установить вовремя, если каждые три недели проводить рентгеновское исследование желудка больного.

Преднизолон в значительной мере способствует прогрессированию атофановой язвы. В некоторых случаях возникают гигантские язвы, напоминающие гигантские язвы старческого возраста.

Одной из предположительных причин ульцерогенного действия преднизолона является иатрогенная недостаточность надпочечников.

Гигантские язвы не сопровождаются гастритом, тогда как в группе, получившей исключительно лишь преднизолон, развивается более-менее тяжелый гастрит, в большинстве случаев без образования язвы. Следовательно, пептическая язва и гастрит обуславливаются двумя независимыми друг от друга механизмами.

ЭКСПЕРИМЕНТАЛЬНОЕ ИССЛЕДОВАНИЕ ПРЕПАРАТОВ ПЕЧЕНИ

И. МАДЬЯР

Эффективность препаратов печени, используемых в терапии хронических заболеваний печени, была исследована в опытах на животных. Эти вытяжки на основе гистологического исследования и определения задержки бромсульфолеина при отравлении четыреххлористым углеродом, по-видимому, оказывают лечебное действие, и их профилактическая дача также понижает степень тяжести поражения четыреххлористым углеродом. Лечебное действие выявляемо равным образом как в остром, так и в хроническом эксперименте и проявляется, прежде всего, в повышении регенерации печени. Это последнее действие выявляемо и при экспериментальном циррозе печени.

Действие препарата, изготовленного из пораженной четыреххлористым углеродом и, предположительно, регенерирующейся печени, более эффективное, чем действие препарата из невредимой печени. Этот экспериментальный результат доказывает существование гуморального фактора, способствующего регенерации печени.

Ввиду того, что стимулирующее регенерацию печени действие, на основе определенных размышлений, не является безусловно выгодным эффектом, исследовался вопрос, оказывают ли препараты печени влияние и на обмен веществ печени. В опытах in vivo и in vitro, в острых и хронических экспериментах равным образом можно доказать, что препарат печени (Солкогепсил) увеличивает потребление кислорода срезами невредимой и патологической печенью.

ОТРАВЛЕНИЕ ЖИРНОЙ КИСЛОТОЙ, КАК ВОЗМОЖНЫЙ ВЫЗЫВАЮЩИЙ ФАКТОР СИНДРОМА ЖИРОВОЙ ЭМБОЛИИ

Дь. САБО, Ж. МАДЬЯР и Р. ЯНКОВИЧ

1. У кроликов летальная доза свободной жирной кислоты не является существенно большой, если она вводится на протяжении более длительного времени при распределении на несколько доз. Значит, *токсическое действие* ее кумулируется.

2. После введения 0,50 мл триолеина на кг веса в легких не наблюдается повышения активности тканевой липазы. Однако в тканях и в норме выявляема очень высокая активность липазы.

3. После введения такой же дозы ни в плазме крови, ни в отдельных тканях (легкие, головной мозг) нельзя выявить повышения содержания свободной жирной кислоты.

4. После внутривенного введения дозы в 0,15 мл на кг веса свободная жирная кислота очень быстро исчезает из кровообращения, и ее большая часть попадает в легкие. Содержание жирной кислоты в тканях, однако, быстро уменьшается и вскоре возвращается к норме.

5. Внутривенно введенная свободная жирная кислота и неэмульгированный нейтральный жир вызывают в легких очень значительный отек и сильную воспалительную реакцию. Однако, после введения свободной жирной кислоты реакция возникает практически моментально, в то время как после введения нейтрального жира реакция возникает значительно позже, когда преобладающая часть введенного жира уже исчезла из легких.

6. Легочная реакция в существенной мере ухудшает газообмен, вернее уменьшает насыщение артериальной крови кислородом.

7. Описанные при человеческом синдроме жировой эмболии вторичные изменения легких, по всей вероятности, вызываются свободной жирной кислотой, медленно и постепенно освобождающейся из нейтрального жира, эмболизованного в легких.

8. Эти изменения легких являются ответственными за артериальную гипоксию, представляющей собой наиболее важный вызывающий фактор синдрома жировой эмболии.

РЕНТГЕНОКИНЕМАТОГРАФИЧЕСКИЙ АНАЛИЗ БРОНХИАЛЬНЫХ ДВИЖЕНИЙ

Т. СЕНЕШ

Рентгенокинематография открыла широкую перспективу исследования закономерностей и анализа механизма двигательных функций перед научно-исследовательской работой и для диагностических целей.

