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T. BAKÁCS, P. GÖMÖRI, M. JULESZ, I. KÖRNYEY, Ö. RAJKA,
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PERIODIC PAROXYSMAL DISCHARGES FOLLOWING EMOLLITION IN THE AREA OF THE MIDDLE CEREBRAL ARTERY

By

P. HALÁSZ, S. GŐDÉNY and J. HIDASI

DEPARTMENT OF NEUROLOGY AND PSYCHIATRY, UNIVERSITY MEDICAL SCHOOL, DEBRECEN

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In a case of cerebral emollition consequent upon occlusion of the right middle cerebral artery a peculiar alternating EEG activity combining individual periodic paroxysmal discharges with repetitive bursts connected by flat intervals was observed. Owing to their functional features and electro-pathological relationships, the two alternating activities may be related in pattern to the two main types of periodic paroxysmal phenomena, i.e. the periodic complexes of the panencephalitis-type and the suppression burst activity representing the pattern of cortical denervation. These EEG phenomena seem to have one and the same mechanism in which, in addition to hypoxia, the special metabolic state of the denervated cortex constitutes the prevalent factor.

The relationship of abnormal EEG phenomena and the underlying pathologic processes of the brain belongs to the principal lines of clinical EEG research. Various EEG patterns, formerly held to be specific of particular entities, have been found to indicate merely some structural or functional constellation arising as a result of the most diverse processes. The synchron bilateral periodic burst phenomena of various types form one of the classical abnormal EEG patterns.

We have here two main types. The first is characterized by alternating runs of a flat scanty activity and high-voltage bursts of paroxysmal elements. "Suppression burst" activity of this kind may also be seen in healthy individuals in deep barbiturate anaesthesia (DERBYSHIRE et al. [1936]) and in deep hypothermia (WEISS and ARFEL [1960]), in diffuse encephalopathy (LESSE et al. [1958], HOEFER et al. [1963]), in the experimentally isolated cortex (BREMER [1935, 1936], KRISTIANSEN and COURTOIS [1949], BURNS [1951], HENRY and SCOVILLE [1952], ECHLIN [1959]), or elicited by ATPase inhibitors (MACCAGNANI et al. [1966]).

The other type is characterized by periodically returning potential complexes of identical morphology, contrasting with the more or less preserved basic activity. This pattern may occur in sclerosing leucoencephalitis (RADERMECKER [1949], COBB and HILL [1950]) as well as in cerebral lesions of hypoxaemic (PAMPIGLIONE [1962]), hypoglycaemic (DODGE et al. [1959]), or traumatic (ZAPPOLI [1959]) origin, and asymmetrically in cases of cerebral infarction or extensive tumours (CHATRIAN et al. [1964]). The EEG phe-

nomena described in spongious encephalopathy (JONES and NEVIN [1954], MAJTÉNYI and NAGY [1963]) and in cerebral lipidosis (COBB et al. [1952]), and reproducible by means of an ATPase inhibitor (BIGNAMI and PALLADINI [1965]) also belong to this type. In this group the periodic bursts are of greater frequency and have a certain similarity to polyphasic spikes appearing in long series interrupted by shorter isoelectric intervals.

In the case to be reported, occlusion of the middle cerebral artery of one side, resulting in extensive emollition, induced a particular asymmetrical paroxysmal activity characterized by an alternation of the periodic burst phenomena referred to above.

Report of the case

The patient M. I. was a 76-year-old woman admitted for left hemiplegia and impairment of consciousness developing gradually in the course of 24 hours. She had been under treatment for hypertensive disease for years. On admission, blood pressure was 140/80 mm Hg, pulse rate 60/min. No signs of cardiac failure were present. She appeared almost semicomatous. Her pupils were of pinpoint size and failed to respond to light. Neurological examination revealed a conjugate deviation and adversion to the left, complete left spastic hemiparesis with faciobrachial prevalence, increased and abnormal reflexes. A few hours after admission she developed left clonic pedal jerks which ceased in response to 0.2 g of mephenytoin. On the subsequent carotis angiogram the middle cerebral artery filled only over a length of 10 mm of its initial part. The CSF withdrawn on the fourth day after admission was yellowish, the cell count was 14/3, protein 4.0 K.U., Pandy's reaction ++. Consciousness gradually cleared up in a few days, but spastic paresis remained as before. Vasodilators, and anticoagulants were prescribed. Angiography repeated one month later revealed no change. In the third month after the onset of the first symptoms she developed generalized motor seizures, two in succession on the same day, with loss of consciousness. She was given 0.3 g of mephenytoin daily and the seizures did not reappear. The stagnation of her general condition preceding the appearance of the seizures gave now way to a successive deterioration. PEG performed during the fourth month of illness revealed an excessive dilatation of the right ventricular system and pneumatization of the right subarachnoid space.

In the fifth month after admission she died of pneumonia.

EEG studies

Four EEG recordings were made; the first three on three successive days after the occurrence of the two seizures in the third month of the disease, and the fourth one month later, five days before death.

The first three tracings were consistent in respect of their main features. The patient who was cooperative but would none the less doze off as soon as left to herself, exhibited a marked difference in electric activity of the two hemispheres. The ground activity of the left side was represented by alpha-beta spindles forming unbroken runs or mixed with polymorphous slow waves below 70 μV. The electric activity of the right hemisphere was characterized by an alternation of two patterns (Fig. 1). Over some sections there

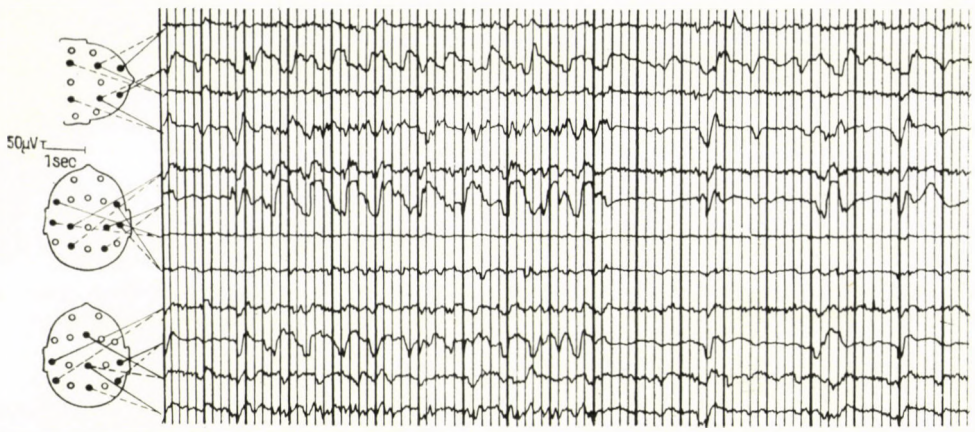

Fig. 1. Two characteristic alternating patterns of electric activity of the right hemisphere. Left side: Serial discharges. Right side: individual periodic complexes. (Continuous recording)

were individual prominent periodic polyphasic burst complexes, in some other sections these complexes formed long runs and were separated by flat intervals from each other and from the periodic complexes. The ground activity between the periodic bursts or runs was definitely poorer in alpha-beta elements than that of the other side. The temporal, particularly the antero-temporal, leads were conspicuously flat and poor in frequency. The periodic paroxysmal bursts were identifiable on the other side too with a definitely lower voltage but with identical phases. The average amplitudes of the periodic and repetitive bursts were above 200 μV. The individual complexes were bi- or triphasic, showing their lowest amplitudes in the bipolar leads obtained by interconnecting the anterior and middle temporal electrodes. In the para-sagittal longitudinal bipolar chains a phase reversal was demonstrable around the frontocentral electrode and there was a diminishing potential gradient in the direction of the parieto-occipital region. In the longitudinal temporal chain the foremost temporal pair revealed, in accordance with the complex, only slight positive deflexions. In the posterior temporal pair there were signs of a diminishing potential gradient and the sagittal leads also revealed a

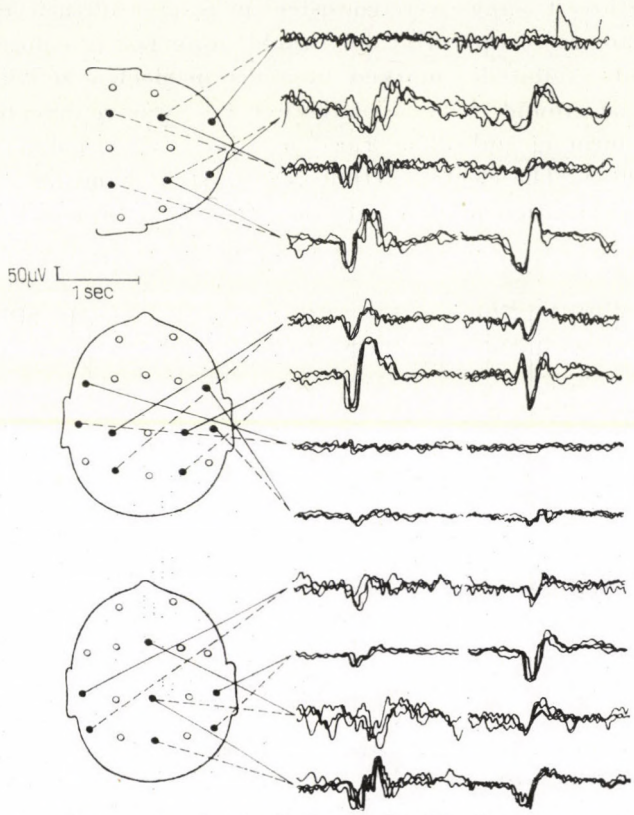

Fig. 2. Periodic complexes appearing in the longitudinal leads on two successive days. Three complexes of each day have been superposed

declining potential gradient in the direction of the occipital region (Fig. 2). In the vertical leads a phase reversal with reference to the phase relationships revealed by the electrode pairs of the longitudinal chains was demonstrable, in other words, potentials of reversed signs were recorded (Fig. 3).

On the fourth EEG recording the periodic and repetitive discharges were no longer present; all leads from the right hemisphere showed flat delta oscillations from which the fast frequencies had disappeared. In the centro-parietal and anterotemporal pairs the activity was flat, nearly isoelectric (Fig. 4). The first three recordings thus revealed an activity of two alternating patterns. In the third recording, the repetitive discharges often recurred several times in succession after short isoelectric periods, in opposition to the first two recordings where they were invariably alternating with the periodic complexes. In the first two tracings there was a nearly full equilibrium between the repetitive discharge and periodic complex discharge sections, more precisely the total duration of repetitive discharges exceeded

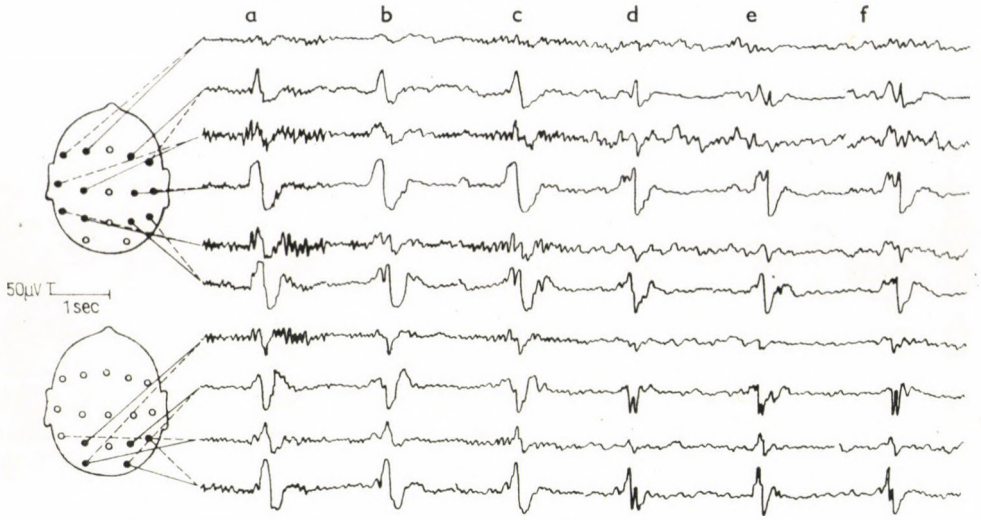

Fig. 3. The individual complexes in the vertical leads. a—b—c: alert state; d—e—f: after intravenous injection of 0.25 g hexobarbital

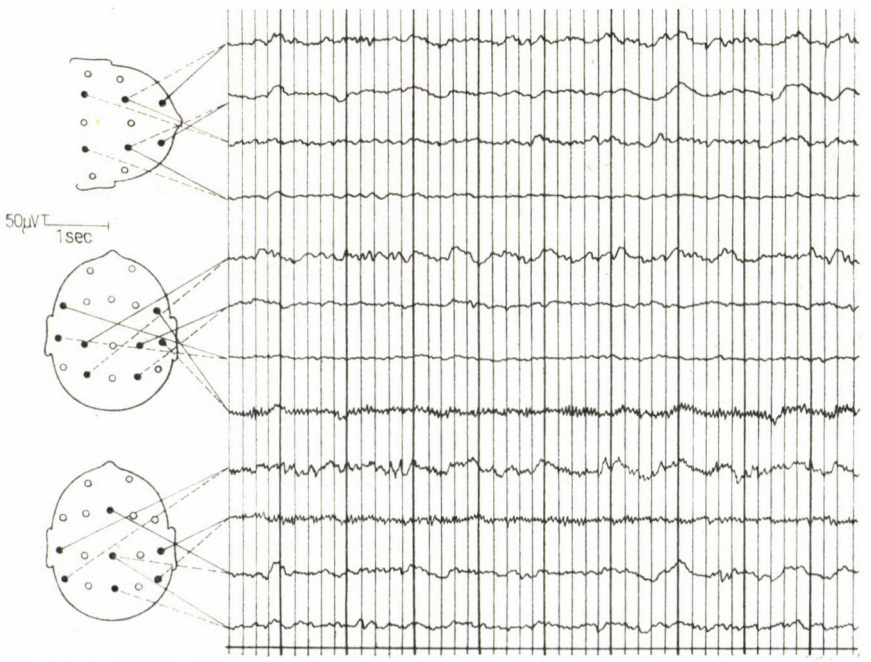

Fig. 4. Recording made five days before death. The complexes had vanished, a flat slow activity has gained predominance

that of the periodic complexes by no more than 30 seconds. In the second recording the total duration of the complexes exceeded that of the repetitive discharges by 2.5 minutes. In the first and second recordings alternation of complexes with repetitive discharges had the following character: in the first recording the repetitive discharges formed two longer runs of one and two minutes, respectively, separated by an interval of one minute. The first longer run was generally followed by shorter repetitive discharges, while after the second, there were only longer complex periods, interrupted by shorter repetitive discharges. In the second recording longer complex periods were alternating with repetitive discharges. If a repetitive discharge was longer than 25 sec., it took at least 55 sec. for the next longer run to appear. The interval between the individual complexes showed a wide variation, from 1.5 up to 15 seconds, but was mostly around 3 to 3.5 seconds.

In the third recording the total duration of the repetitive discharge periods exceeded that of the complex periods by more than 7 minutes, but the duration of the individual repetitive discharges was 20 to 30 seconds as against 50 to 100 seconds of the earlier recordings. The complex periods also showed a decline, not only in their total duration but also in the length of the individual periods which never exceeded 14 seconds.

In the course of the third recording, an intravenous dose of 0.25 g of hexobarbital was given at the rate of 25 mg/10 sec. Parallel with the manifestation of the barbiturate effect the repetitive discharges broke up and ceased altogether, only occasional complexes appeared. In terms of time unit, the total duration of complex periods during sleep was 466 seconds whereas that of the repetitive discharges was as short as 47 seconds and even this coincided with the initial phase of sleep. The patterns of the individual complexes were considerably modified under the influence of hexobarbital, the duration of complexes became shorter, at the same time, components that were not more than a hint in the alert state became more prominent so that the complex became more polyphasic. The constancy of M-shaped chopping of the positive components was particularly remarkable (Fig. 3).

The relationships of the EEG manifestations with the respiration, ECG, mechanogram of the limbs, and movements of the eyeballs and the eyelids were also studied.

Parallel with the polygraphic recordings various sensory stimuli were applied, i.e. photic (single and serial), verbal and electric (single and serial, impulses applied to the body surface).

Neither the appearance of the individual complexes, nor the alteration of the two patterns were affected in any way by the stimuli applied.

Neither the complexes nor the repetitive discharges were accompanied by motor manifestations or by changes in muscular tone.

Post-mortem findings

General features

Necropsy revealed senile marasmus, atrophy of the viscera, multiple decubital ulcers, thromboembolism in the area of the pulmonary arteries of second and third order, consequent upon a thrombosis of the right femoral artery, diffuse bronchitis, bronchiolitis, incipient bronchopneumonia in the right inferior lobe. Nephrosclerosis of arteriosclerotic origin was verified.

Neuropathology

The arteries of the cerebral basis displayed atherosclerosis of medium severity. The 15 mm long section of the middle cerebral artery was free from any lumen-restricting thrombus, there was a plaque of eroded surface in its wall. In the right hemisphere the area surrounding the fossa Sylvii was sunken as a result of earlier ischaemic emollition in the area of the middle cerebral artery. The gross lesion involved the posterior part of the right orbital gyri, the fronto-opercular gyri, the suprasylvian third of the central gyri, the insular cortex, the supramarginal and angular gyri, the whole gyrus T_1 and the anterior third with the temporal pole of gyrus T_2. Loss of subcortical substrate involved the dorsolateral two thirds of the head of the right caudate nucleus, the major part of the fronto-thalamic tract of the internal capsule extending to the lateral wall of the anterior horn. Its farthest extension to the midline was at the level of the optic chiasma, involving the insular, frontal and temporal cortex, external capsule, the entire globus pallidus, the upper half of the knee of the internal capsule, the lateral marginal part of the putamen, the dorsolateral subependymal two thirds of the body of the caudate nucleus. The thalamus and subthalamus showed oedema and a slight broadening. The lesion had spared the structures of the hypothalamus, corpus callosum and midline.

The microscopic changes fell into the three following grades, according to the severity of the parenchymal lesion.

1. The area of the above-described gross changes exhibited colliquation necrosis with a "lattice-cell" reaction and no intact cerebral tissue at any place.

2. Severe but only partial parenchymal lesion in the vicinity of the necrotic changes, characterized by perivascular and intracellular oedema with extensive anisomorphous gliosis and ganglionophagia. The cortical structure was completely disorganized; ganglion cells with hypoxaemic changes of various severity were interspersed with apparently intact ganglial elements.

3. In the most peripheral parts the changes were a fresh perivascular oedema and swelling of the ganglion cells with no gross disorganization.

The left hemisphere was unaffected with the exception of the minor changes described under 3, verified in the central gyri. The left transversal sinus was the site of premortal thrombosis which apart from a slight haemorrhage into the adjacent tissue seemed to have been irrelevant to the primary process.

Relationships between EEG and cerebral lesion

The EEG recordings displaying periodic paroxysmal features date from the third month of disease, six weeks before death. The recordings made five days before death no longer showed the phenomena but were indicative of

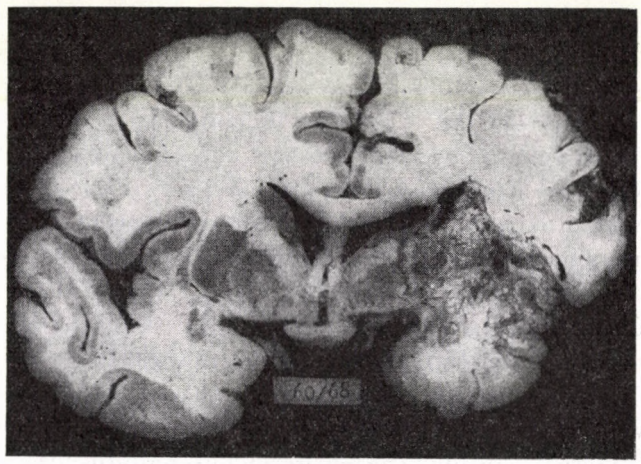

Fig. 5. Emollition of the area of supply of the right middle cerebral artery at the level of the mamillary bodies

the severe impairment of the right hemisphere with its epicentre localized in the surroundings of the fossa Sylvii. This supports the conclusion that the histologic findings reflected a more advanced process than did the previous EEG recordings. This progressive impairment may have involved well-defined morphological changes but we might connect it, with the same justification, merely with a gradual metabolic deterioration of the affected area, which would likewise account for the disappearance of the paroxysmal manifestations.

Voltage and phase analysis of the individual complexes of the periodic paroxysmal bursts was undertaken in an attempt to reconstruct the electric field reflected by them. The measurements were based on the following factors: the frontal and median temporal electrodes apparently correspond to a nearly equipotential area; there is a diminishing potential gradient in the temporo-occipital, centro-occipital and the sagittal rostrodorsal chains; a phase reversal is present in the parasagittal chains around the central electrode and in the vertical pairs in relation to the longitudinal chains, complexes of opposed

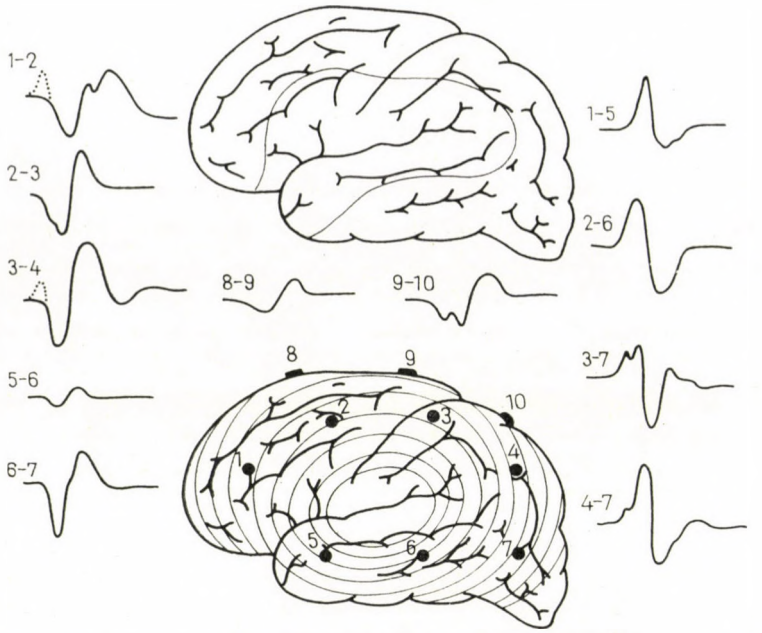

Fig. 6. Upper half: Diagram of the cortical projection of the emollition. Lower half: Potentials derived by means of 1—10 scalp electrodes in the various examined leads and the electric field thus reconstructed

signs become manifest. Fig. 6 shows the potential field reconstructed on these grounds. Its epicentre was found to correspond to the cortical projection of the emollition.

Discussion

The peculiar periodic paroxysmal discharges consequent upon a circumscribed cerebral emollition have raised some intriguing problems.

An EEG pattern of this kind is very rare in circumscribed emollitions of vascular origin. Though the described EEG phenomena bear a certain similarity, in respect of the periodic complexes, to those recorded by HOEFER et al. (Fig. 7, 1963) and by CHATRIAN (Fig. 1, 1964), their distinctive character lies in the fact of combining these periodic complexes with repetitive serial discharges interrupted by flat intervals. These two alternating patterns represent the two main types of periodic paroxysmal discharges referred to in the introduction. They merge more than once into some intermediate pattern; for instance in subacute progressive panencephalitis repetitive discharges made up of paroxysmal slow elements are by no means uncommon (TARISKA [1959], LÓRÁND et al. [1962]) and the inhibitory effect of ATPase may also result in periodic individual bursts, and partly in a "suppression burst" activity (MACCAGNANI et al. [1966]).

The pathomechanism of periodic paroxysmal discharges, whether we consider the "suppression burst" phenomenon or the periodic complexes, is still obscure.

The EEG model of "suppression-burst" is reproducible by partial or complete cortical denervation, achieved by means of undercutting or of a circular isolating incision (BREMER [1935, 1936], KRISTIANSEN and COURTOIS [1949], BURNS [1951], HENRY and SCOVILLE [1952], ECHLIN [1959], KELLAWAY et al. [1966]), and the human counterpart of this EEG phenomenon it is likewise the mechanism of cortical denervation which must be considered in the first place (FISCHER-WILLIAMS [1963]). A similar mechanism has been incriminated for other periodic EEG phenomena as well (CHATRIAN et al. [1964]).

The present case was marked by extensive cortical destruction, cutting off the cortico-cortical interconnections of the adjacent cortical areas. Moreover by involving the capsula interna and other subcortical structures, it also may have interrupted the afferentation of relatively or completely intact cortical areas. All this is compatible with a cortical denervation but leaves us in ignorance about the mechanism by which cortical denervation may lead to the recorded electric phenomena. It seems, however, certain that excitability as well as the refractory period must be involved (ECHLIN [1959], GOLDRING et al. [1961], SHARPLESS and HALPERN [1962], KELLAWAY et al. [1966]). It is obviously an increased excitability which accounts for the periodic complexes reminiscent to a certain extent of epileptic interictal seizure potentials, as well as for the repetitive paroxysmal discharges which, on the other hand, are similar in many respects to the ictal EEG seizure patterns of epilepsy. Certain authors connect the structural background of epileptic seizures with a denervation mechanism (ECHLIN [1959]). This would be in line with the observation that — as in the present case — there is a definite parallelism, though not a strict coincidence, between the development of such an EEG pattern and the onset of the seizures.

The "borderline state" arising in the denervated cortex (KELLAWAY et al. [1966]) represents a metabolic state the lability of which involves a prolonged refractory period and a particular vulnerability to any increased metabolic demand, obviously also after paroxysmal discharges (SHARPLESS and HALPERN [1962]).

It is, furthermore, known that the membrane lability involved by the "borderline state" goes hand in hand with wide variations in the threshold of cortical excitation and in the length of the refractory period. In the present case the individual periodic complexes appeared at different intervals ranging between 1.5 and 11 sec., but mostly around 3 to 3.5 sec. Repetitive periods of some length required a certain interval to reappear, for instance the interval separating two repetitive bursts exceeding 25 sec. in duration was never shorter than 55 sec. The flat intervals seen between the individual or repetitive

discharges represent in all likelihood the refractory periods after the serial paroxysmal discharges as a sign of poor tolerance and increased sensitivity of the affected cortex. It is well in line with these considerations that under the influence of barbiturates which are known to prolong the refractory phase (FORBES et al. [1949]) the repetitive discharges had vanished and only individual complexes remained.

The different theories on the pathomechanism of periodic complexes, particularly of synchronous bilateral complexes described in subacute progressive panencephalitis leave the question unanswered whether the predominant part in their production should be ascribed to cortical or rather to midline subcortical impulses. The proponents of the hypothesis of subcortical origin allege that as a result of cortical lesion the destroyed structures represent a volume conductor for the transmission of subcortical impulses to the surface, moreover, the subcortical mesial structures are released from the control of corticoreticular inhibitory influences (NELSON and LEFFMAN [1963]).

This theory seeks to account for the production of bilateral synchronous EEG patterns in general and it might be a convenient interpretation for the synchronous bilateral stereotype pattern in the present case. There is, however, experimental proof that synchronisation taking effect partly by cortico-cortical pathways, partly via the corpus callosum may be itself account for the production of extensive synchronous bihemispheral patterns even if the mesial diencephalic structures are damaged (MARCUS and WATSON [1966]). In the present case the periodic complexes appeared in the first place on the affected side, the amplitudes having been considerably lower on the contralateral side. This would definitely plead against a mesial origin even when allowance is being made for the possibility that an activation of the lateral areas of the nonspecific thalamic system may produce an asymmetrical cortical reaction (ENOMOTO [1958]).

The phase-relationships of the individual complexes reflect an electric field corresponding to the cortical projection of the softened area whose destruction must have been still in progress at the time of the recordings (as suggested by subsequent diminution of the potentials). Owing to the relativity of potential differences inherent in the bipolar technique it is not possible to establish in retrospect whether this field represents the actual source of potentials or merely an inactive equipotential field. Therefore we can't preclude the possibility that the release of the midline non-specific reticular structures as a result of cortical destruction might have provided the source of the stimuli. However, the assumption of a triggering central impulsation is largely hypothetical. That an interruption of corticoreticular inhibitory impulses should lead to manifestations of this kind is merely a conjecture. Moreover, it is a confirmed fact that denervated thalamic structures — in contrast to the cortex — do not generate periodic impulses (KELLAWAY et al. [1966]).

By the supposition of a subcortical generation of impulses it is difficult to explain why the vanishing of the complexes was associated with the process of destruction of the cortical structures, furthermore, why a volume — i.e. faster — conduction due to destruction of the brain structures failed to reveal itself by a phase shifting of the complexes of the two sides. Actually — as far as the poor resolving capacity resulting from slow paper speed permits to ascertain — these seemed in fact to coincide in time.

A bilateral appearance of potentials with lower amplitudes in the contralateral hemisphere is compatible either with an asymmetrical mesial origin or — a cortical origin being supposed — with spreading to the other side by the commissural system of the corpus callosum. The latter interpretation might apply to the present case since the bulk of the system of callosal fibres had been left unaffected.

The possibility of a cortical origin or of cortical potentials of evoked character is supported by the finding that the hexobarbital sleep had modified the morphology of the complexes by splitting them up in several individual components. Barbiturate-induced modifications in the cortical synaptic interrelations would well account for these findings.

On the ground outlined in the foregoing we tend to interpret the electric phenomena at issue as being of cortical origin and to regard the individual periodic complexes as well as the "suppression burst" activity as a manifestation of a process in a denervated cortical area, at various levels of metabolic impairment, resulting in a "borderline state".