Настоящая работа поставила перед собой целью на основании опытов на животных при помощи рентгенокинематографии исследовать респираторные двигательные явления трахеобронхиальной системы. Было установлено, что закономерности этих движений, характер физиологического или патологического их течения могут быть с поразительной подробностью выявлены анализом рентгенокинематограммы. Нужно подчеркнуть, что отнюдь не достаточно повторно наблюдать явления движения на кинофильме, а непременно нужно на отдельных увеличенных кадрах путем измерения изменений длины, калибра, положения бронхов и т. д. произвести метрический анализ сконструированных таким образом кривых движения.

УЛЬТРАСТРУКТУРНЫЕ И ФЕРМЕНТАТИВНО-ЦИТОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ, НАБЛЮДАЕМЫЕ В ЗОБНОЙ ЖЕЛЕЗЕ КРЫСЫ ПОСЛЕ РЕНТГЕНОВСКОГО ОБЛУЧЕНИЯ

И. ТЁРЁ, Э. БАЧИ, И. ЭКРЁШ, ДЬ. ВАДАС и ДЬ. РАППАИ

В зобной железе крыс, облученных сублетальной дозой рентгеновских лучей, были проведены цитохимические исследования под световым и в электронном микроскопе, а также исследования эстеразы изозима с помощью метода электрофореза на крахмальном геле.

Было установлено, что ретикулярные клетки зобной железы показывают в различном соотношении активность кислой фосфатазы и неспецифической эстеразы. При помощи цитохимических методов были получены данные, указывающие на то, что после облучения в ретикулярных клетках соотношение активностей двух энзимов в сущности не изменяется, повышается, однако, их интенсивность.

При исследовании методом электрофореза на крахмальном геле удалось выявить, что как плотные тельца (dense body), обнаруживаемые в мезенхимальных ретикулярных клетках, так и вакуоли эпителиальных ретикулярных клеток, содержимое которых напоминает нитки жемчуга, показывают активность кислой фосфатазы и, следовательно, их можно рассматривать как лизосомы.

После облучения животных рентгеновскими лучами в мезенхимальных ретикулярных клетках зобной железы выявляемо значительное накопление липоидов.

ЭФФЕКТ АКТГ НА КРОВОТОК В ЯИЧНИКАХ И НАДПОЧЕЧНИКАХ С ОСОБЫМ ВНИМАНИЕМ НА ВНЕНАДПОЧЕЧНОЕ ДЕЙСТВИЕ

Э. ШТАРК и Б. ВАРГА

Измерения с помощью нагретых термоэлементов показали, что в первые 1—2 минуты после внутривенного введения АКТГ повышается кровоток в яичниках и надпочечниках собак. Тот факт, что синтетический АКТГ вызывает такой эффект также и в яичниках собак, лишенных надпочечников, доказывает, что это—вненадпочечное действие. При даче АКТГ ненаркотизированным собакам в виде вливания, даже небольшие дозы гормона оказывают вышеописанное действие, указывая на то, что оно существует и в физиологических, и в частности, в патологических условиях. Изучение механизма действия, распространяющееся на некоторые вазоактивные вещества, указало на возможность того, что здесь гистамин играет роль медиатора.

Исследование предположительной роли синтеза «белка с быстрым обменом» при помощи циклогексимида, — как известно, тормозящего этот процесс, — доказало, что АКТГ вызывает по крайней мере некоторое повышение кровотока, независимо от действия, оказанного на синтез кортикостероидов; кровоток продолжает повышаться еще и после того, как секреция кортикостероидов прекращает усиливаться, причем циклогексимид приостанавливает или уменьшает эти два эффекта с определенным отставанием во времени. АКТГ вызывает также у кошки повышение кровотока в яичниках.

Измерения методом Сапирштейна с помощью радиоактивного рубидия показали, что АКТГ повышает яичниковый кровоток также у хомяка и, хотя лишь в незначительной мере, у крысы.

Обсуждается физиологическое значение этого вненадпочечного эффекта АКТГ.

НЕСКОЛЬКО ДАННЫХ К СРАВНИТЕЛЬНОЙ ЭЛЕКТРОННОЙ МИКРОСКОПИИ КОСТНОГО МОЗГА ПОЗДНИХ ЗАРОДЫШЕЙ И ВЗРОСЛЫХ КРЫС

Б. БУКУЯ и А. БАЛАЖ

С помощью методов световой и электронной микроскопии (фиксирование: альдегидом глютаровой кислоты + осмием; заливка: Дуркупан АСМ; контрастирование: ацетатом уранила + лимоннокислым свинцом) авторы сравнивали цитологию костного мозга 21-дневных эмбрионов и молодых крыс весом в 80—120 г породы Вистар.

Было установлено, что к концу внутриутробной жизни, по сравнению с взрослыми животными

1. наблюдается высокая доля недифференцированных элементов (stem cell, гемоцитобласты), мегакариоцитобластов и базофильных гранулоцитов, а доля эозинофильных клеток более низка;

2. в миелоидном ряде *зрелые* гранулоциты и базофильные клетки показывают подобную структуру, а промежуточные *прекурсоры* отличаются от послеродового состояния (наряду со сравнительно многочисленными митохондриями и ядрами средней зрелости наблюдается небольшое число зернышек небольшого размера, цитоплазма показывает плотный основной тонус).

3. Эмбриональные костномозговые клетки в *общем* содержат больше митохондриев, чем элементы подобного типа взрослых животных.

Для более точного уяснения срока начала миелопоэза и эндогенной репопуляции необходимо проводить электронномикроскопическое исследование костного мозга и крови более молодых (16—20-дневных) эмбрионов крыс.

ДЕЙСТВИЕ ОДНОКРАТНОЙ БОЛЬШОЙ ДОЗЫ ГЛИКОКОРТИКОИДОВ, ВВЕДЕННОЙ НОВОРОЖДЕННЫМ, НА СИСТЕМУ ЛИМФЫ ЗОБНОЙ ЖЕЛЕЗЫ И ВНУТРИСЕКРЕТОРНЫХ ОРГАНОВ, И НА РОСТ У КРЫСЫ И СОБАКИ

Й. ФАХЕТ, Э. ШТАРК и М. ПАЛКОВИЧ

Единственная большая доза гликокортикоидов, введенная через сутки после рождения, вызывает у собаки и крысы фатальную кахексию. Это состояние сильно напоминает «wasting syndrom», наблюдаемый после тимектомии у новорожденных. Вес тела, далее вес зобной железы, селезенки и надпочечников оказались меньшими у животных, выживших после инъекции, а число лимфоцитов был явно пониженным, как в лимфатических органах, так и в крови. Гомологический трансплантат кожи приживал на протяжении значительно более длительного времени и реакция отталкивания была более слабой. На четвертой неделе жизни общий уровень протеинов и трех гаммаглобулиновых фракций сыворотки был пониженным. Повышение уровня кортикостерона крови, вызванное стрессом, было у 4-недельных крыс меньшим, чем у контрольных животных подобного возраста.

В возникновении фатальной кахексии, вызванной введением гликокортикоидов новорожденным, первичная важная роль приписывается нарушению функции зобной железы, однако, не следует упускать из виду ни прямые действия гликокортикоидов, оказанные на прочие периферические лимфатические и внутрисекреторные органы, а также на другие органы.

ДЕЙСТВИЕ И ТОЧКА ПРИЛОЖЕНИЯ ДЕЙСТВИЯ БЛОКАТОРОВ АДРЕНЕРГИЧЕСКИХ НЕЙРОНОВ

Л. ДЬЁРДЬ, М. ДОДА, Е. МОЛНЬАР и К. НАДОР

Сообщаются данные фармакологического исследования полученного в результате многолетней работы избирательного блокатора адренергических нейронов: бромида N-циклогексилбензил-3α-гранатания (N-1024). На основе сопоставления литературных данных с результатами собственных исследований авторов тирамино- и кокаиноподобный, вызывающий выделение адреналина и имеющей различные точки приложения действия антихолинергический эффекты блокаторов адренергических нейронов не представляют собой безусловных сопутствующих явлений активности блокаторов адренергических нейронов. Возвращаясь к первоначальной теории *Хея* и *Уиллея*, а также *Бура* и *Грина*, авторы рассматривают блокаторы адренергических нейронов как соединения, напоминающие местоанестезирующие средства, и оказывающие действие на специальные мембраны.

О СОСТАВЕ ЛИПИДОВ В ЭРИТРОЦИТАХ

С. Р. ХОЛЛАН и Ю. Х. БРЕЙЕР

Изменения состава липидов в эритроцитах были изучены (1) в процессе онтогенеза, (2) под влиянием безжирной диеты и (3) при некоторых заболеваниях с расстройством обмена веществ.

Холестерол, TEFA, липидный P, группы фосфолипидов и общий состав фосфолипидных жирных кислот в эритроцитах были изучены в целях выяснения изменений, происходящих в процессе онтогенеза, изменений, вызываемых безжирной диетой, и наблюдаемых при эссенциальной и вторичной гиперлипидемии, а также при гиполипидемии вследствие акантоцитоза.