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Dr. Péter HALÁSZ } Second Department of Neuropsychiatry, University Medical School, Budapest VIII, Balassa u. 6, Hungary

Dr. Sára GÖDÉNY } Department of Neurology and Psychiatry, University
Dr. József HIDASI } Medical School, Debrecen 12, Hungary

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ÜBER DIE REGULATION DER EXTREMITÄTENDURCHBLUTUNG BEI EXTREMITÄTENLYMPHÖDEM*

Von

F. SOLTI, Júlia NAGY, J. GERGELY, M. ISKUM, I. KRASZNAI, A. HARTAI und M. CSILLAG

I. MEDIZINISCHE KLINIK (DIREKTOR: PROF. DR. I. MAGYAR) DER MEDIZINISCHEN UNIVERSITÄT, UND CHIRURGISCHE ABTEILUNG (CHEFARZT: DR. R. GERGELY) DES JÁNOS-KRANKENHAUSES BUDAPEST

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Bei 8, an Extremitätenlymphödem leidenden Kranken wurden mit Hilfe der venösen Isotopdilutionsmethode Durchblutung, vaskulärer Widerstand, O_2-Aufnahme sowie Minutenvolumenfraktion in der Extremität untersucht. Die Ergebnisse wurden mit den entsprechenden Parametern von 44 kreislaufgesunden Personen verglichen. Die Untersuchungen ergaben, daß sich auf Wirkung einer anhaltenden Lymphstauung in der Extremität die Durchblutung wesentlich erhöht, der Gefäßwiderstand abnimmt, die Minutenvolumenfraktion ansteigt, die O_2-Aufnahme von der Norm jedoch nicht signifikant abweicht. Die Regulation der Extremitätendurchblutung ändert sich entgegengesetzt als in Fällen, in denen es sich um eine Hinderung des venösen Abflusses handelt. Es wird angenommen, daß bei Extremitätenlymphödem ein bedeutender Teil des Blutes — wahrscheinlich wegen der veränderten Regulation der Mikrozirkulation — nicht durch die nutritiven (kurzen) Gefäßbahnen fließt.

Bekanntlich führt sowohl eine anhaltende Insuffizienz des Lymphabflusses (mechanische Insuffizienz der Extremitätenlymphdurchblutung) als auch die Insuffizienz des venösen Abflusses (Verschluß einer größeren Extremitätenvene) zur Entwicklung eines Extremitätenödems. Das lymphstauungsbedingte Extremitätenödem — Extremitätenlymphödem — unterscheidet sich aber von dem durch Veneninsuffizienz verursachten Extremitätenödem in bedeutendem Maße. Das ersterwähnte Ödem, das sich hauptsächlich auf die subkutanen Gewebe lokalisiert, ist des hohen Eiweißgehalts des Exudats zufolge von dichter Struktur. Ein anderer beachtenswerter Unterschied ist, daß während nach durch eine mechanische Insuffizienz der venösen Extremitätendurchblutung verursachten Ödemen häufig Hautulzerationen und Gewebenekrosen entstehen, bei Hautlymphödem Nekrose oder andere trophische Störungen nur selten auftreten.

Aufgrund des Gesagten dürfte angenommen werden, daß in bezug auf die Regulation des Extremitätenkreislaufs zwischen der Lymphstauung und der venösen Stauung ein Unterschied besteht. Während die Einwirkung der venösen Kreislaufinsuffizienz auf die Durchblutung und auf die O_2-Aufnahme der Extremitäten von mehreren Verfassern untersucht wurde, stehen im Zusammenhang mit der Lymphstauung keine diesbezüglichen experimentellen Angaben zur Verfügung.

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Das Ziel vorliegender Arbeit war die Untersuchung der Regulation des Blutkreislaufs bei Extremitätenlymphödem.

Material und Methodik

Die Untersuchungen wurden bei 8 an typischem Lymphödem der unteren Extremitäten leidenden Patienten durchgeführt. In 6 der 8 Fälle meldete sich das Lymphödem bei Erwachsenen nach durch wiederholtes Erysipelas bzw. mykotisches Ekzem verursachter Lymphangitis. In 2 Fällen entwickelte sich das Lymphödem während der Pubertät (lymphoedema praecox). Sämtliche Patienten waren kardial kompensiert. Zwecks Unterstützung der vermuteten



Abb. 1



Abb. 2

Lymphkreislaufstörung wurde bei jedem der 8 Patienten Extremitätenlymphangiographie vorgenommen (intrakutane Zufuhr von Evansblau, Herauspräparierung der sichtbar gewordenen Lymphgefäße des Fußrückens, Injizierung von 5—10 ml öligem Jodetryl). Die lymphangiographischen Aufnahmen wiesen in jedem Fall auf die Störung des Extremitäten- bzw. pelvischen Lymphkreislaufs (lymphatischer Reflux, varikös erweiterte Lymphgefäße, Lymphgefäßverschluß) hin. Abbildungen 1 und 2 veranschaulichen zwei charakteristische lymphangiographische Aufnahmen.

Um die Möglichkeit eines — eventuell gleichzeitig bestehenden — venösen Verschlusses ausschließen zu können, haben wir bei allen Patienten an der ödematösen Extremität auch die Venographie (Phlebographie) durchgeführt. Auf einen venösen Verschluß weisende Zeichen waren in keinem der Fälle vorzufinden. Die wichtigeren Angaben unserer Patienten sind in Tabelle I dargestellt.

Zur Messung der Durchblutung der ödematösen unteren Extremität diente unser venöses Isotopdilutionsverfahren [1]. Mit Hilfe dieser Methode vermochten wir innerhalb einer Fehlergrenze von 5—10% die durch die V. femoralis strömende Blutmenge, d. h. die totale Durchblutung der betreffenden unteren Extremität genau zu messen. Den O_2-Verbrauch der Extremität haben wir anhand des Quotienten der Durchblutung und der arteriovenösen O_2-Differenz ausgerechnet. Die Bestimmung des O_2-Gehaltes der A. und V. femoralis

erfolgte aus durch direkte Punktion entnommenen Blutproben, mit Hilfe des KIPPschen Oxymeters. Der Blutdruck wurde über der A. brachialis mit Auskultationsmethode, mit einem Quecksilbermanometer gemessen. Zur Bestimmung des Herzminutenvolumens diente, nach i.v. Verabreichung von 50 mg Evansblau, die Farbendilutionsmethode. Die Blutproben wurden aus der A. femoralis mittels Punktion sekündlich entnommen. Die Minutenvolumenfraktion der betreffenden unteren Extremität wurde aus der Durchblutung und der Herzminutenvolumenfraktion errechnet. Der in der V. femoralis herrschende Druck wurde im Liegen mit dem MORITZ—TABORAschen Apparat gemessen.

Als Kontrolle dienten die entsprechenden Parameter von 44 Personen mit intaktem Kreislauf.

Bei der Bewertung der Angaben kam der STUDENTsche *t*-Test zur Anwendung.

Ergebnisse

Die anläßlich der Untersuchung der Extremitätendurchblutung unserer acht, an Extremitätenlymphödem leidenden Patienten ermittelten Ergebnisse veranschaulicht Tabelle I.

Tabelle I

Regulation der Extremitätendurchblutung bei Lymphödem

Nr.	Name, Alter, Geschlecht Diagnose	RR mmHg	CO l/min	TVR dyn · sec · cm^{-5}	LBF ml/min	LVR dyn · sec · cm^{-5}	LO_2C ml/min	V_f mm Wasser	LCOF %
1.	B. K. 26 ♀ Lymphoedema praecox	115/80	5,9	0,97×133	640	8,9×1332	15,0	90	11,1
2.	N. O. 77 ♀ Lymphödem. Hochdruck	210/100	5,5	1,56×1332	1300	6,6×1332	22,0	46	23,6
3.	D. SZ. 64 ♂ Lymphödem	170/90	5,0	1,48×1332	650	10,6×1332	18,5	88	13,0
4.	J. O. 32 ♂ Lymphödem	140/80	4,4	1,36×1332	330	19,2×1332	12,5	105	7,5
5.	T. N. 30 ♀ Lymphödem	130/85	5,4	1,11×1332	390	15,4×1332	14,0	100	7,3
6.	P. L. 40 ♂ Lymphödem	135/90	4,7	1,34×1332	400	15,7×1332	13,0	91	8,5
7.	M. V. 39 ♀ Lymphoedema praecox	140/80	5,2	1,27×1332	420	14,3×1332	13,5	88	8,1
8.	J. T. 44 ♀ Lymphödem	125/80	5,8	0,89×1332	580	8,9×1332	7,0	—	10,0

Die bei unseren 8 lymphödematösen Patienten gewonnenen Resultate wurden mit den entsprechenden Parametern der Kontrollgruppe verglichen.

Die wichtigeren Daten der statistischen Analyse haben wir in Tabelle II zusammengefaßt.

Tabelle II

Vergleich der normalen Durchschnittswerte mit den Extremitäten-Kreislaufangaben bei Extremitätenlymphödem

		P_m	CO	TVR	LBF	LVR	LO_2C	V_f	LCOF
n	A	8	8	8	8	8	8	7	8
	B	44	42	42	44	44	44	40	42
$\bar{x}$	A	*103* (7,0)	*5,2* (0,5)	*1,25* (0,12)	*590* (1,4)	*12,5* (2,5)	*14,4* (3,0)	*87* (7,5)	*11,4* (0,9)
	B	*102* (6,2)	*5,0* (0,5)	*1,26* (0,15)	*360* (1,0)	*18,6* (3,1)	*13,8* (3,1)	*72* (6,7)	*7,2* (0,8)
Diff. (B—A)		+1	—0,2	+0,01	—230	+6,1	—0,6	—15	—4,2
s Diff. (B—A)		0,2	0,9	0,11	2,1	3,9	0,4	1,2	2,2
p		>0,8	>0,3	>0,9	*<0,05*	*<0,01*	>0,6	>0,3	*<0,05*

Abkürzungen in Tabellen I und II

n = Anzahl der Fälle
$\bar{x}$ = Durchschnittswerte und Streuung (±)
A = Angaben bei Lymphödem
B = Kontrollwerte
p = Wahrscheinlichkeitszahl
P_m = arterieller Mitteldruck $[P_d + 1/3 \times (P_s - P_d)]$
CO = Herzminutenvolumen
TVR = Gefäßwiderstand im großen Kreislauf
LBF = Durchblutung der unteren Extremität
LVR = Gefäßwiderstand der unteren Extremität
LO_2C = O_2-Aufnahme der unteren Extremität
V_f = Druck in der V. femoralis
LCOF = Herzminutenvolumenfraktion der Durchblutung der unteren Extremität

Wie aus den Tabellen I und II ersichtlich, ist die Extremitätendurchblutung wesentlich höher, und der Extremitäten-Gefäßwiderstand wesentlich niedriger, als die bezüglichen Durchschnitts- bzw. Normalwerte. Die Minutenvolumenfraktion der Extremitätendurchblutung ist höher als normal, die O_2-Aufnahme der Extremität und der Druck der V. femoralis weichen jedoch von den Durchschnittswerten nicht wesentlich ab. Die Werte des Blutdrucks, des Minutenvolumens und des Widerstands im großen Kreislauf waren in der lymphödematösen und der Kontrollgruppe nahezu identisch.

Um die bei Extremitätenlymphödem zustandekommenden Änderungen der Extremitätendurchblutung zu veranschaulichen, haben wir in Abbildung 3 die normalen sowie die bei unseren lymphödematösen Patienten gewonnenen Durchschnittswerte des Minutenvolumens, der Extremitätendurchblutung, des

Extremitätengefäßwiderstandes, der O_2-Aufnahme der Extremitäten sowie der Minutenvolumenfraktion der Extremitätendurchblutung graphisch, in Form von Säulendiagrammen dargestellt.

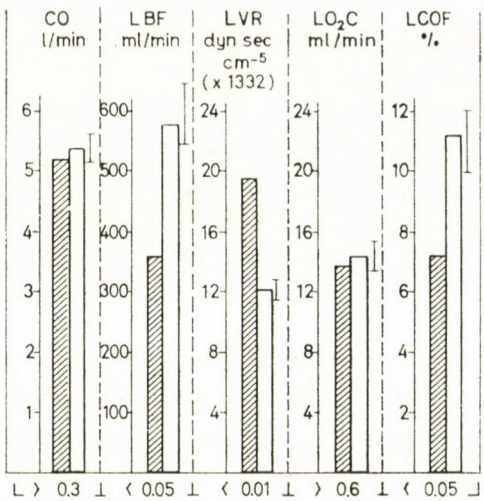

Abb. 3

Besprechung

Die Auswirkung des gehinderten venösen Abflusses auf den Extremitätenkreislauf (Steigerung des venösen Drucks, Venenverschluß) wurde von zahlreichen Forschern eingehend untersucht; nach der Mehrzahl kommt es auf Wirkung einer wesentlichen Steigerung des Venendrucks und der Verlangsamung des venösen Abflusses allmählich zur Verminderung der Extremitätendurchblutung [2—10].

Unsere vorangehenden Untersuchungen ergaben, daß bei nach V. femoralis-Thrombose auftretendem Extremitätenödem in der Extremität die Durchblutung pathologisch niedrig, der Gefäßwiderstand wesentlich gesteigert und die Minutenvolumenfraktion niedriger als normalerweise sind [11].

Puglionisi und Mitarb. [12] vermochten bei thrombophlebitisbedingtem Extremitätenödem die verlangsamte Resorption von ^{22}Na aus dem Muskel, d. h. die Verminderung der Muskeldurchblutung nachzuweisen.

Die Einwirkung der mechanischen Insuffizienz des Lymphkreislaufes auf den Blutkreislauf ist eine noch ziemlich ungeklärte Frage. Calnan und Mitarb. [13, 14] haben in einigen, klinisch als lymphödematös erscheinenden Fällen von Extremitätenödem den Abfluß in der V. iliaca externa gemessen und dabei niedrige Werte vorgefunden. Da jedoch die Lymphographie Normal-

verhältnisse zeigte, während der venographische Befund auf die Hinderung des venösen Abflusses hinwies, kann festgestellt werden, daß es sich in den untersuchten Fällen um venenobstruktionsbedingte Ödeme handelte. Anläßlich der in zwei Fällen durchgeführten Exploration war eine vernarbungsbedingte Abschnürung der V. iliaca externa vorzufinden; nach Lösung der Abklemmung normalisierte sich die Durchblutung der V. iliaca. Die zur Untersuchung des sich in den Extremitäten abspielenden ^{131}J- und ^{131}J-Albumintransports dienenden Lymphgefäß- bzw. Venenobstruktionsversuche von NAVITA [15] führten zur Folgerung, daß die Insuffizienz des Lymphkreislaufs — im Gegensatz zur Venenobstruktion — keine Verminderung der Extremitätendurchblutung verursacht.

Unsere vorliegenden Experimente weisen darauf hin, daß sich bei Extremitätenlymphödem die Regulation der Extremitätendurchblutung bedeutend und auf eigenartige Weise ändert: Die durch die Extremität strömende Blutmenge erhöht sich, der Gefäßwiderstand nimmt ab und die Minutenvolumenfraktion der Extremität steigt an. Beachtenswert ist auch der Umstand, daß diese sich im Laufe des Lymphödems entwickelnde Änderung von entgegengesetztem Typ ist, als die bei durch Veneninsuffizienz verursachtem Extremitätenödem beobachtbare Regulationsänderung [11]. Die angeführten Befunde ermöglichen auch die Erklärung der Tatsache, weshalb sich — der Verminderung der Extremitätendurchblutung zufolge — nach venenobstruktionsbedingtem Extremitätenödem häufig trophische Störungen und Nekrose entwickeln, während bei Extremitätenlymphödem — trotz der hochgradigen Anschwellung der Gewebe — keine trophischen Störungen auftreten.

Nicht weniger interessant ist auch die Analyse jener Regulationsmechanismen, die im Falle einer mechanischen Insuffizienz des Lymphkreislaufs den bedeutenden Anstieg der Durchblutung und die bedeutende Abnahme des Gefäßwiderstandes in der Extremität verursachen. Aufgrund unserer Ergebnisse kann der Mechanismus der Änderung der Extremitätendurchblutung selbstverständlich nicht restlos geklärt werden, einige Angaben erlauben jedoch in bezug auf den Pathomechanismus der beobachteten Kreislaufveränderungen gewisse Folgerungen. Auffallenderweise erhöhte sich bei Lymphödem die Extremitätendurchblutung nahezu auf das Doppelte des Normalwerts, während die O_2-Aufnahme im Verhältnis zur Norm keine signifikante Abweichung zeigte. Daraus ist zu folgern, daß sich auf Wirkung der Lymphstauung in erster Linie die nicht nutritive Fraktion der Extremitätendurchblutung (sog. Shunt-Durchblutung) erhöht. Ein bedeutender Teil des Blutes umgeht somit die nutritiven Kapillaren. Bekanntlich können in gewissen Fällen zwischen den Lymphgefäßen und den benachbarten Venen unmittelbare Verbindungen — lymphovenöse Shunts — nachgewiesen werden. Nach THREEFOOT und Mitarb. [16 17] ist für die Eröffnung der annehmbar präformierten lymphovenösen Anastomosen die Insuffizienz des Lymphabflusses

bzw. der pathologische Anstieg des Lymphdrucks in den betreffenden Lymphstämmen verantwortlich.

Aufgrund dieser Angaben könnte angenommen werden, daß bei Extremitätenlymphödem ein Teil der Lymphe durch Vermittlung der lymphovenösen Anastomosen durch das Venensystem abtransportiert wird. Nach unseren Untersuchungen ist jedoch bei Extremitätenlymphödem die Menge des minütlich durch die V. femoralis strömenden Blutes ca. 200 ml mehr als normalerweise. Einen derart hochgradigen Durchblutungsanstieg kann die eventuelle Eröffnung der lymphovenösen Anastomosen allein nicht verursachen, so daß auch die Rolle anderer Faktoren in Frage kommt. Ein solcher, die gesamte (nicht nutritive) Extremitätendurchblutung steigernder Faktor könnte die lymphstauungsbedingte Entwicklung längerer arteriovenöser Gefäßbahnen sein. Dieser Annahme widerspricht aber der Umstand, daß wir bei unseren an Extremitätenlymphödem leidenden Patienten anläßlich der Messung der Durchblutung der V. femoralis weder eine pulsierende Blutströmung noch Strömungsgeräusche an der betreffenden unteren Extremität zu beobachten vermochten. Eine andere Möglichkeit wäre, daß sich während der mechanischen Insuffizienz des Lymphkreislaufs die Mikrozirkulation der Extremität ändert und ein bedeutender Teil des Blutes aus den Metarteriolen in die Venulen durch die kürzeren Bahnen, die sog. Strömungskapillaren gelangt. Die Anzahl, Öffnung und Schließung der nicht nutritiven, kurzen Mikrozirkulationsbahnen wird durch neurale, humorale und Strömungseinwirkungen reguliert. Die Frage, welchem Ereignis bzw. Faktor zufolge sich die Regulation der Mikrozirkulation ändert, können wir vorläufig nicht beantworten.

Schließlich muß auch die Rolle eines dritten Faktors in Betracht gezogen werden: Auf Wirkung einer anhaltenden Lymphstauung können in der Wand der Kleingefäße aus Eiweißimbibition stammende, degenerative Veränderungen entstehen; im Falle einer mechanischen Insuffizienz des kardialen Lymphkreislaufs lassen sich in der Wand der kleinen Kranzgefäße degenerative Schädigungen nachweisen [18].

Zur Klärung der Problematik, ob sich bei Extremitätenlymphödem infolge der Eiweißtransportstörung der Gefäßwand in den Kleingefäßen degenerative Veränderungen entwickeln und welche Rolle diese Veränderungen in der Änderung der Extremitätenmikrozirkulation spielen, sind weitere Untersuchungen erforderlich.

Ähnlicherweise kann die Frage, auf welche Weise sich auf Wirkung der chronischen Lymphstauung das zwischen der nutritiven und der gesamten Extremitätendurchblutung bestehende Verhältnis ändert, lediglich mit Hilfe weiterer Untersuchungen beantwortet werden.

Für die Durchführung der mathematisch-statistischen Berechnungen wollen wir Herrn J. FISCHER und Herrn P. CSÁKI (Biometrische Abteilung des Mathematischen Forschungsinstituts der Ungarischen Akademie der Wissenschaften) unseren aufrichtigen Dank zukommen lassen.

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Dr. Ferenc SOLTI
Dr. Júlia NAGY
Dr. Miklós ISKUM
Dr. István KRASZNAI
Dr. Anna HARTAI

Budapest VIII, Korányi S. u. 2/a,
I. sz. Belklinika, Ungarn

Dr. Rezső GERGELY
Dr. Miklós CSILLAG

Budapest XII, János Kórház, Ungarn

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EFFECT OF DIETARY CHOLESTEROL ON VITAMIN C METABOLISM IN LABORATORY ANIMALS

By

E. GINTER

INSTITUTE OF HUMAN NUTRITION RESEARCH, BRATISLAVA, CZECHOSLOVAKIA

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A cholesterol-supplemented diet has been found to increase the consumption of vitamin C in guinea-pigs, as reflected by a reduction of vitamin C concentration in the tissues and increase of its urinary output. In rabbits and albino rats, animals which are capable of synthetizing ascorbic acid, cholesterol feeding results in an accumulation of vitamin C in the tissues and an increased elimination in the urine, as a sign of enhanced ascorbic acid biosynthesis. The reactions in either type of animals — capable or incapable of ascorbic acid synthesis — may be connected with the situation enhancing the ascorbic acid demand of the body to which species with a different ascorbic acid metabolism would respond in a different manner. The question whether the mechanism arises from a non-specific stress situation or whether it involves specific interrelations between cholesterol and ascorbic acid metabolism is discussed.

According to recent evidence, high doses of vitamin C reduce the blood cholesterol level in cholesterol-fed laboratory animals [1—4], while chronic vitamin C deficiency leads to an increase of the cholesterol concentration in guinea-pig tissues [5].

How far the opposite is also true, i.e. whether a cholesterol diet affects the metabolism of ascorbic acid, is a question which has received little attention. In search for a suitable experimental model of hypercholesterolaemia [6] we noted the occurrence of vitamin C deficiency marked by haemorrhages in a greater number of guinea-pigs fed a high cholesterol diet than of those being kept on a normal cholesterol diet, while the exogenous supply of vitamin C was the same in both groups. This pointed to the possibility that a massive cholesterol intake might increase the vitamin C demands of the organism. To clarify this point was the aim of the present study, carried out in two different species, one incapable and another capable, of vitamin C synthesis. The guinea-pig was selected as a representative of the first type, the albino rat and the rabbit as those of the second.

Material and methods

Male guinea-pigs of 250 to 450 g initial weight, male Wistar rats of 200 to 250 g initial weight and male albino rabbits of 2000 to 2400 g initial weight were used. The guinea-pigs were offered a modified, vitamin C-free LUNDE diet of the following composition (g): oat flakes 10,000, wheat bran 4,000, dried milk 3,000, butter 2,000, common salt 200, salt mixture 100, cod liver oil 25 ml, vitamin B complex Spofa 100 pills. The salt mixture contained

(in g): $CaCO_3$, 600; K_2HPO_4, 645; NaCl, 335; $MgSO_4 \cdot 7H_2O$, 204; $CaHPO_4 \cdot 2H_2O$, 150; Fe citrate $\cdot 6H_2O$, 55; $MnSO_4 \cdot 4H_2O$, 10; KI, 1.6; $CuSO_4 \cdot 5H_2O$, 0.6; $ZnCl_2$, 0.5. Composition of the "B complex Spofa" pills (mg per pill): thiamine hydrochloride, 2; riboflavin, 2; pyridoxine hydrochloride, 1; Ca panthotenate, 3; nicotinamide, 20. The rats were given the standard LARSEN-type rat food [7], which is also free of vitamin C.

The guinea-pigs and rabbits of the test groups were administered 0.3% of cholesterol, dissolved in the fatty component of the diet. Rats whose tolerance to exogenous cholesterol is far greater, received 2% cholesterol in the diet. Vitamin C was withheld from the animal species capable of ascorbic acid synthesis (rabbits, rats) while to the guinea-pigs it was administered orally by a tube as a solution, 5 mg daily three times a week. The diets (without additional cholesterol) corresponded to the normal growth of all three types of animal. Addition of cholesterol was found significantly to reduce the growth rate of the guinea-pigs and rabbits without, however, affecting the weight curve of the rats.

At the end of the experiment the animals were decapitated 17 hours after the last feeding. The concentration of vitamin C [8], in some cases also that of dehydroascorbic acid [9] were determined in the tissues. In two experimental series, the guinea-pigs and rats were kept in metabolic cages during the entire period and the 24-hour urinary output of vitamin C [8] was determined (in guinea pigs invariably on the day after ascorbic acid administration).

The results were statistically evaluated by STUDENT's *t*-test. The tables list averages $\pm$S.E. Further details of the individual experiments and numbers of animals under investigation will be given below.

Results and discussion

1. Effect of cholesterol on vitamin C metabolism in the guinea-pig

Table I summarizes the results of four separate series of experiments in which the effect of 0.3% additional dietary cholesterol was examined on the ascorbic acid content of the liver, spleen and adrenals in guinea-pigs. The duration of the individual experiments varied between 20 and 140 days. The results showed a diminishing tendency of the tissue of vitamin C concentration under the effect of cholesterol feeding. The decrease of vitamin C in the spleen was significant, regardless of the period of cholesterol diet. Vitamin C concentration in the liver showed no significant reduction unless the period of cholesterol diet exceeded 140 days. In the adrenals the reduction occurred sooner. Cholesterol-fed guinea-pigs accumulated significant amounts of cholesterol in their tissues [6].

In another series of 11 cholesterol-fed guinea-pigs and 9 controls the 24-hr urinary elimination of vitamin C was studied from the 6th to the 72nd day of additional cholesterol feeding. Results presented in Table I show that urinary output of vitamin C increased significantly during the first 25 days of cholesterol feeding, to decline afterwards to the control range.

2. Effect of cholesterol on vitamin C metabolism in the rat

Table II summarizes the results of two series where the effect of cholesterol fed over 87 and 196 days, respectively, on the vitamin C content of the tissues was studied. In the 87-day series, vitamin C concentration in the liver increased, while in the other organs including blood, it was unaffected.

Table I

Vitamin C concentration in tissues (mg per 100 g wet tissue) and urine (mg per 24 hrs) of control and cholesterol-fed guinea-pigs

Duration of experiment (days)	Tissue	Control group	Cholesterol group	Statistical significance
Number of animals		10	10	
	Liver	6.6 ± 0.9	5.1 ± 0.3	—
20	Spleen	20.6 ± 1.6	17.2 ± 0.6	$P < 0.1$
	Adrenals	40.1 ± 2.4	32.3 ± 2.2	$P < 0.05$
Number of animals		7	7	
	Liver	6.1 ± 0.6	5.2 ± 0.4	—
73	Spleen	19.7 ± 1.9	14.2 ± 1.4	$P < 0.05$
	Adrenals	33.6 ± 3.2	27.1 ± 0.8	$P < 0.1$
Number of animals		10	10	
	Liver	3.6 ± 0.3	3.9 ± 0.5	—
80	Spleen	15.2 ± 0.6	11.8 ± 0.6	$P < 0.001$
	Adrenals	24.9 ± 2.4	23.9 ± 2.3	—
Number of animals		6	14	
	Liver	3.7 ± 0.3	2.6 ± 0.2	$P < 0.01$
140	Spleen	11.1 ± 1.3	7.8 ± 0.5	$P < 0.05$
	Adrenals	19.8 ± 1.5	22.9 ± 1.7	—
6—25		0.18 ± 0.02	0.41 ± 0.08	$P < 0.001$
	Urine	(37)	(23)	
37—72		0.17 ± 0.02	0.18 ± 0.02	—
		(46)	(32)	

In contrast, the liver and kidneys of the rats kept on a high cholesterol diet for a period of 196 days exhibited a highly significant accumulation of vitamin C; their urinary vitamin C output was also significantly increased, i.e. to nearly twice the control level.

Cholesterol in blood and tissues was practically unaffected.

3. Effect of cholesterol on vitamin C metabolism in the rabbit

Cholesterol feeding induced a nearly tenfold increase in the blood cholesterol level (1207 mg per 100 ml in the cholesterol-fed group *vs.* 146 mg per 100 ml in the controls). As seen in Table III, in the liver and kidneys the concentration and total contents of vitamin C increased significantly. Though

Table II

Vitamin C level in whole blood (mg per 100 ml), tissues (mg per 100 g wet tissue), and urine (mg per 24 hrs,), in control and cholesterol-fed albino rats

Duration of experiment (days)	Tissue	Control group	Cholesterol group	Statistical significance
Number of animals		10	9	
	Liver	26.3 ± 0.7	29.2 ± 1.2	$P < 0.05$
87	Adrenals	307.4 ±28.9	365.7 ±33.7	—
	Kidneys	18.0 ± 0.7	16.3 ± 1.0	—
	Blood	0.59± 0.04	0.57± 0.04	—
Number of animals		13	5	
	Liver	19.6 ± 0.9	24.5 ± 1.3	$P < 0.01$
196	Adrenals	333.9 ±12.1	364.9 ±10.1	$P < 0.1$
	Kidneys	13.2 ± 0.6	18.7 ± 1.1	$P < 0.001$
	Blood	0.40± 0.03	0.47± 0.07	—
Number of analyses		196	125	
4—184	Urine	0.39 ± 0.03	0.71 ± 0.07	$P < 0.001$

Table III

Vitamin C concentration (mg per 100 g wet tissue) and total content (mg in organ) in tissues of control and cholesterol-fed rabbits

Tissue		Control group	Cholesterol group	Statistical significance
Number of animals		9	7	
Liver	mg per 100 g	15.8 ± 1.7	20.5 ± 1.6	$P < 0.1$
	mg in organ	11.5 ± 1.2	17.8 ± 1.8	$P < 0.02$
Kidneys	mg per 100 g	7.1 ± 0.5	12.0 ± 1.0	$P < 0.001$
	mg in organ	1.0 ± 0.1	2.1 ± 0.3	$P < 0.01$
Adrenals	mg per 100 g	186.1 ± 21.8	108.0 ± 12.0	$P < 0.001$
	mg in organ	1.1 ± 0.1	1.2 ± 0.3	—
Heart	mg per 100 g	3.7 ± 0.2	4.3 ± 0.5	—
	mg in organ	0.3 ± 0.01	0.3 ± 0.03	—
Pool of 4 organs	(mg)	13.9 ± 1.2	21.3 ± 2.2	$P < 0.02$

the vitamin C concentration of the adrenals declined, their absolute vitamin C content was unaffected, due to the fact that the gland nearly doubled its original weight during cholesterol feeding. The total pool of vitamin C in the four organs examined (liver, kidney, adrenals, heart) increased by more than 50%. In contrast, the blood vitamin C level was hardly affected by the diet.

4. Effect of cholesterol on the dehydroascorbic acid level

Dehydroascorbic acid concentration in the tissues of all control animals was very low, sometimes falling to attain the sensitivity threshold of the method of assay. Under the effect of cholesterol feeding, a significant reduction in dehydroascorbic acid concentration was found in the liver of guinea-pigs and rabbits (Table IV). This finding was at variance with the claim of SOKOLOFF et al. that atherosclerosis in both humans and animals is associated with a high blood ascorbic acid level. At any rate, the reduction of hepatic dehydroascorbic acid concentration is an open problem awaiting biochemical interpretation in the light of further research.

Table IV

Dehydroascorbic acid level in liver (mg per 100 g wet tissue) of control and cholesterol-fed guinea-pigs, albino rats, and rabbits

Animal species (Duration of experiment)	Control group	Cholesterol group	Statistical significance
Guinea-pig (140 days)	0.87 ± 0.08 $n = 6$	0.47 ± 0.07 $n = 14$	$P < 0.002$
Albino rat (87 days)	0.67 ± 0.23 $n = 10$	0.58 ± 0.23 $n = 9$	—
Rabbit (72 days)	2.30 ± 0.50 $n = 9$	0.60 ± 0.10 $n = 7$	$P < 0.01$

The results obtained in guinea-pigs clearly indicate that the atherogenous diet may lead to an increased consumption of vitamin C. Guinea-pigs fed a high cholesterol diet reduce the ascorbic acid concentration in their organs while increasing the urinary elimination of the substance. In this context the study of BOOKER et al. [10] is of interest; they demonstrated a transfer of ascorbic acid from cells to plasma in the blood of cholesterol-fed animals. The frequency of haemorrhages in guinea-pigs fed a high cholesterol diet is consistent with this finding and points to an increased consumption of ascorbic acid as a result of excessive cholesterol intake.

Rabbits and rats, capable of ascorbic acid synthesis, respond in a manner different from guinea-pigs to a high cholesterol diet by accumulating significant amounts of vitamin C in their tissues, particularly in liver and kidneys. Rats nearly double their urinary vitamin C output. Since they had no access to any external source of vitamin C, it is clear that the findings must be ascribed to an increased biosynthesis of ascorbic acid resulting from the excessive cholesterol intake. Animals capable of vitamin C synthesis have been known to respond to stress situations involving high vitamin C requirements by increasing the biosynthesis of this substance. Albino rats have been found to increase their urinary output of vitamin C or accumulate it in their tissues after inhalation of dichloroethane [11], furthermore at low environmental temperatures [12], after injuries [13], after administration of sodium ethylenediamine tetraacetate [14], etc. In opposition to these animal species, guinea-pigs, on exposure to similar stress factors, reduce the vitamin C concentration in their tissues [14—17] in the same manner as under the effect of a cholesterol diet.

It is not clear whether it is by producing a non-specific stress situation that cholesterol feeding increases the ascorbic acid demand or whether there is some specific relationship between cholesterol and ascorbic acid metabolism which might be involved in the mechanism. Cholesterol is hepatotoxic to rodents, by inducing fatty liver. By the administration of another hepatotoxic substance, tetrachloromethane, to guinea-pigs, we were able to induce changes similar to those seen after the administration of cholesterol, i.e. an increased urinary elimination of vitamin C and a reduction of its concentration in the tissues [18]. By analogy, it might be presumed that a cholesterol diet would increase the vitamin C requirement by acting on the liver. This would, however, not apply to rats which, as a result of long-term cholesterol feeding, showed an increased biosynthesis of ascorbic acid without developing any demonstrable biochemical or morphological disturbance of the liver. The possible role of atherosclerotic factors being involved in the effect of cholesterol may be equally ruled out since cholesterol-induced atherosclerotic changes develop far later than their effect on ascorbic acid metabolism witnessed in the present study [6].

The increased vitamin C demand as a result of cholesterol feeding raises the possibility of a specific involvement of ascorbic acid in cholesterol metabolism. Chronic vitamin C deficiency in the guinea-pig leads to an accumulation of cholesterol in the tissues and there is indirect evidence that ascorbic acid has a role in cholesterol catabolism [5]. In the light of this hypothesis the consequences of massive cholesterol intake on ascorbic acid metabolism might be interpreted as being due to an increased ascorbic acid demand owing to the enhanced breakdown of cholesterol.

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E. GINTER, CSc. Institute of Nutrition, Bratislava, nam. 4. aprila 7, Czechoslovakia

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EEG ABNORMALITIES IN NORMAL TWINS

By

J. LIPÁK, O. ZSADÁNYI, J. BAZSÓ and O. KLEININGER

DEPARTMENT OF NEUROLOGY AND PSYCHIATRY AND DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY, UNIVERSITY MEDICAL SCHOOL, DEBRECEN

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Sixty normal twins (8 monozygotic and 22 dizygotic pairs) between 5 and 17 years of age were studied. All had been born at term and none had had any disease affecting the nervous system. The investigations included, in addition to EEG tracings in the awake state, anthropologic measurements as well as neuropsychiatric and psychological studies.

EEG abnormalities were found in 38% of the cases (abnormal synchronization in 13%, steep waves in response to hyperventilation in 11%, dysrhythmia in 9%, focal convulsive activity in 5%), significantly higher than in normal single births. Age, birth-weight, order of birth, perinatal complications and zygosity were found to be unrelated to the EEG abnormalities, while being closely, if not significantly, related to subnormality of the nervous system.

The prevalence of EEG abnormalities is ascribed to minor prenatal damage to the brain owing to adverse factors inherent in twin pregnancy.

The problem at issue

The special conditions imposed on twins during intrauterine life (restriction of space, inadequacy of nutrition, birth injuries) put them at a disadvantage in comparison to single-born individuals. In earlier studies (LIPÁK et al. [1968], BAZSÓ et al. [1968]) we could demonstrate numerous anthropologic, neuropsychiatric or psychologic abnormalities in twins. The aim of the present study has been to clarify the relationships of these abnormalities with cerebral bioelectric activity, in view of the fact that EEG studies have thus far been confined to pathological twins with a view to ascertaining the genetic character of epilepsy (LENNOX [1942, 1960], POND [1953], COBB [1950], LÖWENBACH [1939], WILLIAMS [1944]). SCHMIDT et al. (1965) searched for relationships between birth order and EEG pattern. The scarce data derived from normal populations refer in the first line to the question of concordance within individual pairs (DUSTMAN [1965], HENSCHERT [1965]).

The questions to which we sought an answer were thus as follows.

(a) Are EEG abnormalities more frequent in normal twins than in normal single-born individuals, and, if so, of what kind?

(b) What is the explanation of the prevalence of EEG abnormalities in twins and with what other parameters are these changes correlated?

Material

Twins born between 1950 and 1962 at the Department of Obstetrics and Gynaecology, Debrecen University, were selected for study according to set standards. All individuals had been born at term, average duration of pregnancy having been 40 weeks. Both members of each pair were of homogeneous socio-economic and cultural background. None of them had any major nervous lesion, developmental defect or postnatal illness affecting the nervous system.

In this manner 60 twins belonging to the normal population were selected; one pair actually belonged to a set of triplets and the others were twins. Their age ranged between 5 and 17 years with a mean of 10.4 years. 17 pairs were older, 13, younger than 10 years. Of 8 monozygotic pairs 4 were girls, 4 boys. Of 22 dizygotic pairs 9 were both girls, 10 both boys and 3 mixed. As to sex distribution, 13 pairs were female, 14 male, and 3 mixed.

Method

Resting and evoked EEG recordings were made in all children in the alert state with a 16-channel Galileo-apparatus, 3 minutes hyperventilation being used for activation. Normal or abnormal activity was assessed in accordance with GIBBS' atlas (1952). All children without exception were submitted to the following routine studies:

a) Full clinical (paediatric) and ophthalmologic investigation.

b) Neuropsychiatric investigation.

c) Anthropologic study with the evaluation of twelve parameters (including blood group analysis by the method of MARTIN), for the assessment of somatic development and for the identification of zygosity (MARTIN and SALLER [1957]).

d) Intelligence studies by the BINET—BOBERTAG (BOBERTAG [1920]), ALEXANDER (1946) and GOODENOUGH (1926) tests.

e) Evaluation of visuomotor perception by BENDER's test (1957).

f) Evaluation of motor development by the OSERETZKY—GÖLLNITZ-test (GÖLLNITZ [1954]).

Statistical analysis was based on arithmetic means, percentage distribution, and the χ^2-test with 2×2-fold contingency tables.

Results

a) *Frequency and qualitative distribution of EEG abnormalities*

Distribution of cerebral bioelectric activity of 60 normal twins is seen in Table I and Fig. 1.

Table I

Incidence and distribution of EEG abnormalities

EEG	Incidence	
	No.	per cent
Normal EEG	37	62
Abnormal EEG	23	38
abnormal synchronization	8	13
HV-evoked steep waves	7	11
dysrhythmia	5	9
focal convulsive activity	3	5
Total number of twins	60	100

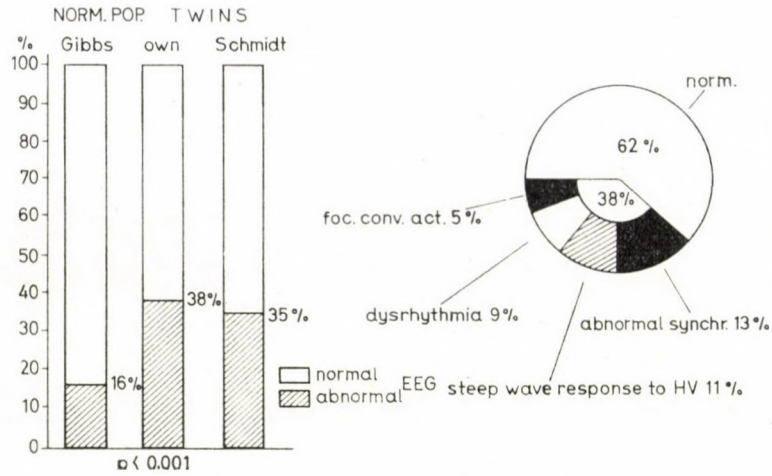

Fig. 1. Incidence and distribution of EEG abnormalities in twins

Activity was normal according to age in 62%, whereas 38% showed abnormalities. Considering that GIBBS—LENNOX (GIBBS et al. [1943]) quote a 16%, and PENFIELD (PENFIELD et al. [1954]) a 5 to 10% incidence of EEG abnormalities in normal single births, the EEG was at least twice as often abnormal in our twins than in a normal population of single births. The difference was highly significant ($p < 0.001$) and agrees with those of SCHMIDT (1965) who found 35% EEG abnormalities in twins.

b) *Correlation of EEG abnormalities with sex, age and birth-weight*

Comparison of children below and above 10 years of age revealed no correlation between age and EEG abnormalities. (Younger than 10 years: $N = 30$, abnormal EEG in 37%; older than 10 years: $N = 30$; abnormal EEG in 40% [$0.95 < p < 0.98$].)

The incidence was higher in girls than in boys ($N = 29$ with abnormalities in 49% as against $N = 31$ with abnormalities in 29%) but the difference failed to reach significance ($0.10 < p < 0.20$).

The twins of lower birth-weight weighed on the average 2250 g at birth, and their twin brothers or sisters of higher birth-weight, 2750 g. The difference was 500 g (18%). EEG abnormalities were present in 40% of the first and in 37% of the second group, a difference of less than 3% and statistically not significant ($0.70 < p < 0.80$). It remained the same when two groups were formed with a birth-weight of 2500 g as the dividing line ($N = 26$, EEG abnormalities in 38% *below* 2500 g as against $N = 34$, EEG abnormalities in 38% *above* 2500 g). A low birth-weight in itself is thus unrelated to EEG

Table II

Correlation of EEG abnormalities with age, sex and birth-weight

Group	Normal EEG		Abnormal EEG		Total		Significance
	No.	per cent	No.	per cent	No.	per cent	
Girls	15	51	14	49	29	100	
Boys	22	71	9	29	31	100	$0.10 < p < 0.20$
Total	37	62	23	38	60	100	
Under 10 years	19	63	11	37	30	100	
Over 10 years	18	60	12	40	30	100	$0.95 < p < 0.98$
Total	37	62	23	38	60	100	
Twins weighing less	18	60	12	40	30	100	
Twins weighing more	19	63	11	37	30	100	$0.70 < p < 0.80$
Total	37	62	23	38	60	100	
Birth weight							
less than 2500 g	16	62	10	38	26	100	
more than 2500 g	21	62	13	38	34	100	
Total	37	62	23	38	60	100	

abnormalities. The present figures are consistent with those quoted by SCHMIDT (*below* 2500 g, N = 56, with abnormalities in 34% as against N = 44, with abnormalities in 36% *above* 2500 g).

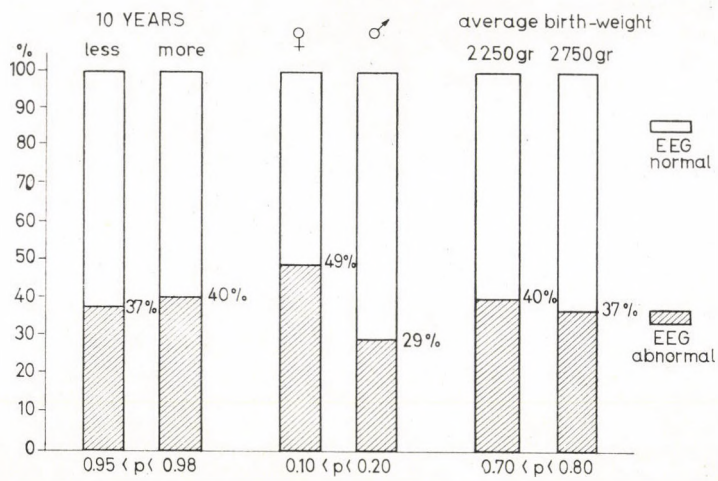

Fig. 2. Correlation of EEG abnormalities with sex, age, and birth-weight

Table III

Correlation of EEG abnormalities with birth order, perinatal complications and subnormalities of the nervous system

Group	Normal EEG		Abnormal EEG		Total		Significance
	No.	per cent	No.	per cent	No.	per cent	
Foetus A	16	53	14	47	30	100	
Foetus B	21	70	9	30	30	100	$0.10 < p < 0.20$
Total	37	62	23	38	60	100	
Perinatal complications							
present	25	67	12	33	37	100	
absent	12	52	11	48	23	100	$0.20 < p < 0.30$
Total	37	62	23	38	60	100	
Abnormal neurological findings							
present	22	53	19	47	41	100	
absent	15	79	4	21	19	100	$0.05 < p < 0.10$
Total	37	62	23	38	60	100	

c) *Correlation of EEG abnormalities with birth order, perinatal complications and neurological abnormalities*

Although the second foetus B delivered after the first A fares worse at birth than its twin, the EEG fails to reflect this; abnormalities having been found in 47% of group A and in 30% of group B. The difference did not reach significance ($0.10 < p < 0.20$), in accordance with the findings of SCHMIDT (A group, N = 50, with abnormalities in 34% as against N = 50, with abnormalities in 36%, in group B). Nor did the EEG abnormalities reveal any relationship with any of the various *complications* of pregnancy (e.g. threatening eclampsia or premature birth), delivery (breach or transverse presentation, Caesarean section) or of the postnatal phase (asphyxia) ($0.20 < p < 0.30$). EEG abnormalities were found in 33% of 37 children where one or more complications had been noted, and in 48% of 23 where there had been none. In opposition to this a close relationship bordering on significance was demonstrable between EEG abnormalities and *subnormality of the nervous system*, including the sense organs ($0.05 < p < 0.10$). EEG abnormalities occurring in 21% of the 19 neurologically intact children attained an incidence of 47% in the group of 41 children with subnormalities of the nervous system. None of the demonstrable neurologic signs (facial asymmetry, slight hypotonia, minimal hyporeflexia) were significant enough to be classed with any definite

neurological syndrome, as the changes were compatible with variations within the normal range. Considering, however, the disadvantageous constellation of twins from the very outset and the great number of subnormalities revealed by psychological tests, we had to regard these neurologic signs likewise as subnormal.

d) *Correlation of EEG abnormalities with zygosity. EEG concordance within pairs*

EEG abnormalities were found in 31% of 16 monozygotes and in 41% of 44 dizygotes. The difference did not reach significance ($0.30 < p < 0.50$). EEG identity within individual pairs was present in 63% of the 8 monozygotic

Table IV

Correlation of EEG abnormalities with zygosity

Group	Normal EEG		Abnormal EEG		Total		Significance
	No.	per cent	No.	per cent	No.	per cent	
Monozygotes							
female pair	6	75	2	25	8	100	
male pair	5	63	3	37	8	100	
Total	11	69	5	31	16	100	Monozygotes and dizygotes
Dizygotes							
female pair	8	44	10	56	18	100	$0.30 < p < 0.50$
male pair	14	70	6	30	20	100	
pairs of identical sex, total	22	58	16	42	38	100	
mixed pair	4	66	2	34	6	100	
dizygotes, total	26	59	18	41	44	100	
Twins, total	37	62	23	38	60	100	

Table V

EEG concordance and discordance within pairs

Group	Concordant EEG				Discordant EEG		Total EEG		Significance
	Norm.	Abnorm.	Total		No.	per cent	No.	per cent	
			No.	per cent					
Monozygotes	4	1	5	63	3	37	8	100	
Dizygotes	9	5	14	64	8	36	22	100	$0.90 < p < 0.95$
Total	13	6	19	64	11	36	30	100	

and in 64% of the 22 dizygotic pairs, there was thus no statistically significant difference ($0.90 < p < 0.95$). These results do not entirely agree with the published data. COBB (1950) and DAVIS (1936) found such a high incidence of EEG concordance within pairs that they relay on it for the identification of zygosity more than on anthropologic measurements. The visually evoked potentials showed significant correlations within pairs in monozygotic, as opposed to dizygotic, twins. LÖWENBACH (1939) and WILLIAMS (1944) found

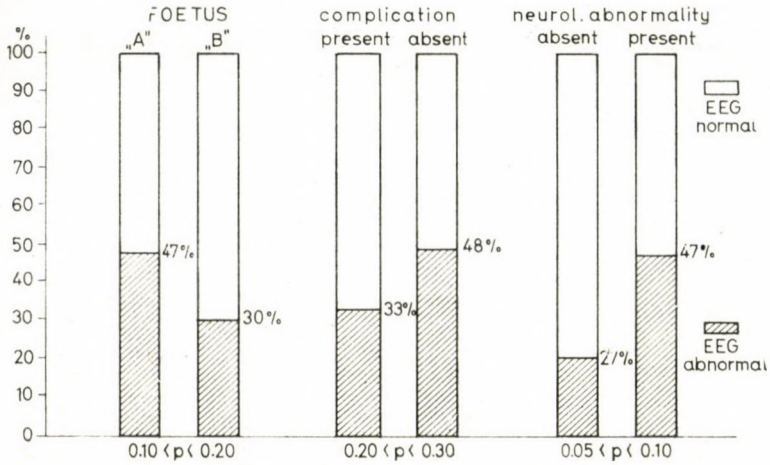

Fig. 3. Correlation of EEG abnormalities with birth order, perinatal complications, and abnormal neurological findings

a fully concordant EEG pattern within monozygotic pairs, particularly in petit-mal epilepsy, even when only one of the twins was epileptic and the other, on clinical evidence, apparently normal. However, the concordance greatly depends on the presence of cerebral injury. Damage to one monozygotic twin reduces concordance and, conversely, damage to both of dizygotic twins increases it. LENNOX, in a survey of 225 epileptic twins (1960) found concordance in 84.5% of intact monozygotes but only in 27% of those with cerebral injury. If cerebral injury is disregarded, the concordance in monozygotes averages 62.1% which is in close agreement with our figure of 63%. On the other hand, the concordance noted by LENNOX in dizygotes was as low as 14.6% which would seem to make our 64% figure excessively high. However, in our material the incidence of injuries was also considerably higher (69%) which sufficiently accounts for the higher concordance.

For the illustration of concordance and discordance within pairs some EEG tracings are presented.

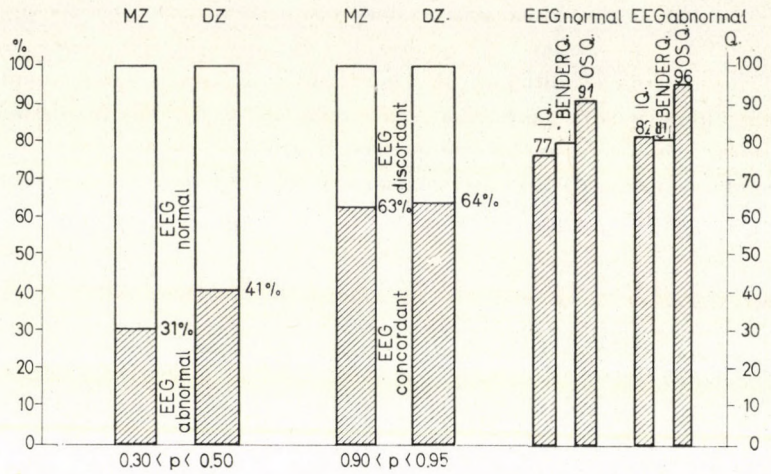

Fig. 4. Correlation of EEG abnormalities with zygosity (left side). EEG concordance and discordance within monozygotic and dizygotic pairs (middle). IQ, Bender-Q and Oseretzky-Q in the groups with normal and abnormal EEG (right side)

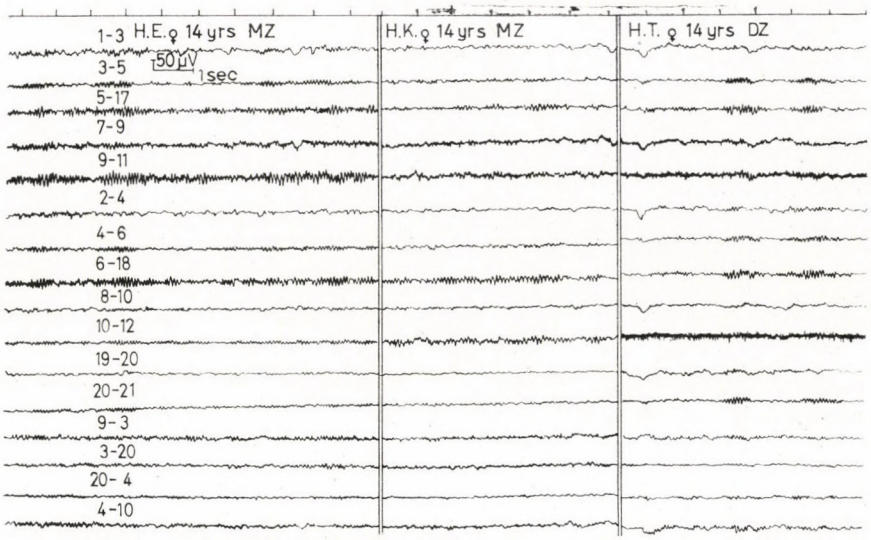

Fig. 5. Waking electric activity of 14-year-old female triplets: Eva and Katalin, monozygotes, Theodora, dizygote. Eva (left): even, regular ground rhythm of 10 to 11 c/s with no side-difference. Wave patterns of slightly higher amplitude in comparison to the others. Katalin (middle): regular alpha-activity of 10 to 11 c/s with spindles. No side-difference. Theodora (right): basic rhythm of 10 to 11 c/s with spindles. No side-difference

Fig. 5 shows the tracings of 14-year-old female triplets. Eva and Katalin (left side and middle of the figure) are monozygotes, Theodora (right side)

is a dizygote. Electric activity was practically identical in all three. All were delivered of transverse presentation.

Eva, foetus A, weighed 1850 g at birth. Apart from hypermetropy, there was no evidence of any abnormality. IQ = 70, Bender-Q = 87, Oseretzky-Q = 91.

Katalin, foetus B, weighed 2100 g at birth. No neurological abnormality. IQ = 71, Bender-Q = 68, Oseretzky-Q = 80.

Theodora, foetus C, weighed 2700 g at birth. Congenital valvular defect. IQ = 86, Bender-Q = 123, Oseretzky-Q = 94.

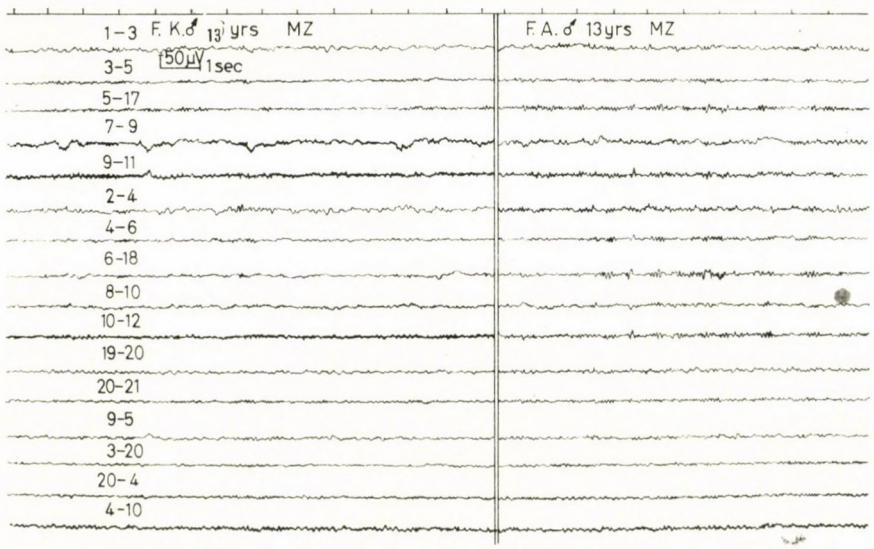

Fig. 6. 13 -year-old monozygotic male twins. Károly (left): regular ground rhythm of 9 to 11 c/s, slightly flattened wave activity. András (right): slightly dysrhythmic basic activity of 9 to 11 c/s. The parieto-occipital (5—17, 6—18) and posterotemporal (9—11, 10—12) leads show some slightly prominent steep waves with a broad basis, distinct from the basic activity

Fig. 6 shows the discordant leads of a pair of monozygotic 13-year-old boys. Károly, foetus A, weighing 2800 g at birth had a normal neurological status. IQ = 42 (by the GOODENOUGH-test only), Bender-Q = 76, Oseretzky-Q = 81. Foetus B, András, was born of transverse presentation and weighed 2950 g at birth. He had a slight facial asymmetry. IQ = 52 (by the GOODENOUGH-test only), Bender-Q = 55, Oseretzky-Q = 82.

The tracings of a dizygotic pair of 5-year-old boys are seen in Fig. 7. Foetus A, Sándor, having been delivered of transverse presentation, with 2400 g weight, exhibited a convulsive electric activity but was normal clinically and never displayed epileptic signs but has still a speech defect with a lips. IQ = 66 (according to GOODENOUGH), Bender-Q = 77, Oseretzky-Q = 70. Foetus B, Árpád, weighing 3000 g at birth after a delivery of transverse pre-

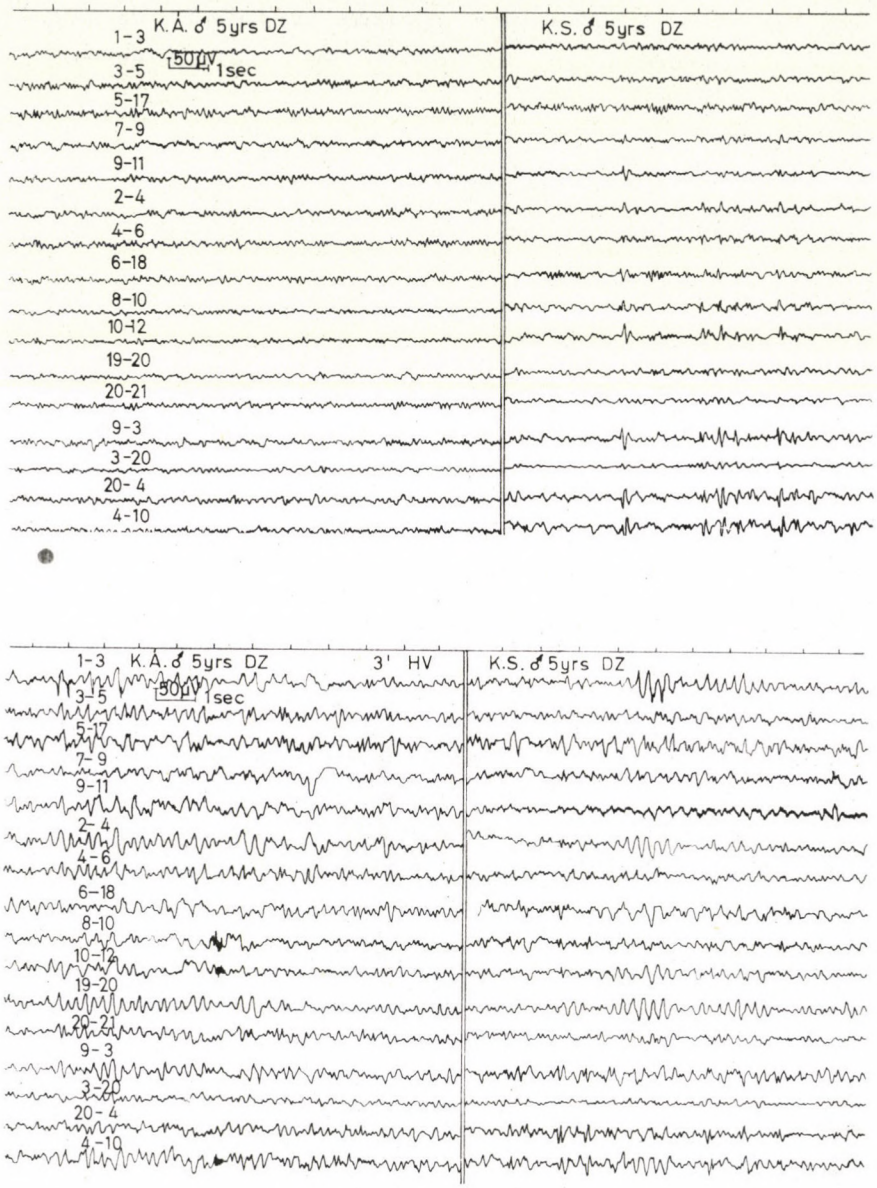

Fig. 7. Tracings of 5-year-old male dizygotes. Árpád (left): slightly irregular basic activity of 7 c/s, with no side-difference. Sándor (right): basic activity of 7 c/s with distinct disorganization. The posteromedian temporal (9—11, 10—12) and coronal (9—3—20—4—10) leads show synchronous high-amplitude steep waves with a broader basis; convulsive activity. The lower figure shows the tracings of the same twins after 3 minutes hyperventilation

sentation had a speech defect similar to that of this twin brother and showed a minor inaccuracy of the right side in pointing at objects. IQ = 63 (according to GOODENOUGH), Bender-Q = 70, Oseretzky-Q = 87.

e) *Correlation of the EEG abnormalities with the IQ, motor development and visuomotor perception*

In the twins with a normal electrical activity, the intelligence quotient (IQ = 77), motor development (Q = 91) and visuomotor perception (Q = 79) did not differ significantly from the findings in those with EEG abnormalities (IQ = 82, Oseretzky-Q = 96 and Bender-Q = 81). There seems to be thus

Table VI

Mean IQ, visuomotor perception (Bender) and motor development (Oseretzky) in the groups with normal and abnormal EEG

EEG	No.	Average IQ	Bender-Q	Oseretzky-Q
Normal	37	77	79	91
Abnormal	23	82	81	96
Total	60	80	80	94

no relationship between the cerebral bioelectric abnormalities and the ratings of the performance tests. In a study of aged monozygotic twins, HENSCHERT (1965) likewise failed to demonstrate any correlation between intelligence and EEG discordance within pairs.