Постоянство липидов в эритроцитах при эссенциальной гиперлипидемии и пределы размера и типа изменений, вызываемых в эритроцитах посредством диеты, указывают на то, что в возникновении изменений участвуют не относительные изменения любого компонента диеты, плазмы или жировой ткани, а другие факторы регуляции.

Обращается внимание на поразительные сходства, наблюдаемые в составе жирных кислот фосфолипидов в зародышевых клетках, в эритроцитах при недостаточности эссенциальных жирных кислот, при акантоцитозе и при некоторых гемолитических заболеваниях. Предполагается, что сходство липидной структуры, проницаемости для неэлектролитов и жизнеспособности этих эритроцитов обуславливается недостаточностью эссенциальных жирных кислот вследствие безжирной диеты, вторичной плохой абсорбцией при акантоцитозе и вторичной местной недостаточностью жирных кислот в условиях повышенной потребности в эритроцитах. При таких условиях происходит эритропоэз «зародышевого типа» с ускорением созревания, который может перескочить несколько фаз деления клеток.

РОЛЬ ЛИМФО- И КРОВООБРАЩЕНИЯ В ПАНКРЕАТИЧЕСКОМ САМОПЕРЕВАРИВАНИИ И РАЗВИТИИ ВНЕПАНКРЕАТИЧЕСКИХ НЕКРОЗОВ

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После вливания J^{131}-альбумина и введения J^{125}-бенгальского красного, — связанного с протеином in vivo — в поджелудочную железу собаки, в лимфе грудного протока были найдены гораздо более высокие концентрации введенных веществ, чем в плазме поджелудочной и воротной вены. В собаке с желчным панкреатитом концентрации липазы и амилазы были также более высокими в лимфе грудного протока, чем в сыворотке воротной вены.

Посредством своей транспортной функции лимфообращение оказывает защитное действие при процессе панкреатического самопереваривания. Определенная опасность, быть может, кроется в факте, что лимфа переносит в кровь в значительной концентрации энзимы, протеины и связанные с протеинами вещества, которые не проходили через печень.

РОЛЬ КАТЕХОЛАМИНОВ В ЦЕНТРАЛЬНЫХ ДЕЙСТВИЯХ АМФЕТАМИНА

А. К. ПФЕЙФЕР, Л. ДЬЁРДЬ и М. ФОДОР

Резерпин и альфа-метил-*m*-тирозин не влияют на повышенную подвижность, вызванную амфетамином, но они в значительной мере понижают стимуляцию обмена веществ амфетамином.

Прениламин и гуанетидин уменьшают оба центральные действия амфетамина.

Между понижением уровня катехоламинов мозга и возможностью воздействия на эффекты амфетамина не наблюдается линейной связи.

Резерпин оказывает тормозящее действие на усиленную подвижность от p-хлороамфетамина.

Независимо от их вызывающего гипермотильность свойства производные амфетамина понижают усиление спазмофилии, вызываемое резерпином и тироксином.

ИССЛЕДОВАНИЕ IN VITRO РОСТА КЛЕТОК, НАПОМИНАЮЩИХ ФИБРОБЛАСТЫ, ПРОИСХОДЯЩИХ ИЗ РАЗЛИЧНЫХ ОРГАНОВ КРЫС, ПОСЛЕ ОБРАБОТКИ ГИДРОКОРТИЗОНОМ

ДЬ. РАППАИ

Автор исследовал на культурах из зобной железы, костного мозга и миокарда крыс рост, способность делиться клеток эксплантатов, напоминающих фибробласты (FL), и в зоне роста эксплантатов густоту клеток после однонедельной обработки культуры гидрокортизоном, а также после обработки гормоном и кратковременным воздействием Кольцемида. Было установлено, что (1) под влиянием гормона число митозов уменьшается, а после совместного добавления гормона и Кольцемида оно существенно повышается. Густота клеток в переводе на единицу площади, однако, уменьшается. (2) Эксплантаты миокарда показали поведение, отличающееся от поведения эксплантатов двух других исследовавшихся органов: по сравнению с контролями число митозов под влиянием любой обработки не изменилось. (3) Гистологическими методами исследования в культурах не удалось доказать активности 11 β-гидроксистероид-дегидрогеназы. Из этих данных можно сделать заключение, что а) гидрокортизон, по всей вероятности, оказывает влияние на отдельные жизненные фазы клеток, а не на число делящихся клеток; б) в культурах, обрабатывавшихся гормоном и Кольцемидом число митозов не повышается в результате синергизма двух средств.

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