Discussion

The first point emerging from the present findings is a high incidence of EEG abnormalities in a normal population of twins as compared to normal single-born individuals. The changes were unassociated with clinical signs. Even in the past history of twins with a convulsive activity there was no indication of epilepsy or of epileptic equivalents. At the interpretation of neurological or EEG findings in twins this fact should not be neglected.

The second conclusion to be drawn connects these EEG abnormalities with cerebral injury as suggested by their correlation to subnormalities of the nervous system. The lesions are obviously slight, owing to the absence of any clinical, particularly focal, manifestation.

The third point concerns the time of origin of the cerebral lesion. The high incidence of EEG changes in twins as compared to single-born individuals rules out the possibility of a genetic determination, but points to the role of environmental factors which tend to reduce the otherwise high concordance in monozygotic twins and to raise the otherwise low concordance in dizygotic twins. This accounts for the fact that the EEG concordance within pairs was practically identical in monozygotic and dizygotic twins.

We may likewise rule out perinatal injuries as possible factors of EEG abnormalities, since they showed no correlation whatever with birth order or perinatal injuries. Postnatal diseases as a factor can also be ruled out, since there were none in the present material.

All this places the origin of cerebral injury into the prenatal period. In the uterus, twins are at a disadvantage as compared to single foetuses. Restriction of surface for placentar implantation, sharing of intrauterine space, nutrition and blood supply are all sources of hazards to the vulnerable nervous system of the foetus and it has been shown that intrauterine nutritional deficiency may give rise to permanent neuropsychiatric abnormalities (LIPÁK 1968). The EEG changes may be ascribed to structural changes of similar origin, generally too small to produce focal manifestations.

It must be emphasized that the prevalence of EEG subnormalities in twins by no means implies their inferiority to single-born individuals. In our material, IQ, motor development and visuomotor perception were entirely unrelated to the EEG changes and in fact we found in the present study more than one pair of twins with a high level of intelligence.

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Dr. János Lipák, Dr. Ottó Zsadányi, Dr. Ottó Kleininger — Department of Neurology and Psychiatry, University Medical School, Debrecen 12, Hungary

Dr. János Bazsó, Department of Obstetrics and Gynaecology, University Medical School, Debrecen 12, Hungary

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RESPONSE TO SALURETICS AND ORAL ANTIDIABETICS IN DIABETES INSIPIDUS AND PSYCHIC POLYDIPSIA

By

F. A. László, L. Czakó, I. Szijj and K. Kovács

FIRST DEPARTMENT OF MEDICINE, UNIVERSITY MEDICAL SCHOOL, SZEGED

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The effects of saluretic drugs (spironolactone, chlorothiazide and chlorothiazide + spironolactone) and of oral antidiabetic agents on fluid- and electrolyte balance have been studied in ten patients with diabetes insipidus with no residual ADH and in four patients with psychic polydipsia.

The diabetes insipidus patients displayed a reduction of urinary output and a moderate increase of specific gravity in response to diuretics. The drugs of all three types produced an increase in sodium excretion. Chlorothiazide had a marked stimulatory effect on potassium excretion, unless given in combination with spironolactone which was found to inhibit this effect. On the other hand, the antidiuretic effect of chlorothiazide was potentiated by spironolactone. Endogenous creatinine clearance diminished during the administration of the drugs, particularly of chlorothiazide. In the case of psychic polydipsia the saluretics had no demonstrable effect on water metabolism.

The subjects with diabetes insipidus exhibited a far more marked antidiuretic response to chlorpropamide than to the saluretics and increased their urinary specific gravity to a higher degree. Excretion of sodium and potassium in the urine and endogenous creatinine clearance remained unaffected. A distinct reduction of urinary output in response to chlorpropamide was seen in the cases of psychic polydipsia as well. CCHC had none of these effects.

On the grounds of the results, chlorpropamide and the combination of chlorothiazide and spironolactone seem to be of use in the long-term management of diabetes insipidus. Their evaluation from this aspect requires further observations.

The successful use of hormone preparations in diabetes insipidus still leaves us with practical problems as regards the long-term management of this disease. ADH administered in injectable form or by nasal insufflation leads to unpleasant side-effects in numerous cases over the years. In 1959, Crawford and Kennedy [10, 29] found the diuretic agent chlorothiazide to reduce urinary output in diabetes insipidus. Having been able to confirm this finding, we found in later studies that in psychic polydipsia urinary output was increased rather than diminished by chlorothiazide [14, 31]. The long-continued use of chlorothiazide results, however, in profound changes of electrolyte metabolism, first of all in potassium depletion. Search for further therapeutic possibilities has revealed that spironolactone and chlorpropamide had an antidiuretic effect in diabetes insipidus.

The aim of the present study was to investigate the effect of some drugs on water balance in diabetes insipidus as also in psychic polydipsia.

Patients and methods

Ten subjects with diabetes insipidus, six males and four females with a mean age of 33 years, and four with psychic polydipsia, two males and two females with a mean age of 23 years, have been studied. The essential features relating to those with diabetes insipidus are shown in Table I. Diagnosis had been established on daily urinary output, specific gravity of urine, concentration test, oral water and salt loading, CARTER—ROBBINS' test, response to ADH. None of the patients had any residual ADH, and there was no evidence of anterior pituitary hypofunction or vasopressin-resistance in any of the cases. The psychic polydipsia patients exhibited a substantial ADH mobilization in response to osmotic stimuli. None of the individuals under study revealed heart, kidney, or liver disease.

The drugs were given for six-day periods, with the patients on a standard diet (Na content, 300 mEq per day). During the first period all medication was withheld, subsequently the effects of the individual drugs on water metabolism were studied. The drugs under study were administered in the following doses. Spironolactone (Aldactone, Searle) 100 mg four times daily; chlorothiazide (Chlorurit, Chinoin) 0.5 g four times daily; chlorothiazide 0.25 g + spironolactone 50 mg, four times daily; chlorpropamide (Diabinese, Pfizer) 250 mg two to three times daily; chlorcyclohexylcarbamide (CCHC, Oradian, Chinoin) 200 mg three times daily. Before changing over to the next drug, the patients were kept drug-free until their daily urinary output had returned to the pre-treatment level. The necessity for a sufficiently long observation made it impossible to study all drugs in each case.

The registered parameters were 24-hr. urinary output, urinary specific gravity, Na and K content, daily creatinine output, endogenous creatinine clearance, serum Na and K levels, and, during the administration of oral antidiabetics, the blood sugar level.

Sodium and potassium were determined by flame photometry, creatinine by the method of FOLIN and WU [18] as modified by BROD and SIROTA [6], and blood sugar by the ortho-toluidine method.

Results

Although all the said parameters have been examined in each instance, the data thus obtained were too numerous to be presented in their totality in respect of each individual case. Registration of daily urinary output seemed to be the most informative. Table II presents the pre-treatment values and the average urine output between the third and sixth day of drug treatment, this being the period necessary for the manifestation of an antidiuretic effect. Spironolactone was thus found — except in Case No. 4 — to reduce 24-hr. urinary output by 30 to 60%. The response to chlorothiazide with and without spironolactone was similar or even more marked. The most significant antidiuretic effect was exerted by chlorpropamide, which (except in Case No. 8) caused a 50 to 80% reduction in daily urinary output. CCHC produced little change, with the exception of Case No. 3, where the daily urinary output fell to 50% of the control. On the other hand, in psychic polydipsia, chlorothiazide and spironolactone caused a moderate increase in urinary output while chlorpropamide had a slight antidiuretic effect. CCHC failed to produce any change in two cases while in Case No. 13 it reduced urinary output to half its original volume.

One of the subjects with diabetes insipidus (Case No. 6) has been selected for detailed analysis as his response to the drugs was found characteristic (Fig. 1).

Table I

Serial No.	Initials	Age (years)	Sex	History
1	M. I.	64	F	Polyuria of unidentified origin appeared 4 or 5 years ago. Daily urinary output 12 to 15 l. Therapy: nasal insufflations of pituitary extract
2	S. M.	26	F	Sudden onset of polydipsia and polyuria (9 l daily) in 1956 subsequent to a febrile disease with frequent stools containing blood (probably dysentery). Therapy: vasopressin-tannate by daily injections owing to intolerance to nasal insufflation
3	U. J.	24	M	Onset of polydipsia and polyuria (9 to 10 l/day) at 3 years of age after an operation for otitis media. Nasal insufflations and vasopressin in itself poorly tolerated
4	C. J.	29	F	Obese since childhood. Present body-weight 124 kg. Gradual onset of polyuria (15 l per day) eight to ten months before admission. Diabetes mellitus, intolerance to ADH. Therapy: chlorpropamide
5	F. P.	40	M	Polydipsia and polyuria (30 to 40 l daily) consecutive to a cranial injury in 1953. Therapy: vasopressin-tannate
6	M. J.	49	M	Onset of polyuria (6 to 8 l daily), after cranial injury with unconsciousness in 1941. No drug treatment
7	B. S.	30	M	Polydipsia since 1958. Primary diagnosis: HAND—SCHÜLLER—CHRISTIAN's disease. Bone defects of skull and upper jaw. Previous X-ray and steroid therapy. Urinary output 13 to 15 l daily. Nasal insufflation of pituitary extract and oral chlorothiazide
8	C. G.	15	M	Gradual onset of polydipsia of unknown origin at 10 years of age. Urinary output 6 to 8 l daily. Intolerance to ADH
9	L. L.	54	F	Sudden onset of polydipsia and polyuria (5 to 6 l daily) after childbirth in 1941. No drug treatment
10	K. Gy.	21	M	Meningo-encephalitis at 4 years of age, followed by polyuria (8 to 9 l daily) and mental retardation. Therapy: vasopressin-tannate

The specific gravity of urine increased in response to the drugs. The value of 1001 to 1002 in the untreated subjects with diabetes insipidus rose to an average of 1005 during treatment, chlorpropamide produced concentrations as high as 1008 to 1010. CCHC failed to affect specific gravity.

In the diabetes insipidus patients on the standard diet, daily mean urinary sodium output was in the range of 200 to 250 mEq in the control period, and exceeded 400 mEq in the first days of chlorothiazide or chloro-

Table II

Serial No.	Initials	Age, years	Sex	Diagnosis	Urinary output (l per 24 hours)					
					Untreated	Spironol-actone	Chlorothia-zide	Spironolac-tone + chlo-rothiazide	Chlor-propamide	CCHC
1	M. I.	64	female	Diabetes insipidus	14.3	7.4	3.3	4.5	4.4	11.1
2	S. M.	26	female	Diabetes insipidus	9.0	6.4	—	6.4	4.8	—
3	U. J.	24	male	Diabetes insipidus	9.8	4.8	4.8	4.1	4.0	4.8
4	C. J.	29	female	Diabetes insipidus, diabetes mellitus	15.0	16.2	14.6	11.7	7.6	17.6
5	F. P.	40	male	Diabetes insipidus	34.0	22.5	24.6	24.0	—	30.3
6	M. J.	49	male	Diabetes insipidus	8.5	3.7	4.8	4.0	1.8	7.6
7	B. S.	30	male	HAND—SCHÜLLER—CHRISTIAN's disease, diabetes insipidus	14.1	8.1	6.7	5.5	3.3	10.2
8	C. G.	15	male	Diabetes insipidus	7.8	5.9	3.9	4.2	5.9	—
9	L. L.	54	female	Diabetes insipidus	5.9	—	2.4	2.3	—	4.8
10	K. Gy.	21	male	Diabetes insipidus	8.5	4.0	2.5	—	2.8	6.3
11	B. K.	20	female	Psychic polydipsia	4.0	3.9	4.4	7.3	3.2	—
12	L. F.	26	male	Psychic polydipsia	5.0	4.8	5.3	5.4	4.1	4.8
13	T. E.	17	female	Psychic polydipsia	5.2	4.1	5.2	5.6	4.5	2.5
14	P. H.	27	male	Psychic polydipsia	5.0	4.2	5.3	5.1	5.4	5.2

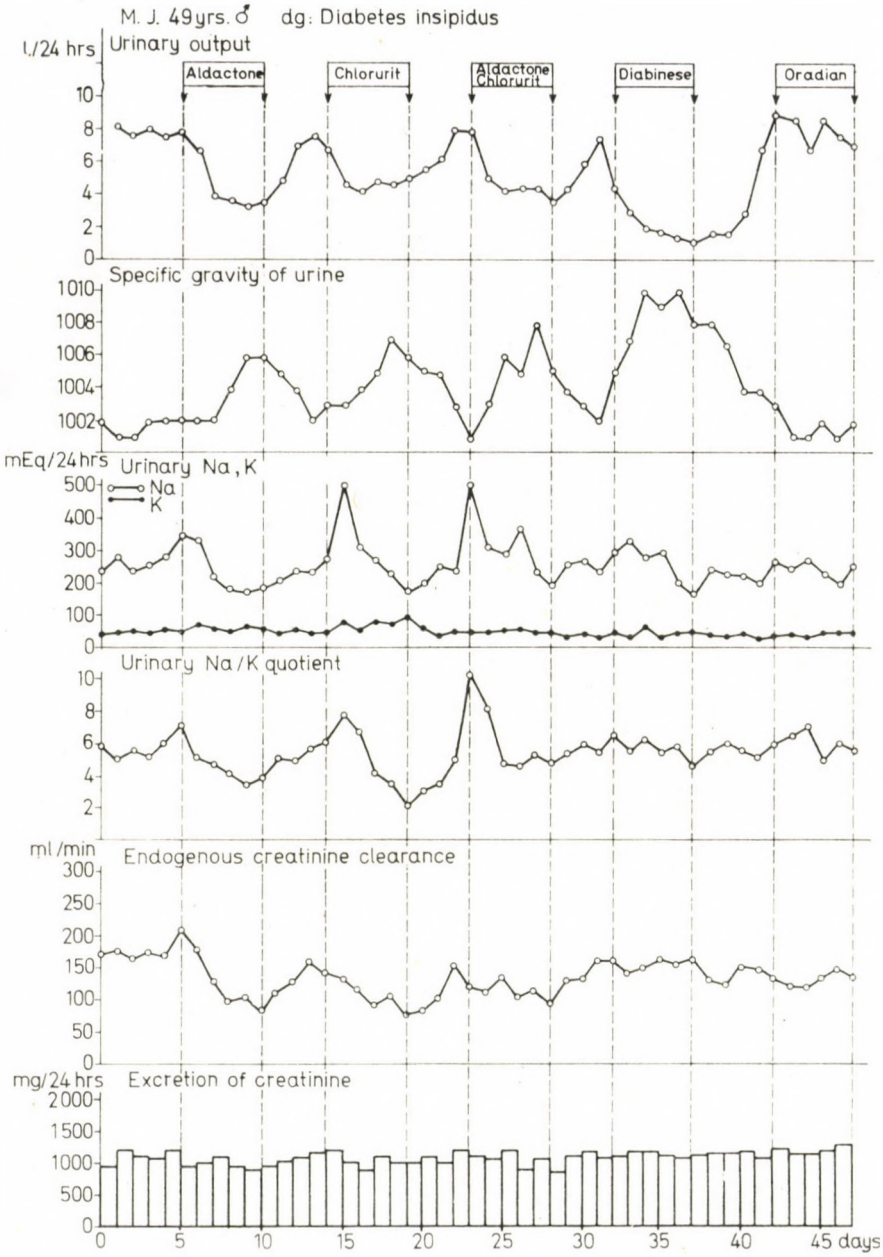
M. J. 49yrs. ♂ dg: Diabetes insipidus
l./24 hrs Urinary output
Aldactone
Chlorurit
Aldactone Chlorurit
Diabinese
Oradian
Specific gravity of urine
mEq/24hrs Urinary Na, K
Na
K
Urinary Na/K quotient
ml/min Endogenous creatinine clearance
mg/24 hrs Excretion of creatinine
0 5 10 15 20 25 30 35 40 45 days

Fig. 1

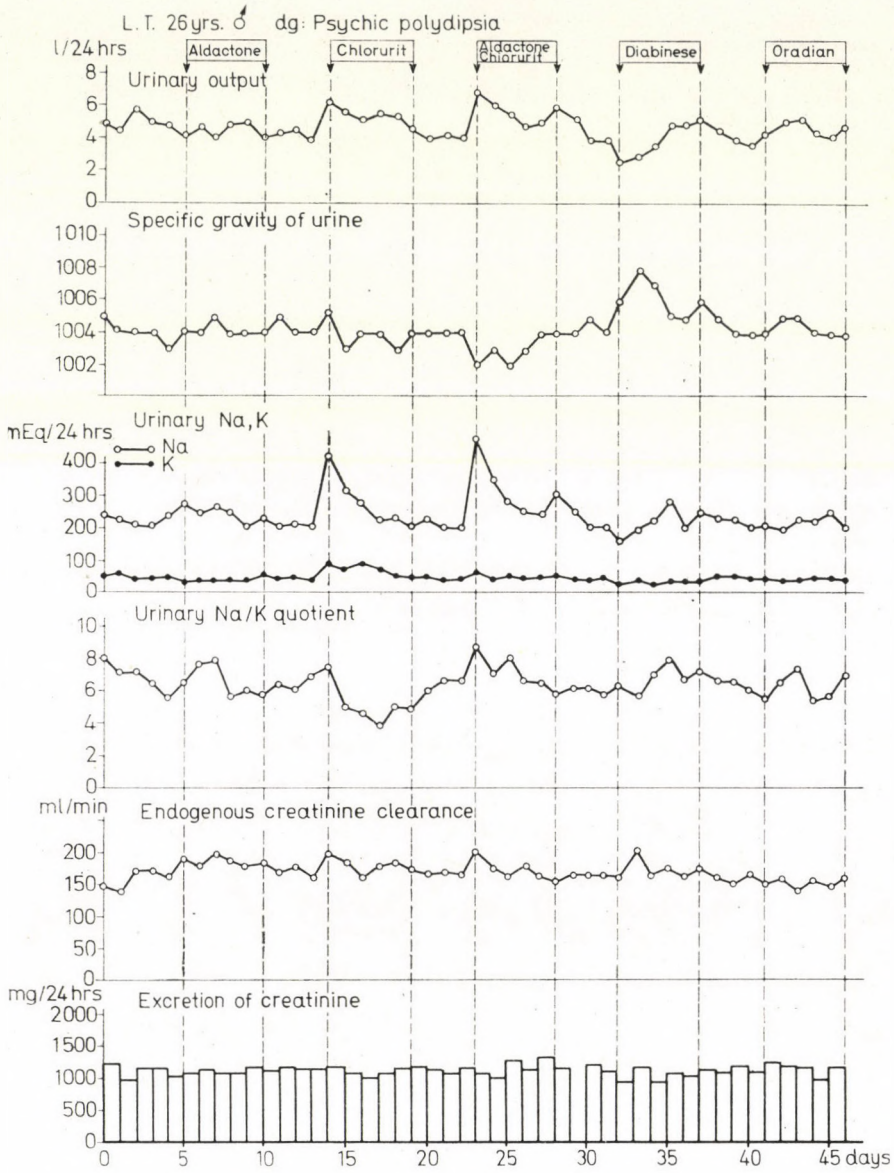

Fig. 2

thiazide + spironolactone administration, requiring a few days to return to its original values. Spironolactone in itself had a moderate effect on natriuresis. Urinary potassium excretion was raised only by chlorothiazide. If, however, it was combined with spironolactone, potassium excretion scarcely exceeded the control values. The oral antidiabetic agents under study had no

noteworthy influence on the urinary excretion of sodium and potassium, and consequently none on the Na/K quotient. There was a transitory rise in this quotient (from 5—7 to 9—10) in response to the combined use of saluretics. Spironolactone and chlorothiazide caused a slight elevation of this figure. This was followed by a moderate reduction on spironolactone and a marked one on chlorothiazide (to 4 and 2, respectively). The changes in the serum sodium and potassium levels have not been presented separately. Only chlorothiazide produced a slight hypopotassaemia, the average level of 5 mEq/l gradually decreasing to 3.8 mEq/l by the fifth day of treatment. Spironolactone inhibited this reduction. Chlorpropamide was found to reduce the blood sugar level of 88 mg to 70 mg per 100 ml.

In creatinine excretion there was no significant change. Endogenous creatinine clearance decreased moderately in response to spironolactone, chlorothiazide + spironolactone, and markedly in response to chlorothiazide (from 150 to 170 ml/min. to 80 to 100 ml/min.).

Data of a subject with psychic polydipsia showing typical responses are presented in Fig. 1. The changes in urinary output have been discussed earlier (Table II). Specific gravity was higher as in the cases of diabetes insipidus even in the drug-free intervals and decreased moderately during the administration of spironolactone + chlorothiazide whereas it increased in the first days of chlorpropamide treatment. Chlorothiazide caused an increase in sodium and potassium excretion, the latter being slightly more protracted. Combined chlorothiazide + spironolactone resulted in increased sodium excretion without affecting that of potassium. The Na/K quotient varied in accordance with these responses. It was slightly reduced by chlorothiazide and slightly increased by spironolactone + chlorothiazide. Endogenous creatinine clearance and excretion of creatinine were little affected by the drugs under study.

Discussion

From the present results it emerges that saluretic agents reduce diuresis, slightly increase urinary specific gravity, enhance sodium excretion and — particularly in case of chlorothiazide — reduce endogenous creatinine clearance in diabetes insipidus. On the other hand, in psychic polydipsia the response to these drugs is confined to an increased sodium excretion with the other parameters remaining unaffected.

The antidiuretic effect of chlorothiazide in diabetes insipidus was described by CRAWFORD and KENNEDY in 1959 [10, 29]. The mechanism of this effect has been extensively studied, but no satisfactory explanation has been found.

It seemed obvious to assume that the diuretics had a direct ADH-like action on the tubular epithelium, an interpretation which in fact had many advocates [7, 15, 28, 37] but which seems to be incompatible with later evi-

dence. It has been found for instance that osmotic stimuli (hypertonic saline, mannitol) fail to enhance the effect [21]. Moreover, these drugs may have an antidiuretic effect even when the kidney fails to respond to ADH, as reported in case of renal diabetes insipidus [9, 12, 37]. The finding that diuretics of the chlorothiazide type produce a urine of slighter osmolality than does the minimum effective dose of ADH [17] is a further evidence against the ADH hypothesis. In earlier papers [14, 31] we also pointed out that chlorothiazide is devoid of an antidiuretic effect in cases of polydipsia with adequate stores of vasopressin, an observation consistent with the results of the present study. Under this aspect it is of interest to note the observation by EARLY and ORLOFF [17] that addition of saluretic agents to an in vitro system fails to enhance the permeability of the ADH-responsive frog bladder.

Although MIGONE and AMBROSOLI [37] found no relationship between the natriuretic effect of diuretics and their antidiuretic activity in diabetes insipidus, the sodium deficiency produced by these drugs is none the less regarded as the principal factor of the response [8, 12, 21, 26, 29, 43]. There is evidence suggestive of sodium depletion being involved in the mechanism. Subjects with diabetes insipidus reduce their urinary output when kept on a low sodium diet even in the absence of any therapy [17, 20, 32]. NaCl deprivation has been found to enhance [17, 20, 21, 32], NaCl loading to reduce [17, 21, 23, 24, 30] the antidiuretic effect of chlorothiazide, and urinary output remains low even after suspending chlorothiazide treatment if NaCl intake is kept at a minimum level [11, 17, 24]. The antidiuretic effect of saluretic agents ceases, however, as soon as NaCl is ingested in liberal amounts [11, 21, 24].

The dietary NaCl intake of the subjects under study was 300 mEq Na per day. The antidiuretic effect in the diabetes insipidus patients may have been more intensive had we kept them on a low-salt diet.

The next problem at issue concerns the mechanism by which Na depletion leads to an antidiuretic effect. It was assumed at first that Na depletion induced by diuretic agents [17, 23, 30] elicits an increased production or release of aldosterone [10, 33] which in turn leads to a reduction of sodium loss [17] and of diuresis. Administration of aldosterone antagonists offers the best approach to this problem. It was in fact with this aspect in view that KENNEDY and CRAWFORD [30] administered spironolactone to rats with diabetes insipidus. The result was a gradual reduction in urinary output. Later workers found no antidiuretic effect of spironolactone in diabetes insipidus, probably because of having used inadequate doses [21, 44]. Subsequent studies have actually shown the drug to potentiate the antidiuretic effect of chlorothiazide [44] instead of inhibiting it [17, 21], moreover to produce an antidiuretic effect of some degree in diabetes insipidus [32, 35], this latter finding being consistent with our own observations.

However, the widely accepted explanation links up the antidiuretic effect of saluretics in diabetes insipidus with changes in renal haemodynamics. Sodium depletion leads to hypovolaemia and thus decreases GFR [23]; if the reduction of GFR attains 30 to 70%, an increase in the specific gravity of the urine and a decrease of urinary output ensue even in case of ADH deficiency [4], in accordance with our observations in rats with polyuria subsequent to destruction of the pituitary stalk [31]. Other workers found, however, no reduction in GFR [9, 35, 39] or none accounting for a reduced urinary output [11]. It is, in addition, hard to reconcile with the theory that administration of chlorothiazide leaves GFR unaffected [5, 25], furthermore that perfusion of the kidney with diuretics fails to raise renal vascular resistance [22]. GILLENWATER [21] rejects the claim of GFR being the responsible factor, if for no other reason then because the changes in the fluid compartment and in electrolyte distribution are too slight to produce a major reduction in GFR. Indeed, in the present study the serum Na level remained unchanged throughout.

Chlorothiazide has been assumed to affect, in addition to the distal tubules, also the proximal ones [11, 13, 42]. To clarify this question we would have to know more about the normal physiological role of the proximal tubules than we actually do [21].

Chlorothiazide as also spironolactone produce sodium depletion accompanied by a fall in serum osmolality which controls water intake by the sensation of thirst. On the other hand, water excretion remains unchanged and, as a result, water depletion ensues [32, 41]. This interpretation takes no account of the generally accepted view which regards the excessive water intake in diabetes insipidus merely as a compensatory process, although certain authors [34, 38] do emphasize the importance of the thirst mechanism. The fact that the changes in serum osmolality and in antidiuresis are by no means congruent, adds to the difficulties of the problem [21].

The various theories thus leave the antidiuretic mechanism of saluretic drugs in diabetes insipidus largely unexplained. The present results have failed to furnish conclusive proof in favour of any of them. It is hoped to gain insight into the fundamental aspects of water metabolism by further studies into the effect of these diuretics.

Chlorpropamide produces changes which considerably differ from those characterizing the effect of saluretics. The antidiuretic effect is particularly marked in diabetes insipidus, the increase in urinary specific gravity is more distinct, the urinary excretion of sodium and potassium as well as endogenous creatinine clearance remain unaffected, and there may be a reduction of urinary output in psychic polydipsia as well.

The hypoglycaemic effect of chlorpropamide is known to result from mobilization of insulin from the pancreas [16, 27]. The mechanism of its anti-

diuretic effect is, however, obscure. ARDUINO et al. [2] assumed chlorpropamide to stimulate the pituitary secretion or mobilization of ADH, or else, to restore the responsiveness of the osmoreceptive centres to physiologic stimuli. This would be, as pointed out by MEINDERS et al. [36] at variance with the finding that chlorpropamide does have an antidiuretic effect in congenital diabetes insipidus, i.e. in the lack of ADH. Our own observations also indicate that chlorpropamide exerts its antidiuretic effect even in cases where no mobilizable ADH is present. On the grounds of studies with intrahypothalamic implantation of chlorpropamide, BERGMANN et al. [3] attribute the antidiuretic effect of this drug to a central suppression of the thirst mechanism. The possibility of a direct effect of chlorpropamide on the kidneys, where it is presumed to produce an ADH-like effect, has been pointed out by ARDUINO et al. [2], MEINDERS et al. [36] and REFORZO-MEMBRIVES et al. [40], an assumption consistent with the observation that chlorpropamide has no antidiuretic effect in vasopressin-resistant renal diabetes insipidus [2, 19], furthermore that it reduces urinary output in normal individuals and, as it has been shown in the present study, in subjects with psychic polydipsia.

In view of these considerations, the study of the antidiuretic effect of diuretic and antidiabetic agents in diabetes insipidus not only involves important pathophysiologic aspects but also opens up new therapeutic possibilities. The present acute experiments are obviously unsuited for the evaluation of a long-term therapy. Although we have had a few patients on maintenance therapy, these observations are not conclusive enough to warrant publication.

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Dr. Ferenc A. László
Dr. László Czakó
Dr. Ilona Szijj
Dr. Kálmán Kovács

I. Belklinika Szeged, Korányi rkp. 12, Hungary

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EFFECT OF LIVER INJURY AND OF INDUCED INFLAMMATION ON THE SERUM GLYCOPROTEIN LEVEL

By

J. FEHÉR, L. JAKAB and Livia TAKÁCS

THIRD DEPARTMENT OF MEDICINE, UNIVERSITY MEDICAL SCHOOL, BUDAPEST

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The serum levels of glycoprotein fractions hexose, hexosamine, sialic acid, and seromucoid in rats treated with croton oil, CCl_4 and their combination have been studied. In croton oil-induced inflammation the hexosamine, sialic acid and seromucoid levels increased, they were significantly elevated in CCl_4-induced liver injury as well. In croton oil-induced inflammation associated with liver injury, the concentrations of hexose, hexosamine and sialic acid were significantly increased in comparison with the values demonstrable in CCl_4-induced liver injury in the absence of inflammation.

The cause underlying the changes in serum glycoprotein (GP) level associated with experimental inflammation and tissue injury has yet to be clarified. Accumulation in the damaged tissue of GP and GG (glycosaminoglycane) has amply been demonstrated (SHETLAR et al. [1959], BERENSON and DALFERES [1960], FISHKIN and BERENSON [1961], HOUCK et al. [1962], HOUCK and JACOB [1964], ANTONOPOULOS et al. [1965], PRODI and LASCHI [1965], RASMUSSEN [1967]), and so has the increase in the serum GP level (SHETLAR et al. [1958], NEUHAUS et al. [1961], NEUHAUS and LIU [1964], VARGA et al. [1966], JAKAB and FEHÉR [1968]). The origin of GP accumulated in the tissues is likewise a point of disagreement. The possibility of increased local synthesis (BERENSON and DALFERES [1960], FISHKIN and BERENSON [1961], HOUCK et al. [1962], PRODI and LASCHI [1965]), of local breakdown, as well as of increased deposition in the tissues (VARGA et al. [1966]) have been raised on theoretical grounds. Our own studies seem to indicate an increased local production (JAKAB and FEHÉR [1968]).

The proponents of increased deposition in the tissues incriminate an enhanced synthetic activity of the liver for the increase in the serum GP level.

It seemed therefore interesting to examine the part played by liver injury and induced inflammation in changes of the serum GP level.

Material and methods

Seventy albino rats of identical breed with body-weights between 180 and 200 g were used. Five groups were set up.

1. Fourteen animals were killed by decapitation without any previous treatment.
2. Ten animals were injected intragluteally into the right side with 0.2 ml of 0.9 % saline on six occasions, i.e. over two weeks and were decapitated on the 14th day.

3. Fifteen animals were injected intragluteally into the left side with a mixture of 0.05 ml croton oil +0.05 ml sunflower oil on a single occasion, and were decapitated five days later (three previous deaths).

4. Seventeen animals were injected intragluteally into the right side with 0.2 ml carbon tetrachloride every second day on six occasions, i.e. over two weeks, and were decapitated on the 14th day (two previous deaths).

5. Fourteen animals were injected intragluteally into the right side with 0.2 ml carbon tetrachloride every second day over two weeks and, simultaneously with the fifth injection (on the 9th day), a mixture of 0.05 ml croton oil +0.05 ml sunflower oil was injected into the left gluteal region, then the animals were decapitated on the 14th day (one previous death).

After decapitation the animals were dissected, the internal organs and the sites of local application of the substances were studied. Histological sections were prepared from the liver and the gluteal subcutaneous connective tissue, and stained with haematoxylin-eosin, PAS and toluidine blue.

In the blood samples the following fractions were estimated: hexose (galactose and mannose) by the orcin reaction according to STARY et al. (1950); hexosamine (galactosamine and glucosamine) by the ELSON—MORGAN reaction according to SWANN and BALÁZS (1966); sialic acid by the diphenylamine reaction according to AYALA et al. (1951); and seromucoid on the grounds of its hexose content, according to WEIMER and REDLICH-MOSHIN (1952).

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

Results

Groups 1 and 2 exhibited no appreciable tissues changes. In groups 3 and 5 there was considerable swelling, oedema of the subcutaneous connective tissue, haemorrhagic inflammation of the left gluteal region with characteristic microscopic features including marked leucocytic and round

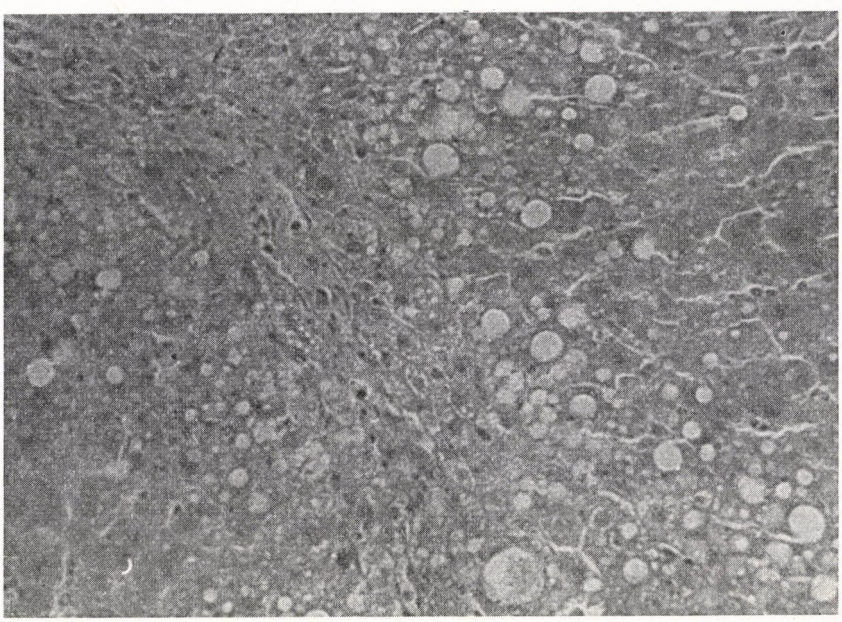

Fig. 1. Rat liver after CCl_4 treatment. Haematoxylin-eosin staining. ×50

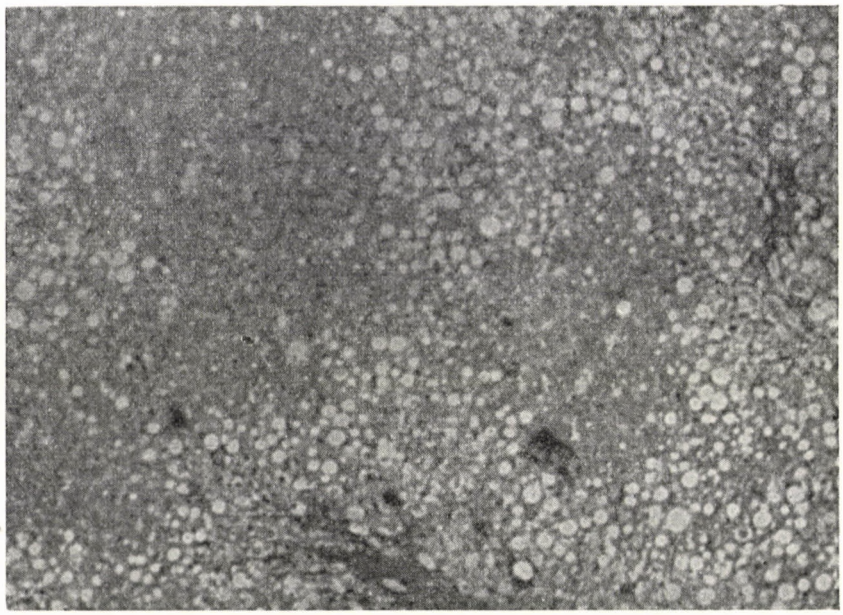

Fig. 2. Rat liver after CCl_4 + croton oil treatment. PAS staining. ×20

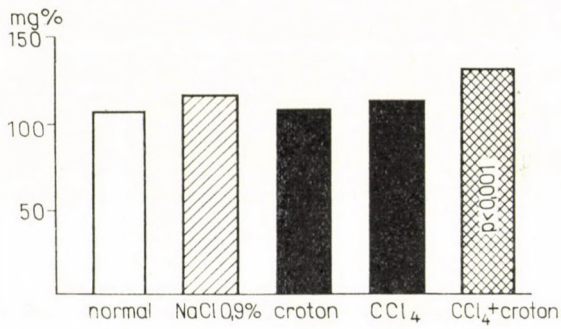

Fig. 3. Serum hexose level in induced inflammation and liver injury

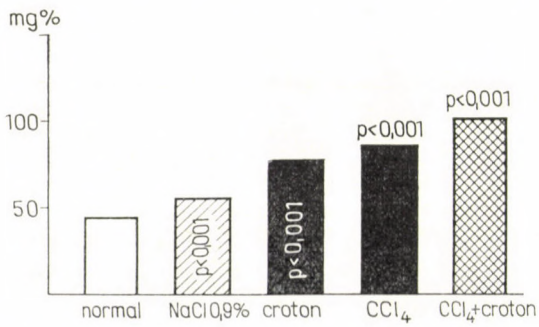

Fig. 4. Serum hexosamine level in induced inflammation and liver injury

cell infiltration with significant accumulation of fibroblasts, histiocytes and mastocytes.

Approximately 50% of the animals of groups 4 and 5 were jaundiced. The histologic pattern of the liver was that of fibrosis with pseudolobulation. There was a significant broadening of the portobiliary connective tissue with round cell infiltration (Figs 1 and 2).

The serum GP fractions exhibited the following changes (Figs 3, 4, 5, 6 and Table I): The hexose level (Fig. 3) tended to rise; the significance of this elevation in comparison to the control and carbon tetrachloride groups was confined to the experimental inflammation associated with liver injury.

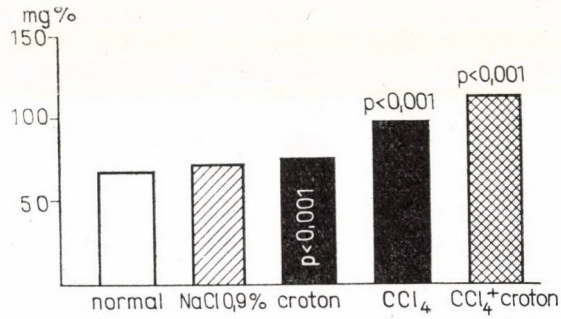

Fig. 5. Serum sialic acid level in induced inflammation and liver injury

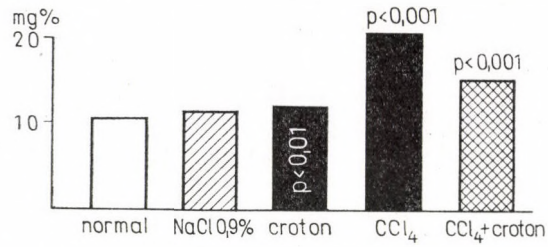

Fig. 6. Serum seromucoid level in induced inflammation and liver injury

The hexosamine level was significantly higher in the NaCl-treated than in the normal rats. A further, likewise significant increase was noted in association with inflammation as well as in liver injury. Croton oil together with liver injury induced a further, highly significant increase (Fig. 4).

The sialic acid level (Fig. 5) was left unaffected by saline. A highly significant elevation was demonstrable in croton oil-induced inflammation as compared to the normal controls and in liver injury as compared to the saline-treated animals. In liver injury associated with tissue destruction, the elevation was highly significant also in comparison to liver injury alone.

The seromucoid level (Fig. 6) was significantly increased in the case of inflammation, and still more so in carbon tetrachloride injury. Tissue destruction after liver injury produced no further change in the seromucoid level.

Table I

Serum glycoprotein concentrations in liver injury and in induced inflammation

		Untreated controls	0.9% NaCl	Croton oil	CCl_4	Croton oil + CCl_4
	n	14	10	12	15	13
Hexose mg%	$\bar{x}$	103.7	110.7	112	106.5	$124^{\square+++}$
	s	18.5	13.8	18.6	31.3	17.2
Hexosamine mg%	$\bar{x}$	40.5	54.0^{+++}	75.3^{+++}	$83.1^{+++}_{\times\times\times}$	$97.4^{+++}_{\times\times\times}$
	s	5.3	10.2	11.8	6.7	$6.7^{\square\square\square}$
Sialic acid mg%	$\bar{x}$	66.6	70.5	74^{+++}	$96^{+++}_{\times\times\times}$	$115.7^{+++}_{\times\times\times}$
	s	7.4	9.8	6.7	14.3	$20.3^{\square\square\square}$
Seromucoid mg%	$\bar{x}$	10.4	11.1	11.8^{++}	$20.3^{+++}_{\times\times\times}$	$18.6^{+++}_{\times\times\times}$
	s	1.6	1.6	1.1	2.6	2.7

$+$ significance against untreated controls
$\times$ significance against NaCl-treated animals
$\square$ significance against CCl_4-treated animals
n = number of animals
$\bar{x}$ = average
s = standard deviation

Discussion

Physiological saline caused no significant increase in the level of the GP fractions, with the exception of that of hexosamine. The increase in this fraction was presumably due to non-specific inflammation. Croton oil induced an elevation of the serum GP level while the increase in hexose was not significant.

Carbon tetrachloride did elevate the GP level with the exception of that of the hexose fraction. The seromucoid level was also significantly increased (showing the highest elevation of all groups). In CCl_4-induced liver injury the levels of hexose, hexosamine and sialic acid increased further in response to inflammation. PIUKOVICH et al. (1964), studying the changes in the sialic acid, hexose and haptoglobulin levels after CCl_4 treatment, exploratative laparotomy and partial hepatectomy in the rat, found an increasing tendency of the GP levels; this was less distinct after partial hepatectomy. They attributed a role to the liver in the change. NEUHAUS and LIU (1964) and VARGA et al. (1966) drew similar conclusions. The results of SHETLAR et al. (1969), BERENSON and DALFERES (1960), FISHKIN and BERENSON (1961) suggested that in inflammatory changes the damaged tissue is a site of increased GP synthesis. According to HOUCK et al. (1962) and to HOUCK and JACOB (1964), in the inflamed tissues the collagen and acid GG concentrations

are reduced at the onset while that of the locally synthetized GP poor in sialic acid is increased. The hexosamine concentration reached its peak on the seventh day.

SHETLAR et al. (1964) demonstrated labelled glucosamine earlier in the liver than in the serum proteins of rats and rabbits and suggested that, in addition to the liver, other tissues of the body might also be involved in the production of serum GP glucosamine. WHITE (1965) obtained similar results with labelled galactosamine. According to MOLNÁR et al. (1965) in the rat the incorporation of labelled glucosamine into serum GP takes place in the microsomal fraction of the liver cells. Sialic acid is also demonstrable in the submicrosomal fraction. HELGELAND (1965) reported on similar observations.

On the evidence of the studies of WHITE et al. (1965) isotope glucosamine is incorporated into the GG which is produced in the damaged tissues even after hepatectomy, but in this case its activity in the serum proteins is low. These authors regard the liver as the principal site of GP synthesis. WARREN (1966), though sharing this view, pointed to the lung, kidney, cerebral cortex, trachea and intestines as additional sites of glucosamine production. After hepatectomy α_1-acid GP is demonstrable in serum.

Thus, the data point to serum GP being partly, if not prevalently, synthetized in the liver. However, the turnover of serum GP involves a wide range of different substances. Each serum globulin fraction binds carbohydrates of different structure and in various amounts. A variety of GPs has been isolated and identified from the plasma of mammalians but with a few exceptions, their metabolism and biological significance are unknown. Certain serum GPs, in particular the immunoglobulins, are known to be of extrahepatic origin.

Seromucoid is closely connected by the majority of authors with the function of the liver. The heterogeneity of this perchloric acid-soluble fraction has been shown by SCHMID et al. (1964) and ANDERSON (1965). Production of α_1-acid GP (orosomucoid) by the liver cells has been confirmed by WEIMER et al. (1965) in liver cell cultures. HERRING (1964) isolated from bovine bone tissue a sialoprotein which closely resembled orosomucoid in its structure. There is evidence suggesting that bone sialoprotein has the bone itself as its site of production. This would be of basic importance by documenting that mesodermal tissues are also involved in the production of GP similar in structure to serum GP.

All this points to the wide diversity of serum GP, particularly as regards metabolism and individual functions, and their production in tissues. It is certain that some of their fractions, e.g. α_1-acid GP, are synthetized in the liver. Local production of GP by mesodermal tissue seems equally certain.

Our earlier and the present results favour the theory of extrahepatic production, elevated serum GP levels having been found even in the presence of grave liver injury. Obviously, allowance must be made for the fact that

pathologic conditions are bound to affect the normal anabolic and catabolic processes. There are no data as to whether the GPs, being formed in the tissues, find access to the circulation and, if so, in what manner.

Further studies concerned with the part played by the liver parenchyma and interstitial tissue in GP metabolism under normal and abnormal conditions, are in progress.

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Dr. János Fehér
Dr. Lajos Jakab
Livia Takács

Budapesti Orvostudományi Egyetem
III. Belklinika,
Budapest VIII, Mező I. u. 17

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PATHOGENESIS OF ANAEMIA IN TRANSPLANTED AKR LEUKAEMIA*

By

T. BURGER and L. G. LAJTHA

FIRST DEPARTMENT OF MEDICINE (DIRECTOR: PROF. T. JÁVOR), UNIVERSITY MEDICAL SCHOOL, PÉCS

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Using a spleen cell suspension of animals suffering from spontaneous AKR-leukaemia, lymphoid leukaemia with an acute course was induced in AKR—C3H hybrid animals; the condition proved to be a model suitable for studies. ^{59}Fe uptake decreased in the erythrocytes as the disease was progressing and the development of anaemia ran parallel. The increase in ^{59}Fe uptake by the leukaemic liver suggested the role of leukaemic cell infiltration in the phenomenon. The higher mortality of the irradiated leukaemic bone marrow grafted animals as compared to normal marrow grafted ones showed that the protective effect of leukaemic bone marrow was lower. At 15 days after irradiation these normal bone marrow and leukaemic bone marrow grafted groups showed a significant difference in 72-hour iron uptake. The high ^{59}Fe uptake of the liver of irradiated chimeras strongly suggested the relative integrity of its storing capacity and its resistance to radiation injury. There was an increase in spleen weight and in the number of visible superficial spleen colonies in the leukaemic bone marrow-grafted groups as compared to normal bone marrow grafted animals. The difference may be explained by the contribution of leukaemic blast cells in forming additional colonies.

Haematologists have long been interested in the problems of anaemia in leukaemia and it is certain that not only the disease itself but the therapeutic measures are also responsible for the symptom. Investigation of the problem in patients has obviously many limitations, the first being that it is impossible to make observations without treatment. In spite of the difficulties there are some important data available for further investigation. According to BERLIN (1966) the mechanism of anaemia in leukaemia is a failure to produce an adequate number of red cells, a shortening of red cell life span (it is supposed that there is an intrinsic defect in red cells), an increasingly ineffective erythropoiesis and multiple small haemorrhages, or even massive haemorrhages, and, finally, the effects of therapeutic drugs on the rate of red cell formation (BERLIN 1966, FREIREICH et al. 1967, BURGER et al. 1966a, VIDEBAEK 1962, NATHAN and BERLIN 1959, BURGER et al. 1966b). Transplantation of leukaemia by spleen cell suspension, and the rapidly ensuing disease and severe anaemia were convenient for experiments.

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Materials and methods

Female C3H—AKR F1 hybrid mice between 9 and 12 weeks of age were used in all experiments. In iron kinetic experiments, blood samples were taken under ether anaesthesia, after which the animals were killed by cervical fracture.

Two types of experiment were carried out.

(1) Ferrokinetic studies at 5, 10, 15, and 20 days after the transplantation in healthy animals, transplanted with 10^5, 5×10^5, 10^6, leukaemic spleen cells.

(2) Normal and leukaemic bone marrow grafting into previously whole body irradiated animals with 850 rad. Ferrokinetics were tested 9 and 15 days after grafting, both for 6 and 72 hour uptake by blood, spleen, liver and femoral marrow.

Routine haematological parameters

Haematocrit values were determined by the micro-test tube method. White cells and cell distribution in blood smears were determined with traditional methods. The removed spleen was placed in Bouin's fluid (picric acid 75%, formaldehyde 20%, acetic acid 5%) for counting the number of macroscopically observable spleen colonies 9—10 days after grafting with 5×10^4 bone marrow cells. Cytological investigation of spleen colonies was performed after Romanovsky—Giemsa differential staining.

Ferrokinetics

Individual mice were given intraperitoneal injections of 0.5 μC of ferric citrate (specific activity, 20—25 μC/μg) 6 and 72 hours before killing them by bleeding from the right axillary vessel under ether anaesthesia. ^{59}Fe concentration was determined in 0.1 ml samples of whole blood collected in disposable PVC tubes; radioactivity of each sample was measured in a well-type (gamma guard, Tracerlab) scintillation counter. Radioactivity in spleen, liver and the two femurs was measured in similar tubes. Spleen and liver were weighed previously. The total volume of blood was determined indirectly by taking it to be 7% of body weight. The ^{59}Fe uptake of the organs, total blood and plasma was expressed in per cents of radioactivity per 200 sec.

Bone marrow and spleen cell grafting

The cell suspension was prepared by removing the femurs and the spleen from healthy C3H—AKR F1 hybrid animals and from moribund leukaemic animals (inoculated with 10^5 leukaemic cells 18—20 days before). The end of the bones was opened by removing the joints with scissors. A gauge 1 hypodermic needle fitted to a tuberculin syringe which contained 1 ml of Hanks' solution, was introduced and the marrow plug forced out of the bone into a bijou bottle. The removed large, fragile leukaemic spleens were placed into Petri dishes containing Hanks' solution. After a spleen was cut and slightly pressed, it was easy to prepare cell suspensions with scissors. Both suspensions were then shaken by hand for one minute.

The number of cells in the final suspension was estimated with a cell counter. The number of injected normal bone marrow cells, and also that of leukaemic bone marrow cells, was found to be 5×10^4, and that of leukaemic spleen cells 10^5, 5×10^5, 10^6. The cell suspensions were prepared under sterile circumstances. Bacteriological tests were negative in every case. Injections were made intravenously into the lateral tail vein of the test mice.

Irradiation technique

Irradiation was carried out with a 300 kVp Resomax X-ray set operating at 19 mA half-value thickness of 1.9 mm Cu, at a source-to-surface distance of 70 cm for 850 rad (35 rad/min).

The controls and the animals with leukaemic cell grafts received standard food and drinking water *ad libitum*, whereas the drinking water given to the irradiated animals contained 100 mg tetracycline/liter.

Results

The transplantation of leukaemic cells established a leukaemic line in the F1 hybrid animals. After the injection of 10^5 leukaemic spleen cells (90% lymphoblasts), leukaemia developed in 18 to 22 days. By this time the animals had become sick, anaemic, and their peripheral blood contained 20,000 to 30,000 leukocytes, with 90% lymphoblasts. The spleen, liver and thymus, and the lymph nodes were enlarged. Fig. 1 shows the number of dead animals in relation to time.

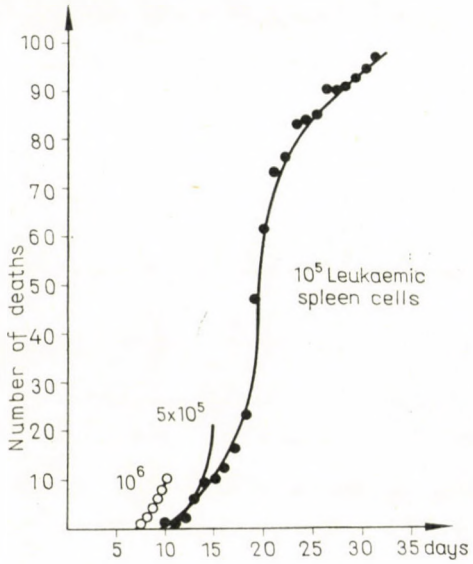

Fig. 1. Cumulative death curve of C3H–AKR F1 hybrids grafted with 10^5, 5×10^5 and 10^6 AKR leukaemic spleen cells

If a larger quantity of leukaemic spleen cells (5×10^5 and 10^6) was given, the leukaemic state developed earlier and the animals died earlier.

The animals of the leukaemic line developed leukopenia with relative lymphocytosis 10 days after transplantation. The bone marrow of the dying animals and of those used for transplantation contained 90% lymphoblasts. The same bone marrow pattern was observed in the animals with 5×10^5 and in those with 10^6 leukaemic cell graft. In peripheral blood the total leukocyte count increased before death, and morphologically there was a lymphoblastic preponderance (Fig. 2).

The haematocrit value and ^{59}Fe uptake by the blood, plasma and organs was estimated 5, 10, 15 and 20 days after the leukaemic spleen cell transplan-

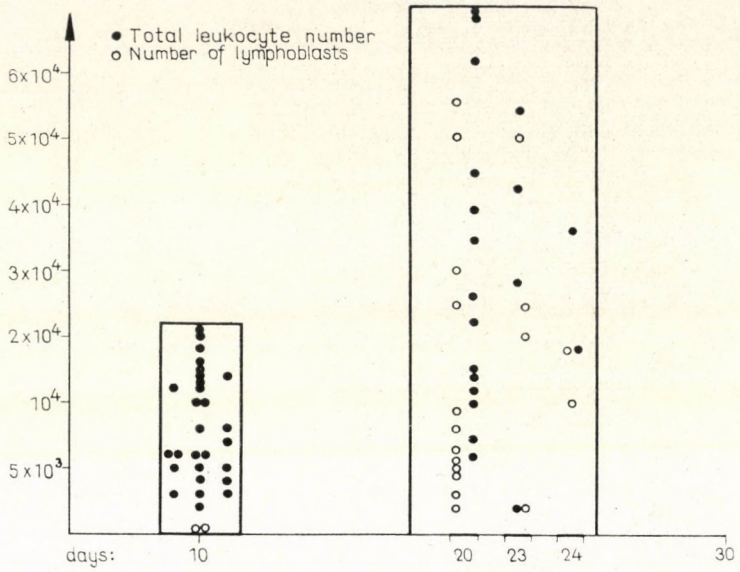

Fig. 2. Leukocytes and lymphoblasts in animals grafted with leukaemic spleen cells as measured on the 10th and 20th—24th days

tation. Fig. 3 presents the decreasing haematocrit values on the above mentioned days in the animals injected with 10^5, 5×10^5 or 10^6 cells.

Table I contains the 72-hour iron kinetic data. ^{59}Fe utilization decreased gradually after the transplantation of leukaemic spleen cells. On the 5th day

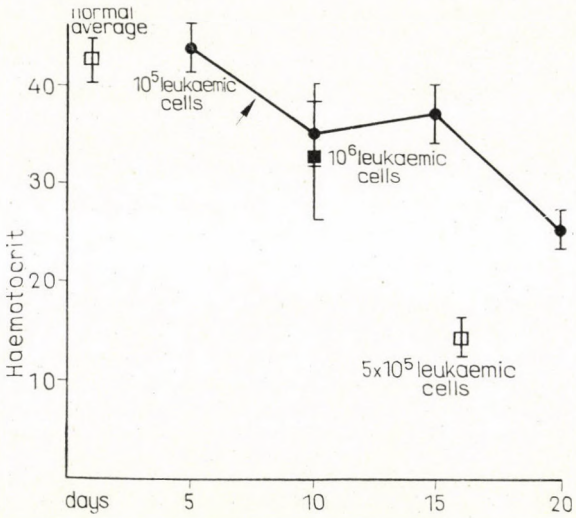

Fig. 3. Haematocrit value in animals grafted with 10^5, 5×10^5 and 10^6 leukaemic spleen cells

Table I

AKR leukaemic spleen cells grafted into C3H – AKR F1 hybrids
Iron kinetic data 72 hours after ^{59}Fe administration

Number of animals		11	8	10	11	7	5
Number of transplanted cells		10^5 Spleen cells				5×10^5	10^6
Days (after transplantation)		5	10	15	20	15	10
Activity of total blood volume in per cent		43.00 ± 5.00	24.67 ± 21.40	10.05 ± 2.91	5.28 ± 4.28	1.55 ± 0.22	4.81 ± 1.87
Total plasma activity		0.48 ± 0.06	0.64 ± 0.05	0.72 ± 0.02	0.75 ± 0.04	0.44 ± 0.05	3.51 ± 0.04
Spleen	activity per cent	1.90 ± 0.90	3.10 ± 0.30	3.81 ± 1.23	4.25 ± 1.02	5.90 ± 2.93	6.23 ± 1.06
	Weight (mg)	135.50 ± 14.30	395.25 ± 22.15	731.33 ± 138.50	876.25 ± 54.60	535.0 ± 52.30	516.80 ± 41.50
Liver	activity per cent	13.50 ± 2.35	18.60 ± 3.45	26.03 ± 4.52	31.90 ± 6.45	34.73 ± 5.53	33.26 ± 7.15
	Weight (mg)	1355 ± 84.24	1960.5 ± 80.25	2551 ± 103.45	2672.2 ± 108.35	3738 ± 103.00	3570 ± 515.0
Control	activity per cent	Blood	47.99 ± 15.50	Spleen	1.50 ± 0.01	Liver	9.25 ± 1.59
	Weight (mg)				103.06 ± 16.90		1121.80 ± 74.95

there were no significant changes in comparison with the normal animals. The severe disturbances of ^{59}Fe utilization developed earlier in the animals which had received 5×10^5 and 10^6 leukaemic spleen cells. Fig. 4 shows these changes. ^{59}Fe uptake decreased exponentially with time. In the latter animals plasma activity was high, and the direct and indirect serum bilirubin levels were increased (1.5 mg each per 100 ml). ^{59}Fe uptake and spleen and liver weight increased parallel with the time after transplantations (Table I and Figs 4 and 5).

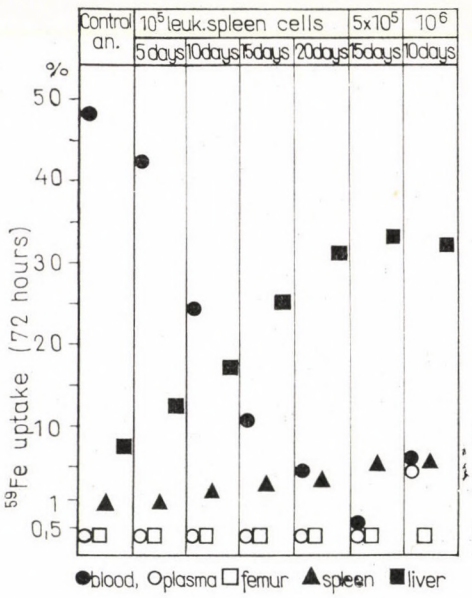

Fig. 4. ^{59}Fe uptake of blood, plasma, femur, spleen and liver in animals grafted with 10^5, 5×10^5 and 10^6 leukaemic spleen cells

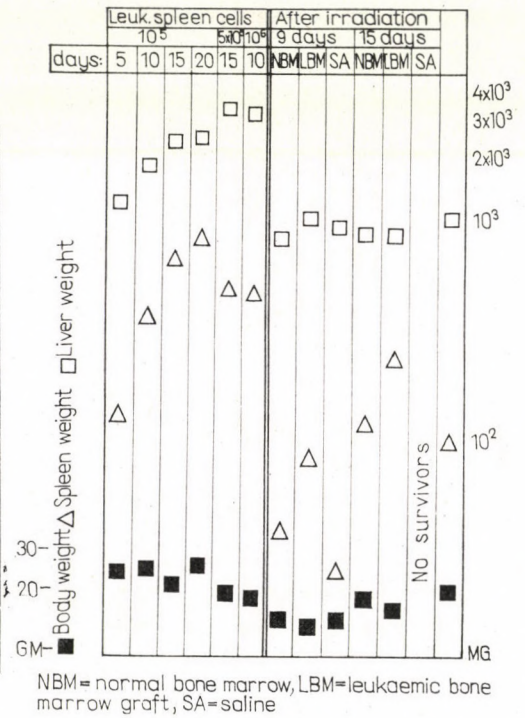

Fig. 5. Total body, spleen and liver weight in animals grafted with leukaemic spleen cells and in whole-body irradiated animals grafted with NBM or LBM

Mortality in the irradiated groups is shown in Fig. 6. Mortality in the groups with leukaemic bone marrow (LBM) grafts and in those treated with saline (SA) was much higher than in the group with normal bone marrow (NBM) grafts. The difference was more significant in the groups observed for 15 days. In the NBM-grafted groups observed for 9 days, only 5 out of 20 animals died, but in the LBM-grafted groups 23 out of 60 died. In the saline-treated groups 30 animals out of 40 reached the 9th day after supralethal total body

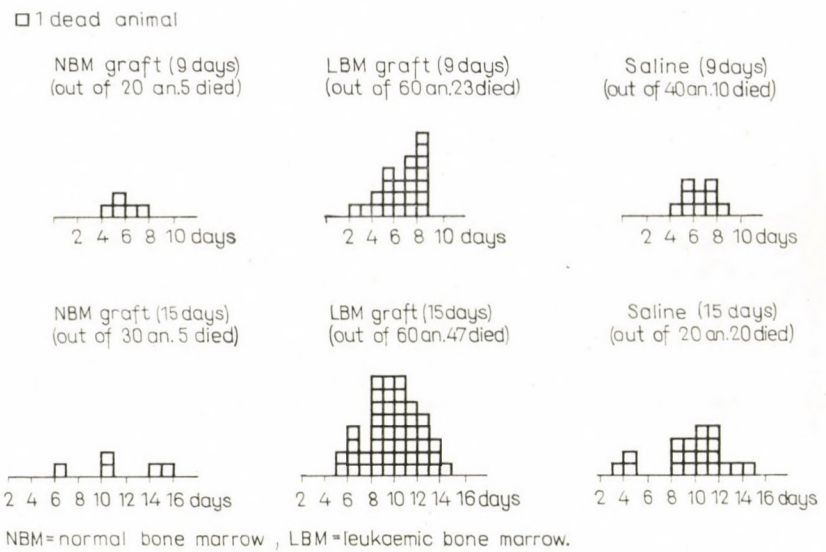

Fig. 6. Mortality of animals having received whole-body irradiation with 850 rad and subsequently grafted with normal bone marrow (NBM), and leukaemic bone marrow (LBM). Ungrafted saline-injected controls

irradiation. More striking were the differences in mortality of the animals observed for 15 days. Of the 30 NBM-grafted animals, 5 died. In the LBM groups only 16 out of 60 animals reached the 15th day. In the SA-treated groups there were no survivors.

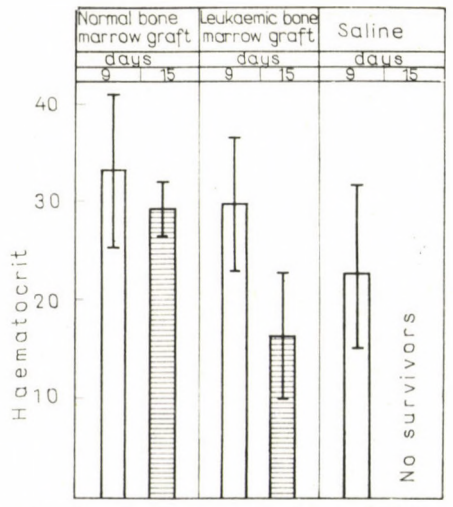

Fig. 7. Changes in haematocrit value between the 9th and 15th day in the irradiated animals grafted with NBM, LBM, and ungrafted saline-injected controls

Table II

Iron kinetic data of irradiated animals grafted

	9 days (after irradiation)									
	6^h ^{59}Fe uptake					72^h ^{59}Fe uptake				
	blood	plasma	femur	spleen	liver	blood	plasma	femur	spleen	liver
normal BM	14.94 ±1.91	11.12 ±2.08	0.67 ±0.25	5.95 ±2.68	14.13 ±1.87	3.90 ±1.05	0.31 ±0.02	0.50 ±0.05	2.62 ±0.14	18.80 ±2.06
	9 animals (1 series)					9 animals (1 series)				
leukaemic BM	14.60 ±3.69	11.60 ±2.27	0.46 ±0.10	6.20 ±3.77	11.20 ±2.81	4.25 ±1.28	0.33 ±0.10	1.05 ±0.02	3.18 ±1.09	18.57 ±3.46
	24 animals (3 series)					9 animals (1 series)				
saline	8.46 ±2.89	8.39 ±5.37	0.49 ±0.23	0.47 ±0.25	15.15 ±3.94	Ø	Ø	0.32 ±0.05	0.66 ±0.22	17.00 ±1.74
	15 animals (2 series)					15 animals (2 series)				

The haematocrit value estimated on the 9th day underwent a decrease in both groups by the 15th day; the decrease was more than threefold in the LBM as in the NBM group (Fig. 7). Table II contains the iron kinetic data of the irradiated animals. Total blood and plasma activities were similar in the NBM and LBM groups, but significantly lower in the SA groups on the 9th

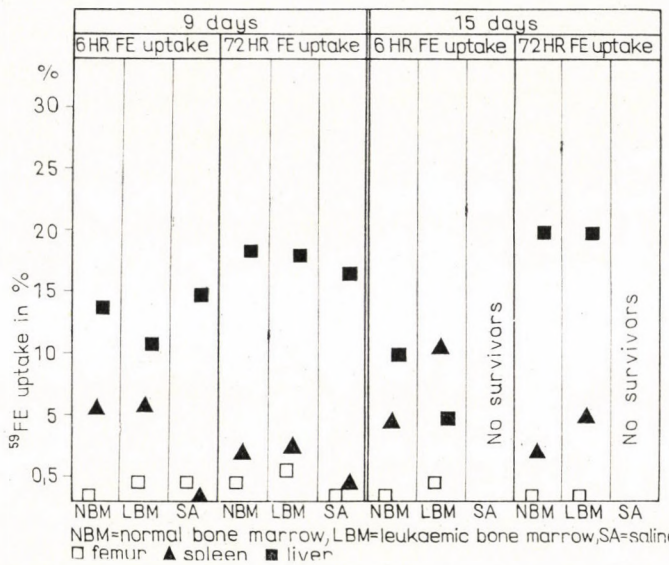

Fig. 8. ^{59}Fe uptake of blood in irradiated animals grafted with NBM, LBM, or in ungrafted saline-injected controls

with normal and leukaemic bone marrow

15 days (after irradiation)									
6^h ^{59}Fe uptake					72^h ^{59}Fe uptake				
blood	plasma	femur	spleen	liver	blood	plasma	femur	spleen	liver
7.43 ±1.62	4.47 ±1.51	0.23 ±0.05	4.99 ±0.27	10.42 ±5.35	22.86 ±4.10	0.78 ±0.15	0.42 ±0.10	2.58 ±0.19	20.65 ±3.48
5 animals (1 series)					20 animals (3 series)				
11.89 ±4.62	10.46 ±5.81	0.47 ±0.02	11.25 ±2.30	5.19 ±2.30	5.64 ±4.47	1.53 ±0.45	0.30 ±0.04	5.42 ±1.30	20.52 ±3.25
3 animals (1 series)					13 animals (5 series)				
No survivors					No survivors				
—					—				

day. In the 15-day groups there were significant differences in 72 hour ^{59}Fe uptake (Table II and Fig. 8). Of the organs, ^{59}Fe uptake was the highest in the liver. The femur did not take up ^{59}Fe in any of the irradiated groups (Fig. 9 and Table II).

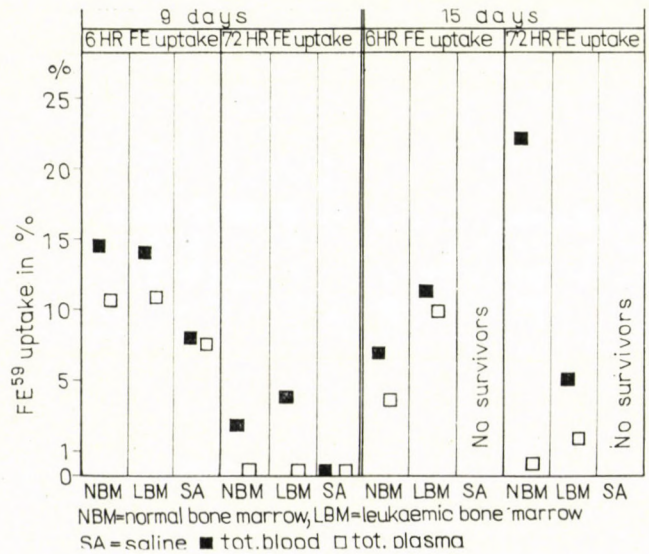

Fig. 9. ^{59}Fe uptake of femur, spleen and liver, in irradiated animals grafted with NBM, LBM, and in ungrafted saline-injected controls



Fig. 10. Spleen colonies in animals grafted with 5×10^4 normal bone marrow cells



Fig. 11. Spleen colonies in animals grafted with 5×10^4 leukaemic bone marrow cells

The spleen was significantly heavier in the LBM-grafted animals, while the liver showed no change in weight. Body weight decreased in all groups (Fig. 4 and Table III).

The average number of spleen colonies in the NBM groups was 10.48 (55 mice) and in the LBM-groups 16.50 (44 mice; Table IV). On inspection there was no difference in the form of colonies between the two groups (Figs 10 and 11).

Differentiation of the cells in spleen colonies was difficult. With conventional histological staining methods it was practically impossible to distinguish between erythroid precursors and lymphoblasts. In the 9-day groups the number of normoblasts might have been less in the LBM grafted spleen colonies than in the NBM grafts; the difference was greater in the 15-day-old

colony-forming cells, as in the spleen colonies of NBM-grafted animals there were 20% normoblasts, as against 1% in the LBM-grafted animals.

It has been attempted to differentiate erythroid precursors from lymphoblasts with ALEXANDER and PICKWORTH's haemoglobin staining technique modified by DOHERTY et al. This study has not yet been concluded and all that can be said in advance is that the colonies in LBM-grafted animals contained only 10% haemoglobin positive cells.

Table III

Total body, spleen and liver weight of whole-body irradiated animals grafted with NBM and LBM

	9 days after irradiation			15 days after irradiation		
	Body weight	Spleen weight	Liver weight	Body weight	Spleen weight	Liver weight
normal BM graft	16.21 ±1.50	39.88 ±5.34	923.50 ±250.0	19.55 ±3.51	127.0 ±20.40	993.9 ±95.40
	21 animals			20 animals		
leukaemic BM graft	15.16 ±1.83	87.62 ±48.70	1143.80 ±198.00	18.46 ±2.93	245.0 ±49.35	957.60 ±102.15
	24 animals			13 animals		
saline	16.23 ±1.61	26.66 ±3.34	1028 ±22.40	No survivors		
	15 animals					

Table IV

Number of spleen colonies of whole-body irradiated NBM and LBM grafted animals (9—10 days after irradiation)

	NBM graft	Number of mice	LBM graft	Number of mice
5×10^4 cells	9.30 ± 7.44	18	18.66 ± 5.58	12
	12.00 ± 1.91	10	10.00 ± 2.59	8
	10.00 ± 3.39	8	20.80 ± 9.38	10
	8.70 ± 4.91	9	16.33 ± 8.65	6
	12.40 ± 2.12	10	16.75 ± 7.69	8
Average number of colonies	10.48		16.50	
Total number of animals		55		44

Conclusions

Leukaemic cells were transplanted in Fl hybrid mice. One leukaemic cell line was used in all the experiments. Cytological tests were applied to verify that the cell donors were leukaemic.

The ^{59}Fe utilization test also supported the well-known fact that Fe uptake decreases as the disease progresses. The development of anaemia went parallel with the decrease in ^{59}Fe utilization. The degree of increase of ^{59}Fe uptake by the liver and the spleen suggested the role in the phenomenon of leukaemic cell infiltration. Recently, WALTERS et al. (1967) have demonstrated the ability of lymphoma cells to incorporate iron into haem. The biosynthesis of haem in leukaemic cells indicates that the iron storage of leukaemic organs may not be due to storage by RES cells only.

The high mortality of the irradiated and LBM-grafted animals showed that the protective effect of LBM was weaker, and recovery was diminished or absent in these animals. The difference in haematocrit value between the NBM and LBM-grafted animals was in agreement with this observation. At 15 days after irradiation these two groups showed a significant difference in 72-hour iron uptake, which is consistent with the above findings.

^{59}Fe uptake, as observed 9 days after irradiation, was very low in both groups. The high ^{59}Fe uptake of the liver strongly suggests the relative integrity of its storing capacity and its resistance to radiation injury.

The higher spleen weight in the LBM-grafted groups was in keeping with the higher number of spleen cell colonies. The increase in number of colonies with time was found to be significant in the LBM-grafted animals.

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Dr. Tibor BURGER | First Assistant to Professor,
First Dept. of Medicine, University Medical School Pécs, Hungary
L. G. LAJTHA | D. M. Ph. D., Director of Paterson Laboratories
Manchester, 20, Great Britain

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HARMFUL SIDE EFFECTS INDUCED BY A PIPERAZINE DERIVATIVE IN EXPERIMENTAL ANIMALS

I. OPHTHALMOLOGICAL OBSERVATIONS

By

P. MEZEY, G. J. KÖTELES, A. TAKÁTS and P. JOBBÁGYI

FIRST DEPARTMENT OF OPHTHALMOLOGY, UNIVERSITY MEDICAL SCHOOL AND "FRÉDERIC JOLIOT-CURIE" NATIONAL RESEARCH INSTITUTE FOR RADIOBIOLOGY AND RADIOHYGIENE, BUDAPEST

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Prolonged treatment with 1,4-bis-(phenyl-isopropyl)-piperazine · 2HCl known to induce cataract in humans, caused pathological alterations in the eye of rats and mice. The compound seemed to elicit a photosensibilizing effect even in animals. In the treated groups, significant changes in the conjunctiva, the cornea and the fur of the animals were observed. Although dense cataracts similar to those seen in humans failed to develop, morphological alterations of the lens were observed which were ascribed to a disturbance in fibre formation.

MEZEY (1967) was the first to call attention to the fact that prolonged administration of the minor tranquillant 1,4-bis-(phenyl-isopropyl)-piperazine · 2HCl induced cataract in humans; this observation was supported by a number of clinical cases (VARGA et al. 1968). Therefore, animal experiments were performed to decide whether any eye lesions developed after the chronic application of the compound.

Material and methods

The compound 1,4-bis-(phenyl-isopropyl)-piperazine · 2HCl was dissolved in physiological saline, at a concentration of 2.5 mg per ml for the purpose of injecting rats, and in water at a final concentration of 0.15 mg per ml to be used as a drinking water for mice. Fresh solutions were prepared twice monthly. The solution was stored in dark, at +4°C.

At the beginning of the experiments the rats were 6 weeks old and of 100—120 g weight, the mice 3 weeks old. The rats were injected with the solution subcutaneously 5 times weekly for a year. Simultaneously, the respective groups were exposed to ultraviolet (UV) rays, 5 times weekly for a year. Subsequently the observation was continued for another half year. The mice were given drinking water with piperazine for 1 year.

The experimental groups consisting of 5 rats each were as follows. I: untreated control; II: UV irradiated; III: 5 mg per kg piperazine daily; IV: 5 mg per kg of piperazine and UV irradiation daily; V: 10 mg per kg of piperazine daily; VI: 10 mg per kg of piperazine and UV irradiation, daily.

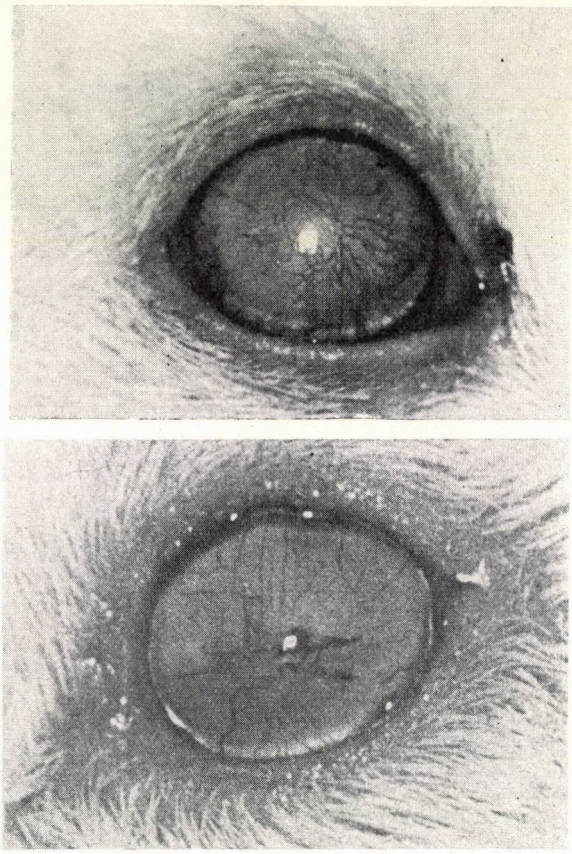
The groups of mice, consisting of 10 animals each, were, I: UV irradiated; II: drinking water containing piperazine; III: drinking water containing piperazine + UV irradiation.

UV irradiation was performed by a Siemens Hanau S 500 mercury tube. The animals were kept in wire cages, at a distance of 1 meter from the lamp. Radiation was performed laterally, without fixing the animals, during the first 3 months for 5 minutes and later for 10 minutes, daily.

The ophthalmological lesions were examined by ophthalmoscope and slit-lamp. For the observation of slight changes, the pupil was dilated with 1% atropine solution.

Results

The conjunctiva, the cornea and the lens were regularly examined during treatment. In both the rats and the mice treated with piperazine the conjunctiva became hyperaemic, swollen and covered with dilated vessels, and in most cases hypersecretion and photosensitivity occurred. While in both rats and mice the cornea in the control groups was clear, and in the UV-



a

b

Fig. 1. Cornea of rat (a) and mouse (b) exposed to combined chronic piperazine and UV treatment. Vascularization and infiltration extending to the whole corneal area. In (b) haemorrhages along the vessels in several areas

irradiated animals slightly vascularized, in the majority of piperazine-treated and even more in piperazine and UV-treated animals it was diffusely vascularized and extensively infiltrated, with a leukoma in some animals.

In some cases haemorrhagic plaques were observed along the dilated vessels. On the epithelium, superficially or immediately below it, tiny vesicles appeared, either in groups localized in various sectors or covering the whole corneal surface. In a few cases, a slight linear pattern was observed among the vesicles (Fig. 1). The incidence of corneal lesions in rats as a function of

the duration of treatment is demonstrated in Fig. 2, which shows also the more harmful effect of combined UV and piperazine treatment. The different dates of appearance reveal differences in individual sensitivity. After the first year, i.e. a certain latency period, a significant increase in frequency of the corneal lesions was to be noted.

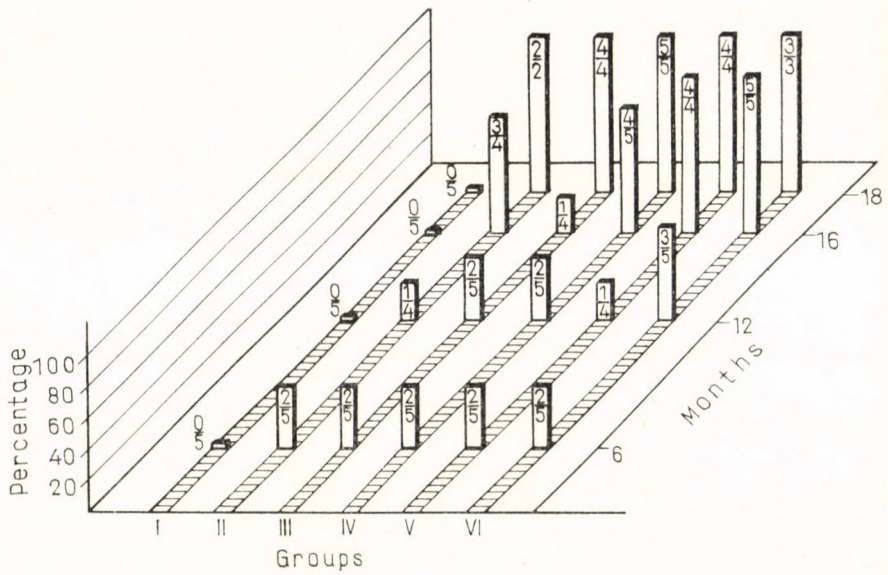


Fig. 2. Frequency of corneal lesions in rats treated with piperazine and UV irradiation, as a function of time. The groups are as follows. I: untreated control; II: UV irradiated; III: 5 mg per kg of piperazine daily; IV: 5 mg per kg of piperazine and UV irradiation; V: 10 mg per kg of piperazine daily; VI: 10 mg per kg of piperazine and UV irradiation. In the fractions the numerator is showing the number of damaged animals against the total number given in the nominator

Lens lesions in rats could be seen where at least some parts of the cornea had remained clear and the deeper parts of the eye could be examined by slit-lamp. In these cases, the equatorial parts of the lens were circularly demarcated in a grey colour from the other tissues. This slight lesion was the more remarkable, since neither a similar change nor colour differences were observed in the control groups. However, crude lens lesions were not found *in vivo*. Therefore, 1 year after treatment from the mice the lens was removed and with its anterior surface turned downwards examined by the slit-lamp. This position allowed to observe the lenses laterally and to detect lesions not appearing on frontal inspection. During the examination the lenses were preserved from exsiccation by constant wetting with physiological saline. The lenses from the untreated control animals seemed to be clear, with a slightly opalescent equatorial part. Apart from this, no lesion was found in the UV-treated

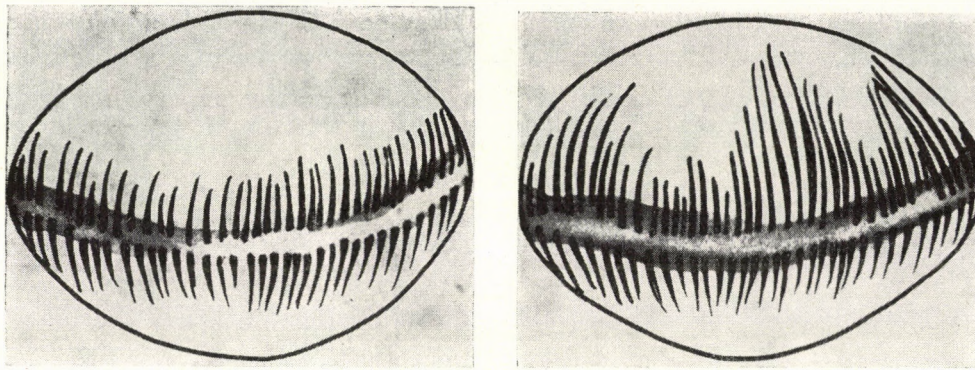


Fig. 3. Schematic diagram of slit-lamp findings in the lens of mice treated with piperazine and UV. For details, see text

mice (Group I). In Group II, a distinct cloudy opalescence was seen in the equatorial part and from this, diffuse linear opacities of various length and thickness started towards the anterior and posterior poles, which gradually coalesced with the clear parts. In Group III, an intensive homogeneous equa-

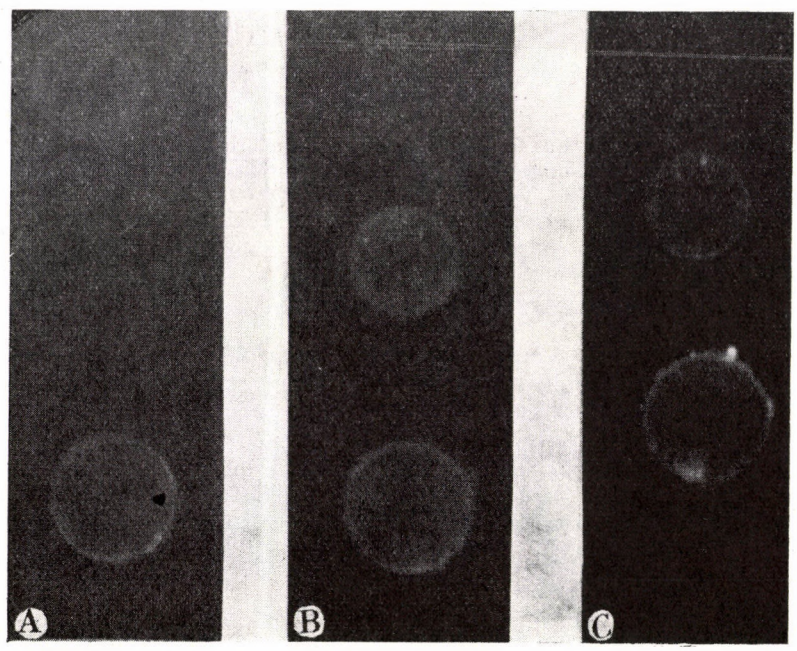
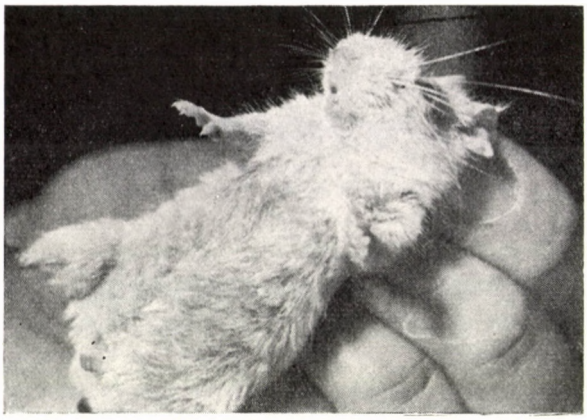


Fig. 4. Isolated lenses of mice. The upper lenses originated from untreated animals, the lower ones from treated animals
A: UV treatment. B: piperazine treatment. C: piperazine and UV treatment



a



b

Fig. 5. Piperazine and UV treated mice. (a) Changes in fur; (b) ulcers in the cervical region

torial opacity could be observed. A linear pattern with longer and shorter, thinner and thicker grey stripes resembling a comb could be detected, directed mainly towards the posterior cortex. Various degrees of these alterations are demonstrated schematically in Fig. 3; isolated lenses are shown in Fig. 4.

To demonstrate the epithelium-damaging effect of the piperazine compound, photographs of treated animals are shown in Fig. 5. Prolonged piperazine and UV treatment caused a marked loss of fur in the rats and the mice, near the end of the treatment. The change seemed to be independent of the route of administration.

Discussion

A number of substances have been found to be cataractogenic such as the phenothiazines (Barsa et al. 1965, Margolis et al. 1965, Petrohelos et al. 1968), naphthalene (Pirie 1968), steroids (Sundmark 1966, Furst 1966)

and certain sulphonamides (RIBELIN et al. 1967) and also the piperazine derivative at issue (MEZEY 1967). The mechanism of the effect is not clear. In the present study, the first lesions appeared on the cornea; they were morphologically similar to a keratitis punctata. Significant differences in the degree of corneal lesions were observed between the animals treated with UV and with UV and piperazine. It has to be mentioned that in the human cases of piperazine cataract no corneal lesion was observed, but there were similarities in the different sensitivity of individuals and also in that the lesions developed after a long latency period. In the animals the lesions were slighter than in humans, in spite of the treatment of animals having been started in early life and continued for a longer time as compared to the average life span of animals than it occurred in humans. According to literary data, the skin and the fur of albino animals are not sensitive to UV radiation (LOGAN and WILHELM 1966). In our experiments UV light did not cause any change in the fur, in contrast with combined UV and piperazine treatment. It was therefore concluded that the piperazine derivative exerted a photosensibilizing effect.

The significant corneal lesions themselves might have appreciably contributed to the prevention of so marked lens lesions as those found in humans. Normally, only a small part of the UV irradiation to which the eye is exposed reaches the lens, and the oedematous, infiltrated and densely vascularized corneal tissue must have further reduced the amount of penetrating UV radiation. In the experimental group treated with UV and piperazine, slight lens lesions still occurred. UV radiation of wavelengths other than those applied by us, e.g. the near UV and visible range of radiation present in sunshine, might have a more intensive effect in the presence of the piperazine compound. This assumption is the more likely because, according to several chemical structural relations, the cataractogenic tranquillants are similar to the photosensitizing dyes. It is, however, also possible that the slight lens lesions are characteristic of the initial stage, and similarly to the human piperazine cataract, they would have turned into more dense opacities after a longer time. Of course, not only the amount of UV irradiation interferes with the development of the lesion, but first of all the amount of piperazine compound accumulated in the lens and damaging either its cells or its capsule. Considering the biology of the lens, the accumulation of even minute amounts of some substance might provoke the appearance of fibres of pathological morphology and composition. The linear opacities running from the equator towards the poles seemed to support this hypothesis.

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Dr. Pál MEZEY, Budapest X, Kőbányai út 45
Dr. György József KÖTELES, Budapest XXII, Pentz Károly u. 5
Dr. Attila TAKÁTS, Dr. Péter JOBBÁGYI } Budapest VIII, Illés u. 15

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HARMFUL SIDE EFFECTS INDUCED BY A PIPERAZINE DERIVATIVE IN EXPERIMENTAL ANIMALS

II. BIOCHEMICAL EXAMINATION OF THE LENS: EFFECT ON LYMPHOCYTES

By

G. J. KÖTELES, A. DÖKLEN, P. MEZEY and A. TAKÁTS

FIRST DEPARTMENT OF OPHTHALMOLOGY, UNIVERSITY MEDICAL SCHOOL AND "FRÉDERIC JOLIOT-CURIE" NATIONAL RESEARCH INSTITUTE FOR RADIOBIOLOGY AND RADIOHYGIENE, BUDAPEST

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Prolonged treatment of rats with 1,4-bis-(phenyl-isopropyl)-piperazine · 2HCl failed to change either the protein, ribonucleic acid and acid-soluble ribose contents, or RNA synthesis in the lens. On the other hand, ^{14}C-amino-acid incorporation into the proteins increased as compared to the controls. Mitosis of lymphocytes of piperazine-treated animals was disturbed on phytohaemagglutinin induction *in vitro*. As to the pathomechanism, two explanations are offered. The first assumes a disturbance in permeability due to the damage of the lenticular capsule or the membranes of cells and fibres. The second might be an injury at the nuclear level of mechanism of fibre differentiation from epithelial cells.

Introduction

As reported in a previous paper, chronic administration of 1,4-bis-(phenyl-isopropyl)-piperazine · 2HCl, a compound cataractogenic in humans, was found to induce in animals visible lesions in the lens, suggestive of a disturbance of fibre formation (MEZEY et al. 1970). These lesions were neither clinically nor histologically so advanced as those in humans (VARGA et al. 1968, RADNÓT and VARGA 1969). Still, after the clinical observation period, the crystalline lenses of the animals were subjected to biochemical investigations of both analytical and functional nature, in order to elucidate whether the initial metabolic disturbance induced by the compound and leading to cataract formation could be observed in the lens. In addition, in view of an analogous damaging effect of some piperazine derivatives on blast transformation of human lymphocytes *in vitro* by phytohaemagglutinin (NASJLETTI et al. 1965), the mitotic capacity of lymphocytes in the treated animals was also studied.

Material and methods

Compound 1,4-bis-(phenyl-isopropyl)-piperazine · 2HCl was dissolved in physiological saline, at a concentration of 2.5 mg per ml. A fresh solution was prepared twice monthly and stored in the dark at +4°C. At the beginning of the experiments the rats were 6 weeks of

age and weighed 100—120 g. The animals were injected subcutaneously with doses of 10 mg per kg body weight, 5 times weekly for a year. The respective group was exposed to ultraviolet irradiation 5 times weekly, also for a year, by a Siemens Hanau S 500 mercury lamp. The animals were accommodated in wire cages, at a distance of 1 meter from the lamp. During the first 3 months irradiation lasted 5 minutes, afterwards 10 minutes, daily.

The present examinations were performed after a year of treatment followed by another half year of observation period. Isolated lenses and lymphocytes from the peripheral blood were investigated in untreated controls, in a piperazine-treated, and in a piperazine + UV treated group.

As described earlier (ANTONI et al. 1963; KÖTELES et al. 1963) biochemical analyses and estimation of the protein, ribonucleic acid (RNA) and acid-soluble ribose contents were performed with colour reactions after isolation according to the modified Schmidt—Thannhauser method. For studying RNA and protein synthesis, the lenses were incubated in Parker's TC 199 medium, at 37°C for 3 hours, in the presence of 0.1 μCi per ml of ^{14}C-valine (Amersham), and ^{14}C-uridine, respectively. After incubation, the fraction containing the RNA nucleotides was separated according to the modified Schmidt—Thannhauser method, the lens proteins according to SIEKEVITZ (1952). Radioactivity in aliquot samples was determined by a Friesecke—Hoepfner gas-flow counter. RNA content was estimated by the orcinol colour reaction, and the protein content by the method of LOWRY et al. (1951). Specific activity of the samples was given in cpm per μg RNA-P and cpm per mg protein.

Investigation of lymphocytes *in vitro* was performed as follows. The rats were exsanguinated from the abdominal aorta under sterile conditions. The blood without any anticoagulant was placed into an incubator of 37°C for 30 minutes, then the coagulate was removed. Under such circumstances, about 0.5—1.0% of the erythrocytes remained in the serum. The lymphocytes remaining in the serum were used for preparing cultures. Parker's TC 199 medium was added to the serum containing the cells, in a ratio of 1 : 3. The lymphocyte count then was $0.5—1.0 \times 10^6$ per ml. Blast transformation of lymphocytes was induced by 0.002 ml per ml of phytohaemagglutinin P (Difco). After 68 hours the cultures were treated with 0.01 mg per ml of demecolcine and after another 4 hours, in the 72nd hour of cultivation, chromosomal preparations were made and stained with Giemsa solution.

Results

Biochemical study of the lenses

After one year treatment and another half year of observation of the rats, the acid-soluble ribose, protein, and ribonucleic acid-phosphorus contents in the lenses were estimated.

Results are shown in Fig. 1. The average protein, ribonucleic acid and acid-soluble ribose contents were about the same in the different groups.

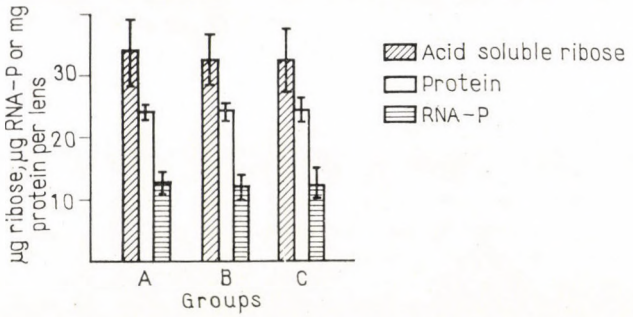


Fig. 1. Acid-soluble ribose, protein and ribonucleic acid phosphorus contents in rat lenses. A: untreated control, B: chronic treatment with piperazine derivative; C: chronic treatment with piperazine derivative and UV irradiation

Table I

Labelling of RNA and protein of rat lenses incubated in vitro

	Labelling time: 3 hours; percursors used: ^{14}C-uridine and ^{14}C-algal protein hydrolysate		
	Untreated control	Piperazine treated groups	Piperazine and UV treated
Specific activity of RNA	27.1^{+}	33.9	28.4
	$\pm$11.3^{++}	$\pm$12.3	$\pm$7.7
cpm per μg RNA-P	12.6—41.2^{+++}	14.0—49.3	19.3—38.7
	6^{++++}	6	6
Specific activity of protein	67.7^{+}	120.9	155.2
	$\pm$30.1^{++}	$\pm$85.6	$\pm$154.2
cpm per mg protein	38.2—123.5^{+++}	26.6—263.7	24.6—416.0
	7^{++++}	6	7

$^{+}$ average value; $^{++}$ standard deviation (S.D.); $^{+++}$ estimated limit; $^{++++}$ number of determinations.

Next, RNA and protein synthesis in the isolated lenses was studied *in vitro* (Table I). The specific activity values for RNA in the various groups did not differ from each other, if studied at the level of total RNA. In the piperazine-



Fig. 2. Chromosomes from phytohaemagglutinin induced peripheral lymphocytes of untreated rats

treated as well as in the piperazine + UV-treated cases the average protein activity was higher than in the controls, and the S.D. values were appreciably higher in the treated cases. This reflected the different rate of ^{14}C-amino-acid incorporation; certain lenses took up the activity like the controls while others incorporated it to a much higher extent. It should be emphasized that UV irradiation by itself did not influence RNA and protein synthesis in the lenses.

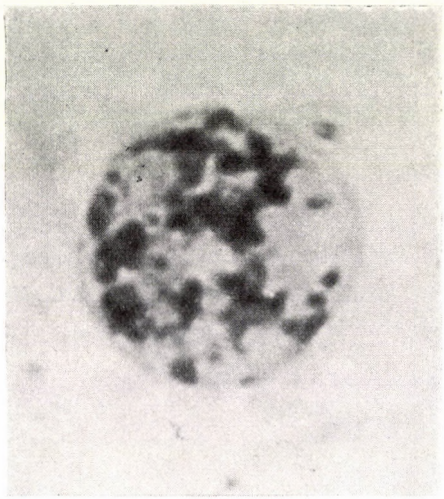
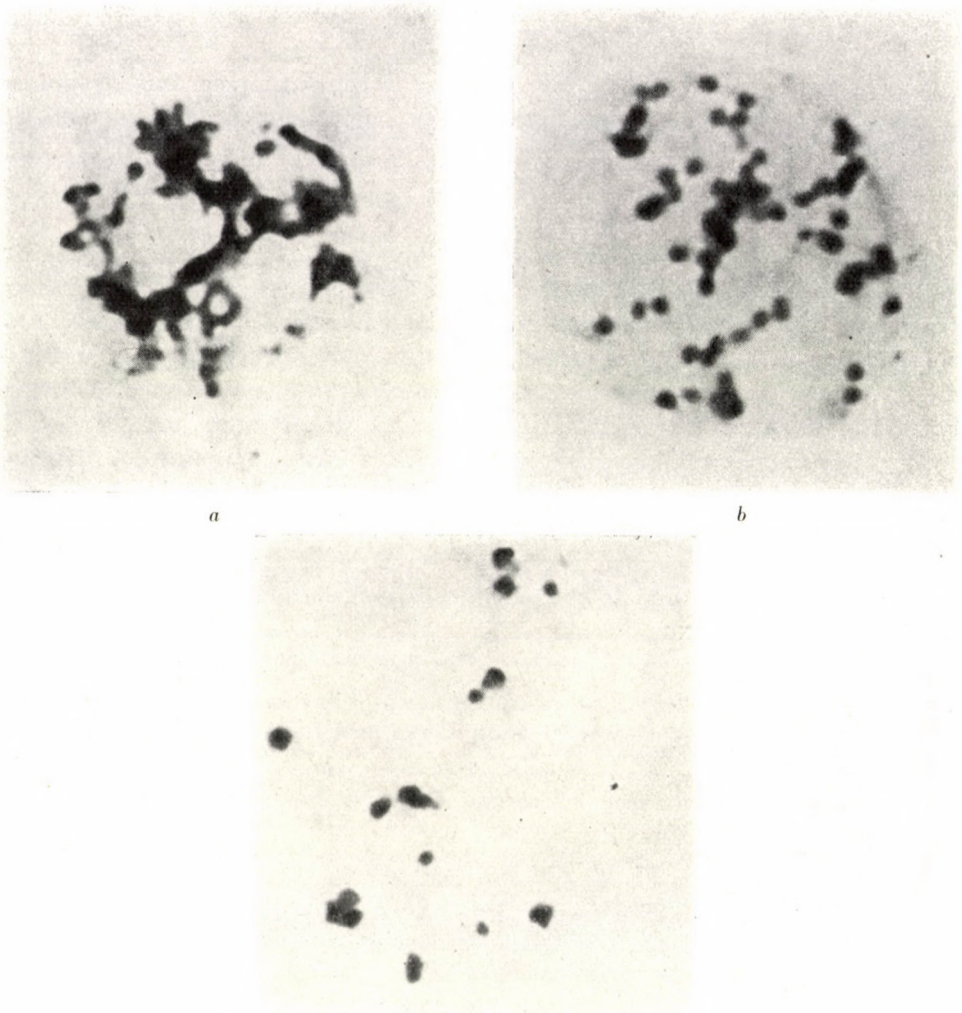


Fig. 3. Nucleus in prophase from phytohaemagglutinin-treated peripheral blood culture of piperazine-treated rat

Division of lymphocytes induced by phytohaemagglutinin, in vitro

In the cultures prepared from the peripheral lymphocytes of rats treated for a year with piperazine derivative, serious chromosomal lesions were observed in the dividing cells. In the lymphocyte cultures from both the piperazine- and piperazine + UV-treated animals, mitotic frequency decreased and numerous injured nuclei were observed in the preparations. Fig. 2 shows chromosomal preparations from lymphocytes transformed by PHA from normal, untreated rats of the same age. Fig. 3 demonstrates a nucleus in the prophase from treated animals. The inhomogeneous staining of the developing chromosomes, which was suggestive of quantitative and qualitative alterations in the chromatin substance, renders the damage well visible. While the outlines of the chromosomes were well observable in the unstained preparations, they did not stain with basophile stains.

Fig. 4 shows chromosomes from the lymphocytes of animals pretreated with piperazine, in the order of the lesion's seriousness.



Figs 4a, b, c. Chromosomes from phytohaemagglutinin-induced lymphocytes of piperazine-treated rats. For details, see text

In Fig. 4a the effacement of the chromosomal structure is well shown, the normal spiral structure cannot be recognized anywhere. It appears as if the basophilic chromosomal deoxyribonucleic acid would not fill up completely the whole length of the chromosome. In Figs 4b and 4c neither the structure nor the shape of the chromosomes can be recognized. The chromosomal substance staining with basic dyes shows a spotlike aggregation. In the lymphocyte cultures of animals pretreated with piperazine + UV no chromosomes taking the stain were seen, only some sporadical dividing cells with polyploid chromosomal sets.

Discussion

The findings have raised the problem of the pathomechanism of piperazine-induced lens lesions. Although no such advanced, dense lens opacities as found in humans were seen in the experimental animals, the data obtained still allow some assumptions.

The opalescence might be caused by the flow of water or other kinds of molecules into and out of the lens due to changes in the permeability of the capsule or of the fibre membrane. In connection with the effect of piperazine derivatives on the cell membrane it has been shown that they may be bound to biological membranes. FLETCHER and YUSA (1967) demonstrated that a piperazine derivative inhibited reversibly the penetration of influenza virus into tissue culture cells. Accordingly, piperazine bound to the capsule or the fibre membranes even in small amounts may be assumed to damage them.

According to the general opinion, during cataract formation, or in the case of a developed cataract, low molecular RNA and proteins are lost from the lens, presumably due to the permeability alteration of the fibres or the capsule (VAN HEYNINGEN 1962, MARAINI and GIOVANNI 1967). Therefore, in the course of the present study, we examined first of all the amount of acid-soluble nucleotides, i.e. the amount of acid-soluble ribose, as a component of small molecular weight, as well as the protein and RNA content. The findings spoke against any marked permeability change, since the amount of the said components of the lens was practically equal in the treated and the control cases. The observed slight lens alterations may be assumed to represent phenomena characteristic of the initial phase of cataract development which, similarly to the piperazine cataracts in humans, might have turned into a more dense opacification after some longer period. This hypothesis was supported by the lack of differences in RNA synthesis between the different groups while the specific activity in the course of protein synthesis sometimes exceeded that of the controls. The considerable scattering of the values was remarkably high even in the control groups, and still more significant in the treated groups.

The other alternative, a disturbance of fibre differentiation, supplies a more plausible explanation since in the treated animals a serious damage of the dividing peripheral lymphocytes was seen. This damage, manifesting itself in the nucleus, may affect also the epithelial cells of the lens from which the lens fibres start to differentiate. It was possible to induce by PHA a division of the lymphocytes, thus these were capable of DNA synthesis but in the 72-hour culture the developing chromosome structure was found to be altered. The cause of the change could be 1) an alteration in the synthesis of macromolecules, i.e. changes in the composition of the chromosomes with

a decrease of their resistance manifesting itself during mitosis; 2) the resistance of the whole cell, or the nucleus may decrease so that cytolysis occurs during the 72-hour incubation *in vitro*. It has to be mentioned that the nuclei of non-dividing cells were not intact in structure either, as their basophilic staining became weak. Our experiments thus suggest that a significant decrease in the resistance of lymphocytes in the course of PHA-induced division must have occurred in the piperazine-treated animals. This effect may have concerned the functions of non-dividing cells, too, and may mean a general inferiority of the affected cells. It is of course possible that the cellular damage may manifest itself only in the case of such complex events like division. It is known that in certain conditions such as lymphoid leukaemia, the cells react similarly to PHA treatment (SANDBERG et al. 1962).

Comparing the above findings with the damage of the cornea and the lens, it is presumable that piperazine may damage many kinds of cell by interfering with the synthesis of macromolecules or with the cell membranes. The damage of peripheral lymphocytes induced by piperazine derivatives has been described by other authors, too (NASJLETTI et al. 1965). In this respect it is a noteworthy observation that chlorpromazine, in itself a cataract-inducer, is also inhibiting the PHA-stimulated mitosis of lymphocytes (PISCIOTTA and WESTRING 1967). The effect on chromosomes or genes could thus be explained that, similarly to other compounds, the piperazine derivatives adhere to the DNA of nuclei. It has been shown by the use of mammalian cells *in vitro* and animal viruses that the effect of other photosensitizing agents manifests itself through the nucleic acids, probably through the DNA (MIURA and OHBA 1967, GITTELSON 1967, COLOMBO et al. 1965).

The photosensitizing compounds might alter the protein structure too. It is known that electron transfer induced by light effect may occur between dyes and SH-containing proteins (FUJIMORI 1964; MCLAREN and SHUGAR 1964). As regards the light effect and the phenothiazines, it is particularly interesting that under a radiation effect the phenothiazines form free radicals which react with the reactive groups of proteins for a long time after irradiation (KALKWORF and DIEBEL 1967, WOLLEMANN 1968). This possibility also arises in the case of piperazine derivatives. It is also possible that the granular formations observed histologically (RADNÓT and VARGA 1969) are due to the accumulation of the compound itself, or the complex formed from the compound and the macromolecules of the lens, proteins and/or nucleic acids. Whichever of the said alternatives prevails, the metabolism of the lens may change into a pathological direction.

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Dr. György József KÖTELES
Dr. Anna DÖKLEN } Budapest XXII, Pentz Károly u. 5
Dr. Pál MEZEY, Budapest X, Kőbányai út 45
Dr. Attila TAKÁTS, Budapest VIII, Illés u. 15

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THE METABOLISM OF [4-^{14}C]DEHYDROEPIANDROSTERONE BY HUMAN SKIN IN VITRO

I. TRANSFORMATION IN VITRO OF [4-^{14}C] DEHYDROEPIANDROSTERONE INTO ANDROST-4-ENE-3,17-DIONE, TESTOSTERONE AND ANDROSTERONE BY NORMAL HUMAN MALE AND FEMALE SKIN SLICES

By

I. Faredin, K. Kókai, I. Tóth, Á. G. Fazekas and M. Julesz

FIRST DEPARTMENT OF MEDICINE AND INSTITUTE OF BIOCHEMISTRY, UNIVERSITY MEDICAL SCHOOL, SZEGED

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Abdominal skin slices from normal female and male patients were incubated with [4-^{14}C] dehydroepiandrosterone in order to study the biosynthesis of steroids with high androgenic activity. Both female and male skin were found capable of synthetizing androst-4-ene-3,17-dione, 5α-androstane-3,17-dione, androsterone and testosterone from [4-^{14}C]-dehydroepiandrosterone. The theoretical and practical significance of these results has been discussed.

It was shown previously that segments of human female skin obtained from the pubic and abdominal areas intensively metabolized [4-^{14}C] dehydroepiandrosterone ([4-^{14}C] DHA) *in vitro* (Faredin et al. 1966, 1967a). Incubation with [4-^{14}C] DHA of pubic skin slices from an agonadal male patient resulted in the formation of 15 metabolites of which 11 were identified including androst-4-ene-3,17-dione, testosterone and androsterone (Faredin et al. 1967b). Cameron et al. (1966) have shown that human male skin slices converted [7α-^{3}H] DHA into testosterone. These observations were confirmed by Gallegos and Berliner (1967) and Berliner et al. (1968) using human male abdominal skin slices.

The aim of this work was to obtain qualitative data concerning the metabolism of [4-^{14}C] DHA in normal human male and female abdominal skin tissues with special regard to the formation of androst-4-ene-3,17-dione, 5α-androstane-3,17-dione, androsterone and testosterone.

Materials and methods

Skin tissues. — Skin specimens were obtained during appendectomy from a female and a male patient. The female patient was 29 years old, her temperature was 38.1°C prior to surgery, the W.B.C. was 11,600. She had had regular menstrual cycles since 14 years of age and the operation was performed on the 13th day of the cycle. The 35 years old male patient had a W.B.C. of 21,000 and a temperature of 37.7°C. The skin samples were freed of adipose tissue and chopped with scissors into cubes 1—2 mm in diameter.

Chemicals. — [4-^{14}C] DHA (specific activity: 7.8 μC/μM) was purchased from The Radiochemical Centre (Amersham, England) and purified on an alumina column prior to use. The purity was further checked by thin layer chromatography on alumina layer in System "C".

NAD, NADP and ATP were products of Sigma Chemical Co. The reference steroids were purchased from Organon and Koch—Light Laboratories. Reagents used were of analytical grade. Solvents were redistilled before use.

Incubation. — Male (1.0 g) and female (1.0 g) skin slices were separately incubated with 1,122,880 dpm [4-^{14}C] DHA in 10 ml Krebs—Ringer phosphate solution, pH 7.4, containing 200 mg/100 ml glucose (KRPG), 10^{-3} *M.* NAD, NADP and ATP, in air atmosphere, at 37°C, for 5 hours. Radioactive DHA was dissolved in 0.2 ml propylene glycol in both flasks prior to incubation.

When skin slices were inactivated for control purposes, this was accomplished by boiling the tissue slices in KRPG for 10 minutes, after which the slices were transferred into a flask containing fresh medium and substrate for incubation.

To control the possible spontaneous degradation of [4-^{14}C] DHA during the incubation, a blank experiment was performed in parallel, incubating [4-^{14}C] DHA without skin tissue.

Extraction. After incubation, skin tissue and medium were extracted separately. Skin tissue was extracted first with absolute ethanol and in the second step using a 2 : 1 mixture (v/v) of acetone and water. The incubation medium was extracted with dichloromethane and ether (FAREDIN et al. 1967a). All the extracts were combined and evaporated to dryness at 45°C under vacuum. Recovery of radioactivity was 87.6% in the male and 85.7% in the female skin incubate after extraction.

Chromatography. — Column chromatography. The dry residue of the extract was dissolved in 2 × 5 ml benzene and chromatographed on a 3 g alumina column (E. Merck, Brockmann III/IV activity). Radioactivity in the column eluates was measured by a Packard Tri-Carb Liquid Scintillation Spectrometer (Model 3375) operating with 87% efficiency for ^{14}C. Distribution of radioactivity in the column eluates is shown in Fig. 1. The column eluates were pooled into fractions I—VIII, according to the major peaks of radioactivity (FAREDIN et al. 1967a). Recovery of radioactivity was 96.2% (male) and 99.2% (female) after column chromatography.

From fractions I and II, 5α-androstane-3,17-dione and androst-4-ene-3,17-dione were separated from the residual [4-^{14}C] DHA by chromatography on a 5.5 g alumina column (E. Merck, Brockmann III/IV activity). Solvent systems were, fractions 0—25 benzene, fractions 26—54 0.05% ethanol in benzene, fractions 55—74 0.10% ethanol in benzene. 20 ml fractions were collected. The quantity of unmetabolized [4-^{14}C] DHA was 450,750 dpm (40.1%) in the male and 344,980 dpm (30.7%) in the female skin incubate.

From column fraction IV, androsterone and testosterone were isolated.

Thin-layer chromatography was performed on 250 μ thick layers of silica-gel-G (Merck) and alumina (Al_2O_3-G, Merck). The following solvent systems were used:

Silica-gel plates:

TLC-"1a": cyclohexane–ethyl acetate (60 : 40, v/v)
TLC-"1b": dichloromethane–ethyl acetate (40 : 10, v/v)
TLC-"1": benzene–absolute ethanol (92 : 8, v/v)
TLC-"2": benzene–ethyl acetate (3 : 2, v/v)

Alumina plates:

TLC-"A": n-hexane–ethyl acetate–glacial acetic acid–absolute ethanol (210 : 30 : 2 : 0.5, v/v/v/v)
TLC-"C": n-hexane–ethyl acetate–glacial acetic acid–absolute ethanol (180 : 60 : 2 : 1, v/v/v/v)
TLC-"E" n-hexane–ethyl acetate–glacial acetic acid–absolute ethanol (20 : 210 : 1 : 10, v/v/v/v)

Steroids were detected on TLC plates by exposure to iodine vapour (MATTHEWS et al. 1962) and UV absorption. Radioactive areas on the chromatograms were located using a Packard Radiochromatogram Scanner (Model 7201). Extraction of steroids from TLC plates was performed with methanol by means of a zone extractor.

Mass measurement of steroids. 17-ketosteroids with saturated A-ring were determined by the Zimmermann reaction using the Allen equation (ALLEN 1950). The quantity of steroids with Δ^4-3-keto-configuration was measured spectrophotometrically at 240 mμ. The mass of 5α-androstane-3,17-diol was measured by forming sulphuric acid chromogen. Readings were taken at 280, 320 and 360 mμ after keeping the mixture in a boiling water bath for 5 minutes (FAREDIN et al. 1967b). Quantities were calculated by using the Allen equation.

Identification and radiochemical purity of steroids. Isolation and identification of individual steroids from the column fractions were carried out by first running the major radioactive peaks in an adequate system with authentic carrier steroids. The chromatograms were scanned for radioactivity. Radioactive zones containing the authentic nonradioactive carriers were eluted and the steroids subjected to further chromatography and derivative formation.

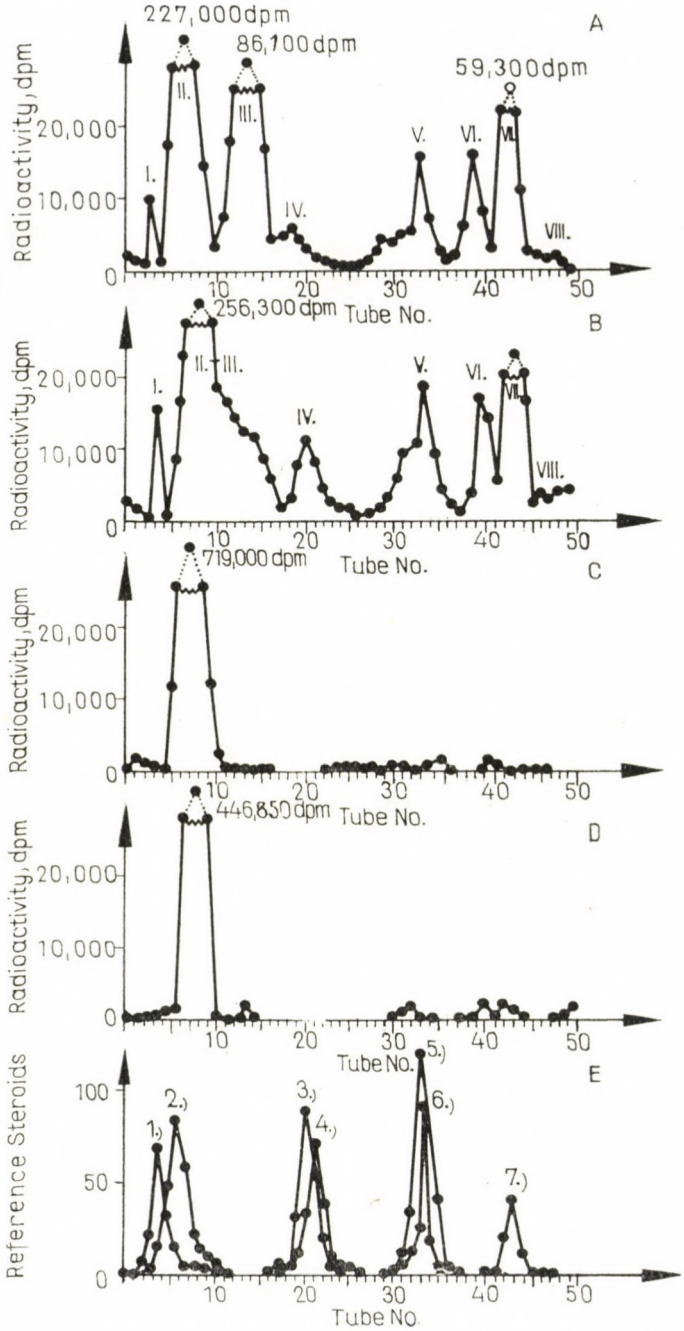

Fig. 1. Distribution of radioactivity in the eluates of the alumina column. Numbers I—VIII represent peaks of radioactive steroids with increasing polarity. Graph A: Normal female skin, Graph B: Normal male skin, Graph C: Boiled skin, Graph D: [4-^{14}C] DHA substrate, Graph E: Reference steroids: 1. 5α-androstane-3,17-dione; 2. Androst-4-ene-3,17-dione; 3. Androsterone; 4. Testosterone; 5. Androst-5-ene-3β,17β-diol; 6. 7-keto-DHA; 7. 7α-hydroxy--DHA

Purification of steroids was continued until the specific activity (dpm/μM) remained constant after two successive chromatographies and derivative formation.

Derivative formation. Acetylation and hydroxyl-group oxidation of the isolated steroids were performed according to the procedures outlined by BUSH (1961).

Reductions by sodium borohydride were carried out as follows. 5α-androstane-3,17-dione was reduced to 5α-androstane-3,17-diol using 1 ml $NaBH_4$ solution (57 mg in 1 ml methanol) at 0°C for two minutes. Androst-4-ene-3,17-dione was reduced to testosterone using a $NaBH_4$ reagent previously stored at 0°C for 60 minutes. Reduction was carried out at 0°C for 2.5 minutes, the yield was 60—70%. The reduction was stopped with 1.4 ml of 1 *N* HCl. Extraction was performed by using 2×5 ml dichloromethane, the extracts were combined, washed with 3 ml 5% Na_2CO_3 solution and 2×3 ml distilled water. The extract was evaporated to dryness *in vacuo*.

Results

As Figure 1 shows [4-^{14}C] DHA was actively metabolized by normal female and male human skin slices *in vitro* (Graph "A": normal female skin, Graph "B": normal male skin). Graph "C" indicates that when skin tissue had been inactivated by boiling before incubation no transformation of [4-^{14}C] DHA occurred under the same conditions in the absence of active skin

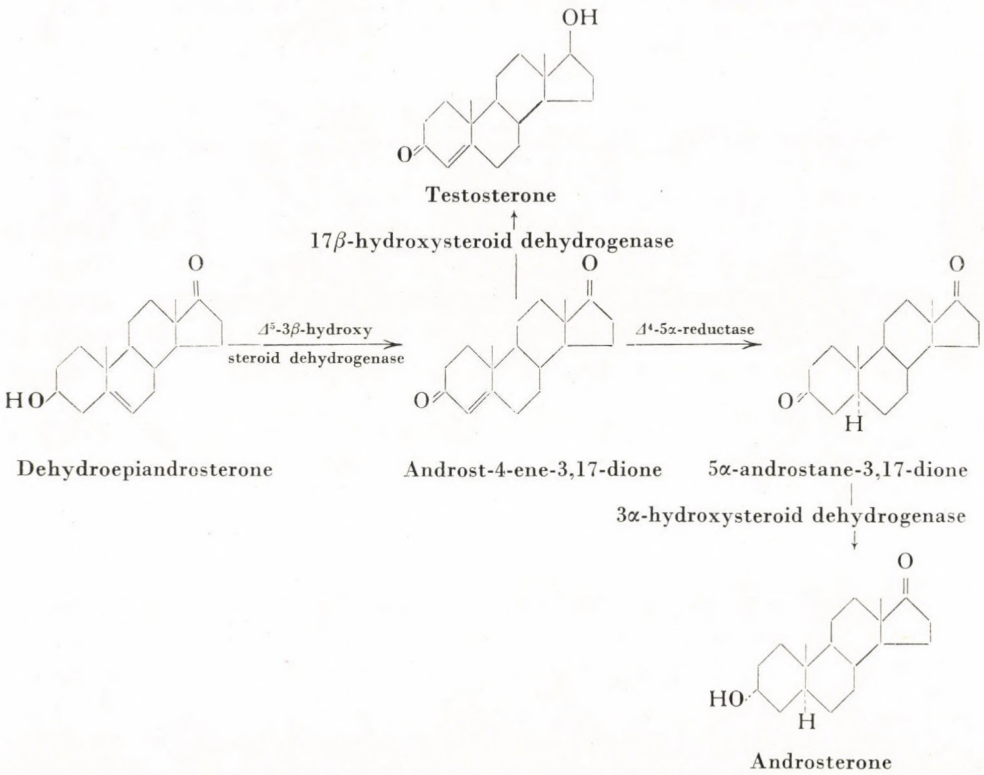

Fig. 2. Metabolism *in vitro* of [4-^{14}C]-dehydroepiandrosterone in female and male skin

tissue. That these were true metabolic products of the substrate is shown in Graph "D" in which it is seen that only [4-^{14}C] DHA was recovered when this substrate had been incubated in the absence of tissues. Graph "E" shows the elution of authentic steroids from the column.

By the analysis of the metabolites of DHA the most active androgenic steroids, i.e. 5α-androstane-3,17-dione, androst-4-ene-3,17-dione, androsterone and testosterone were found in column peaks I, II and IV. These steroids were identified by thin layer chromatography and derivative formation as presented in Table I. It can be seen that normal female and male skin tissue was capable of synthetizing 5α-androstane-3,17-dione, androst-4-ene-3,17-dione, androsterone and testosterone.

Discussion

In previous papers (FAREDIN et al. 1966, 1967a) it was shown that normal female pubic and abdominal skin slices were capable of transforming [4-^{14}C] DHA into androst-4-ene-3,17-dione and 5α-androstane-3,17-dione. In these experiments no testosterone formation was observed. More recently it has been found that pubic skin slices of an agonadal male patient formed, among other metabolites, significant amounts of androst-4-ene-3,17-dione, 5α-androstane-3,17-dione, androsterone and testosterone from [4-^{14}C] DHA (FAREDIN et al. 1967b). In control experiments where [4-^{14}C] DHA was incubated without skin tissue or if boiled skin tissue was used, no metabolism of the DHA substrate could be detected.

The present results point to the fact that normal female and male skin tissue is capable of synthetizing androst-4-ene-3,17-dione in nearly equivalent radioactivities. It is known that androst-4-ene-3,17-dione is a "key-compound" (DORFMAN and UNGAR 1965) from which various androgenic steroids can be formed. The formation of androsterone and testosterone shows that androgenic steroids can be synthetized not only in the endocrine organs (adrenal cortex, ovaries and testes) but also in a peripheral tissue such as the skin (Fig. 2). The conversion of labelled DHA by male skin tissue into testosterone (CAMERON et al. 1966) and into androst-4-ene-3,17-dione was demonstrated previously (FAREDIN et al. 1966, 1967a, b; GALLEGOS and BERLINER 1967; BERLINER et al. 1968; KIM and HERRMANN 1968).

WOTIZ et al. (1956) detected several metabolites by autoradiography after [4-^{14}C] testosterone had been incubated with human skin *in vitro*; one of these was identified as androst-4-ene-3,17-dione by chromatography and infrared spectrophotometry. GOMEZ and HSIA (1968) studied the metabolism of [4-^{14}C] testosterone and [4-^{14}C] androst-4-ene-3,17-dione in human skin; testosterone was converted into androst-4-ene-3,17-dione, 5α-dihydrotestosterone, 5α-androstane-3,17-dione, epiandrosterone and androsterone,

Table I

Isolation and identification of 5α-androstane-3,17-dione, androst-4-ene-3,17-dione, androsterone and testosterone from incubates of female and male skin tissue with [4-^{14}C] DHA

Steroid identified	Carrier μg	Chemical reaction	Derivative	TLC-system	Female skin			Male skin		
					R_f	S.A.** dpm/μM	Total dpm incorp./g***	R_f	S.A.** dpm/μM	Total dpm incorp./g***
5α-androstane-3,17-dione	243	—	Free steroid	TLC-"1b"	0.63	12,200		0.65	22,250	
		—	Free steroid	TLC-"A"	0.42	11,500	9,557	0.43	21,720	17,970
		$NaBH_4$-reduction	5α-androstane-3,17-diol	TLC-"E"	0.65	11,190		0.63	20,950	
Androst-4-ene-3,17-dione	253	Acetylation	Free steroid	TLC-"1"	0.50	17,814	16,332	0.48	19,704	17,430
		$NaBH_4$-reduction	Testosterone	TLC-"2"	0.43	18,472		0.44	19,217	
Dehydroepiandrosterone*	280	—	Free steroid	TLC-"C"	0.34	35,450	344,980	0.37	47,160	450,750
		Acetylation	DHA-acetate	TLC-"A"	0.62	35,530		0.61	46,440	
Androsterone	296	—	Free steroid	TLC-"2"	0.54	14,232		0 53	33,227	
		Acetylation	Androsterone-acetate	TLC-"1a"	0.74	15,393	15,729	0.72	30,874	29,994
		CrO_3-oxidation	5α-androstane-3,17-dione	TLC-"1"	0.64	15,103		0.62	29,422	
Testosterone	295	—	Free steroid	TLC-"2"	0.45	1,730		0.46	3,634	
		Acetylation	Testosterone-acetate	TLC-"1a"	0.54	865	886	0.55	2,134	2,068
		CrO_3-oxidation	Androst-4-ene-3,17-dione	TLC-"1"	0.50	922		0.52	2,019	

* [4-^{14}C] dehydroepiandrosterone substrate
** S.A. = specific activity
*** Total dpm incorporated per g tissue

and [4-^{14}C] androst-4-ene-3,17-dione was transformed into testosterone. Etiocholanolone (3α-hydroxy-5β-androstane-17-one) and etiocholane-3,17-dione (5β-androstane-3,17-dione) were not detected among the metabolites. Incubation in our laboratory of [4-^{14}C] androst-4-ene-3,17-dione with female skin slices resulted in the formation of 5α-androstane-3,17-dione, androsterone and testosterone (FAREDIN et al. 1968). In accordance with the results of GOMEZ and HSIA (1968) we could not observe the formation of etiocholane--3,17-dione or etiocholanolone. Thus, formation of androsterone in human skin is equally possible from DHA, androst-4-ene-3,17-dione, and testosterone.

According to the present results, human skin contains Δ^5-3β-hydroxysteroid dehydrogenase, 17β-hydroxysteroid dehydrogenase, Δ^4-5α-reductase and 3α-hydroxysteroid dehydrogenase activity. These investigations do not show which component of the skin is responsible for the metabolism of DHA. BAILLIE et al. (1965, 1966) demonstrated various hydroxysteroid dehydrogenases in the sebaceous glands of human skin. JULESZ and HORVÁTH (1965) found intensive formazan formation around the hair follicles, the sebaceous glands and sweat glands when studying the activity of Δ^5-3β-hydroxysteroid dehydrogenase. BERLINER et al. (1961a, b, 1968) showed that fibroblasts participate actively in steroid biotransformations.

The significance of the steroid metabolic capacity of human skin is not known. OERTEL and TREIBER (1969) demonstrated various C_{19}- and C_{18}-steroid metabolites in skin extracts of normal male patients after intravenous administration of [7α-^{3}H]-dehydroepiandrosterone-[^{35}S]-sulphate. POCHI and STRAUSS (1969) found DHA and androst-4-ene-3,17-dione to cause sebaceous gland stimulation and this finding suggests a role for these weaker androgens in the control of sebaceous gland function. A further study of the utilization of steroids in human skin might furnish some more information concerning skin changes in endocrine diseases and in the pathogenesis of hirsutism and acne.

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Dr. Imre FAREDIN, Dr. István TÓTH, Dr. Miklós JULESZ — First Department of Medicine, University Medical School, Szeged, Korányi rakpart 12, Hungary
Dr. Károly KÓKAI, Dr. Árpád G. FAZEKAS — Institute of Biochemistry, University Medical School, Szeged, Hungary

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ELECTROCARDIOGRAPHIC ALTERATIONS IN METABOLIC CARDIAC NECROSIS AND THE INFLUENCE OF AMILORIDE ON THESE CHANGES

By

S. VARGA, H. G. CLASSEN and B. SOLYMOSS

INSTITUT DE MÉDECINE ET DE CHIRURGIE EXPÉRIMENTALES UNIVERSITÉ DE MONTRÉAL, MONTREAL (CANADA)

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During the development of nonocclusive metabolic cardiac necrosis (produced in the rat by conjoint treatment with fluorocortisol, disodium phosphate and corn oil), the duration of the PR, QRS and QU periods was prolonged, the P, R and S amplitudes were increased and the T waves were altered; U waves were regularly observed. These manifestations preceded the histologic and macroscopic cardiac lesions and a correlation existed between them and previously observed electrolyte and acid–base disturbances. The most pronounced electrocardiographic deviations occurred generally in the presence of macroscopic lesions and were sometimes associated with arrhythmias, blocks and bigeminia. Amiloride, a potassium-sparing agent, while failing to prevent the increased amplitudes of the P, R and S waves, normalized the other changes and inhibited cardiac necrosis.

The "Electrolyte-Steroid-Cardiopathy with Necrosis" (ESCN) is a convenient experimental model for the study of nonocclusive or metabolic cardiac necrosis. It can be elicited in different animal species by certain halogenated steroids given in combination with sodium salts [1]; the cardiotoxicity of this treatment is enhanced by the concurrent administration of lipids [2]. A decrease of myocardial potassium and an increase of sodium precede the development of the cardiac lesions; in the blood an early hypopotassaemia, hypernatraemia and extracellular metabolic alkalosis is observed [3, 4]. Amiloride, a non-steroid diuretic agent, prevents the development of electrolyte and acid–base disturbances [3, 4] and protects the rat against the infarcts and mortality characteristic of the ESCN [5]. Since both the biochemical alterations and the cardiac lesions might influence the electrocardiogram, we evaluated the chronologic development of metabolic cardiac necrosis by electrocardiographic means. We also attempted to define the effect of amiloride on the ESCN with the same technique.

Materials and methods

A total of 460 female Sprague—Dawley rats of the Holtzman Farms (Madison, Wisc., U.S.A.), averaging 100 g (range 90 to 110 g) and maintained ad libitum on Purina Laboratory Chow and tap water, was used. Fluorocortisol acetate (750 μg micro-crystals in 0.2 ml water) was injected subcutaneously once daily whereas the following agents were given twice daily:

disodium phosphate (1 mM in 2 ml water), cor oil (1 ml) by gavage and amiloride hydrochloride (200 μg in 0.2 ml water) subcutaneously.

In a preliminary experiment, 50 rats were divided into 5 equal groups. One served as control; each of the others received one of the following substances: fluorocortisol, disodium phosphate, corn oil or amiloride for 5 days to test their effect when administered separately.

In the main experiment, 410 rats were treated as indicated in Table I, and examined on the 2nd, 3rd, 4th and 5th days; some groups were larger than others to compensate for the higher mortality expected in the rats receiving cardiotoxic treatment.

The ECG was recorded under light ether anaesthesia using a Simpliscriptor EH 100 (Litton Medical Products, U.S.A.). The animals were placed on their back on wooden boards and their paws secured by rubber bands. Twenty-six gauge needles were placed subcutaneously and served as electrodes for standard limb leads. Similar electrodes were located at the midstream anteriorly (V_{ant} corresponding to V_2) and at the anterior axillary line lateral to the xiphoid process (V_{lat} corresponding to V_5) as chest leads. All records were taken with a paper speed of 100 mm/sec and the sensitivity was adjusted so that 1 mV was equivalent to 10 mm deflection. The ECG was evaluated by means of a magnifying lens.

The severity of the cardiac lesions in the animals dying during the experiment and in those subsequently sacrificed was estimated in terms of an arbitrary scale of 0—3 [1].

Results

In tests on the control rats, the recording technique revealed a clear distinction between the P and QRS components in leads II, III, V_{ant} and V_{lat}, but the P and T waves of lead I were often iso-electric. The electrical axis deviated to the right, averaging $-85°$ (Figs 1A, 2A). The electrocardiogram resembled the normal human ECG in its basic pattern except that: (a) the T wave always rose in direct continuity with the S wave, hence an iso-potential ST segment was not visible; (b) voltages were lower; (c) intervals were shorter due to the higher heart rate (Figs 1A, 2A). The animals given disodium phosphate, corn oil or amiloride alone during 5 days showed an essentially normal ECG.

The alterations produced by fluorocortisol, administered alone or in combination with one or more of the above compounds, are presented in Table I. This shows the heart rate and duration of the PR, QRS and QU periods, the severity of the cardiac lesions and the mortality in the different groups.

Compared with the controls (group 1), the average heart rate of the animals treated with fluorocortisol (group 2) gradually increased and became significantly higher by the 4th day ($P < 0.01$). The electrical axis showed a similar deviation to the right. No change occurred in the duration of the different periods, but a progressive rise was observed in the P, R and S amplitudes in all leads, e.g., the normal average R_2 of 0.55 mV increased to 0.81 mV on the 3rd day ($P < 0.001$). Similarly, the S_2 advanced from 0.18 to 0.35 mV ($P < 0.001$) and the P_2 from 0.10 to 0.21 mV on the 4th day ($P < 0.001$). The descending segment of the T wave became more oblique and, after 3 days treatment, a U wave was frequently seen.

Animals given fluorocortisol and corn oil conjointly (group 3) had a higher heart rate on the 5th day ($P < 0.01$) than the fluorocortisol-treated

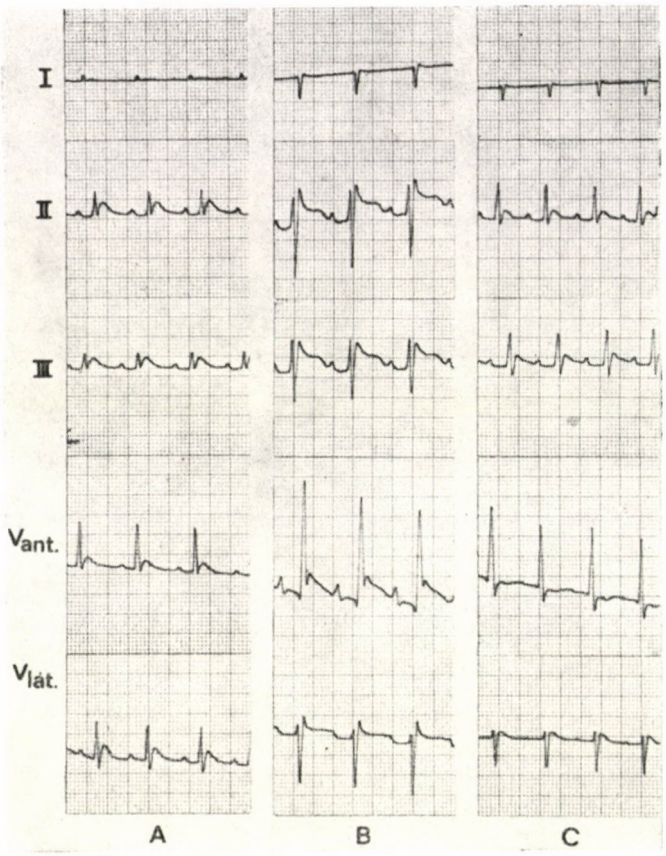

Fig. 1. A: ECG of normal rat; I, II, III standard limb leads and two precordial leads; B: ECG alterations five days after combined treatment with fluorocortisol, disodium phosphate and corn oil; C: Preventive effect of amiloride on the ECG alterations in the ESCN

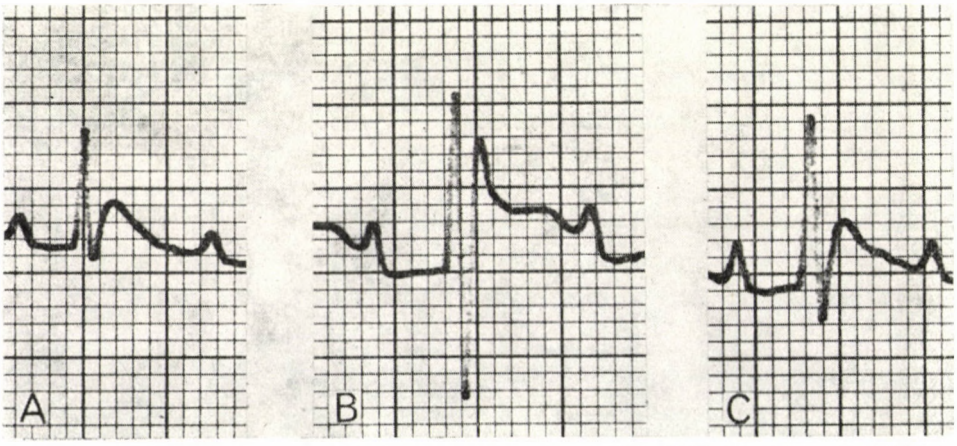

Fig. 2. Lead II ECG (C×magnification). A: Normal rat; B: ECG alteration of ESCN on the 5th day; C: Prevention of ECG changes in ESCN by amiloride

Table I

Group	Treatment	Day	No.	Rate (min)	PR (msec)	QRS (msec)	QU (msec)	Infarct (0–3)	Mortality (%)
1			60	426.1 ± 5.3	45.88 ± 0.49	19.01 ± 0.14	67.60 ± 0.6	—	—
2	Fluorocortisol	2	10	438.1 ± 6.6	46.01 ± 0.68	18.44 ± 0.28	67.90 ± 1.05	—	—
		3	10	451.5 ± 8.8	46.70 ± 1.01	19.00 ± 0.25	69.30 ± 1.16	—	—
		4	10	466.3 ± 11.0	47.60 ± 0.99	18.30 ± 0.51	67.70 ± 1.33	—	—
		5	25	467.9 ± 6.7	46.87 ± 0.46	19.16 ± 0.35	68.16 ± 1.03	—	—
3	Fluorocortisol + corn oil	2	10	464.2 ± 9.8	45.34 ± 0.63	18.39 ± 0.31	66.12 ± 1.32	—	—
		3	15	486.2 ± 9.7	47.00 ± 1.24	18.26 ± 0.25	66.26 ± 0.80	—	—
		4	15	498.6 ± 6.9	44.66 ± 0.83	18.20 ± 0.14	66.13 ± 0.88	—	—
		5	25	499.1 ± 7.3	46.39 ± 0.75	18.52 ± 0.24	66.04 ± 1.03	—	—
5	Fluorocortisol + disodium phosphate	2	10	418.0 ± 6.3	49.90 ± 0.22	19.20 ± 0.28	72.40 ± 1.26	—	—
		3	15	433.3 ± 9.9	49.33 ± 0.99	19.13 ± 0.19	74.50 ± 1.44	—	—
		4	15	433.7 ± 12.2	51.92 ± 2.11	20.33 ± 0.47	77.60 ± 3.20	0.1	—
		5	25	444.2 ± 10.6	50.16 ± 2.56	19.83 ± 0.28	71.87 ± 1.24	0.2	—
4	Fluorocortisol + disodium phosphate + corn oil	2	10	410.8 ± 13.3	53.20 ± 1.04	19.70 ± 0.30	77.10 ± 1.59	—	—
		3	20	442.3 ± 12.0	50.66 ± 1.15	20.78 ± 0.32	81.26 ± 3.05	0.2	5
		4	20	435.0 ± 9.9	55.06 ± 1.08	21.56 ± 0.20	77.93 ± 2.22	0.8	20
		5	40	423.1 ± 14.8	54.54 ± 1.34	22.54 ± 0.77	82.62 ± 3.20	1.3	35
6	Fluorocortisol + disodium phosphate + corn oil + amiloride	2	10	442.3 ± 7.6	45.10 ± 1.20	17.40 ± 0.30	69.40 ± 0.42	—	—
		3	20	491.4 ± 8.9	43.26 ± 0.57	17.94 ± 0.71	66.63 ± 1.06	—	—
		4	20	506.9 ± 8.3	42.64 ± 0.59	17.52 ± 0.22	64.64 ± 0.98	0.1	—
		5	25	500.1 ± 8.3	44.39 ± 0.60	17.73 ± 0.17	64.39 ± 0.84	0.1	4

rats (group 2). Otherwise, the ECG in these two groups was similar in all respects.

Signs of cardiotoxicity became evident when fluorocortisol and disodium phosphate were administered concurrently (group 4). A prolongation of the PR ($P < 0.05$) and QU ($P < 0.05$) periods, as compared with those of the animals receiving fluorocortisol alone (group 2), was observed on the 2nd day. The same extension in the QRS period ($P < 0.01$) was noted on the 4th day (Table I), associated with a more angular T wave and the presence of U waves. A comparable increase was observed in the amplitudes of the P, R and S waves. On the 4th and 5th days atrial fibrillation and third degree block were recorded in 5 animals with or without macroscopic cardiac necrosis.

As expected, the most severe lesions were produced by the full cardiotoxic treatment (group 5). Compared with those of the group given fluorocortisol and disodium phosphate (group 4), the ECG of these animals showed a further increase in the duration of the PR ($P < 0.05$) and QU ($P < 0.05$) periods on the 2nd day as well as of the QRS ($P < 0.01$) period on the 3rd day (Table I). The T wave took on a spike-like appearance on the 5th day; the same increase in the P, R and S amplitudes and the presence of U waves was again observed (Figs 1B, 2B). Between the 3rd and 5th days atrial fibrillation, second and third degree block and bigeminia were also recorded in 10 animals. On the 3rd day cardiac necrosis became visible in some animals; by the 4th and 5th days, many more had similar lesions. In general, the most significant electrocardiographic alterations were found on the 5th day in animals manifesting signs of necrosis, compared with those (in the same group) not displaying macroscopic lesions: the heart rate dropped from 462 to 406/min ($P < 0.05$), the PR period was prolonged from 51 to 56 ($P < 0.05$), that of the QRS from 21 to 23 ($P < 0.01$) and that of the QU from 77 to 86 msec ($P < 0.05$).

All severe changes in the ECG following the full treatment (group 5) were inhibited when amiloride was also given (group 6). The duration of the PR, QRS and QU periods was normal, and the T waves showed the normal convex appearance (Figs 1C, 2C). Neither fibrillation, block nor bigeminia was observed, and cardiac necrosis as well as mortality was almost completely prevented. However, the amplitudes of the P, R and S waves remained elevated and, compared with that of the controls (group 1), the heart rate was higher ($P < 0.001$).

Discussion

The correct interpretation of the electrocardiogram of small mammals should take into consideration the size, weight and heart rate of the animals, the heart's generally vertical axis and great mobility when functioning, certain physiological peculiarities of cardiac activity and, of course, the recording

technique [6, 7, 8, 9]. Our findings are in agreement with observations [7, 8] showing that the large deflection which follows the QRS complex is a T wave and that there is no iso-potential ST segment in the electrocardiogram of rats. In our controls, the duration of the PR, QRS and QU corresponded to the data of others [7, 10, 11] allowing for certain slight, inevitable variations. There are discrepancies in the literature concerning the duration of the QT period [6—11]. These might be explained by the fact that the U waves are not always distinct from the T waves; in such cases, the QT actually corresponds to a QU period. Though differentiation is generally possible with the aid of a magnifying lens [9], we measured the duration of the QU periods, in order to avoid any misunderstanding.

The correlations between the electrocardiographic alterations and the cardiac lesions produced by methylchlorocortisol and NaH_2PO_4 were studied by ZSÓTÉR et al. [10]. Although the development of this cardiopathy was not aggravated by additional lipid administration as in the present experiments, our results reveal many similarities between the electrocardiograms of these two variants of the ESCN.

The early electrocardiographic alterations in the ESCN are probably due to electrolyte and acid–base disturbances. In hypopotassaemia the amplitudes of the P and U waves as well as the QRS complexes are increased and the duration of the PR, QRS and QU periods is prolonged [12, 13, 14]. Since fluorocortisol was shown to produce hypopotassaemia (3.9 mEq/l [3]), the increase of the P and QRS amplitudes and the appearance of U waves in group 2 are undoubtedly related to a disturbed electrolyte metabolism. The prolongation of the PR, QRS and QU periods after conjoint treatment with fluorocortisol and disodium phosphate (group 4) can be explained by the accentuation of hypopotassaemia (3.5 mEq/l [3]). There is a correlation also between the very severe hypopotassaemia observed in earlier experiments (2.8 mEq/l [3]) and the most pronounced changes recorded after the full cardiotoxic treatment (group 5).

The ECG could also be influenced by hypernatraemia (148 mEq/l [3]) and extracellular metabolic alkalosis (pH 7.5, standard bicarbonate 31 mEq/l, base excess +8, pCO_2 43 mm Hg [4]), which are further biochemical characteristics of the ESCN [3, 4]. High sodium concentrations decrease the duration of the QRS period [12] and therefore might counteract the effect of hypopotassaemia. Either by conditions of alkalosis, with or without hypopotassaemia or by hypopotassaemia in the absence of alkalosis, similar electrocardiographic alterations were produced in dogs [15].

The electrographic changes were apparent by the 2nd day, thus preceding the macroscopic and histologic cardiac lesions. Treatment with fluorocortisol and disodium phosphate (group 4) resulted in the prolongation of the PR and QU periods, which were further extended when lipids were also given (group 5).

At this time, only fine ultrastructural changes were visible, the cardiac lesions becoming manifest under light microscopy by the 3rd day [16]. Their macroscopic appearance, between the 3rd and 5th days, was usually associated with the most pronounced electrocardiographic deviations. However, in some animals not manifesting morphologic lesions similarly severe ECG changes were found; these were probably due to electrolyte disturbances [3, 4].

In analyzing the different form variants of T waves, WERTH [7] found an excessively high and sharp deflection of the T wave, a characteristic sign of hypoxia. This type of T wave was frequently seen on the 5th day after conjoint treatment with fluorocortisol, disodium phosphate and corn oil (Fig. 2B).

The administration of amiloride prevented the development of many of the electrocardiographic changes in the ESCN. It also completely inhibited the development of any type of arrhythmia. Of special importance is the fact that, without causing hyperpotassaemia, it normalized or even shortened the duration of the PR, QRS and QU periods in every treated animal (Fig. 2C). The inhibitory effect of amiloride on these electrocardiographic alterations and on the development of arrhythmias deserves further attention.

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Dr. S. VARGA
Dr. H. C. CLASSEN
Dr. B. SOLYMOSS
Institut de Médecine et de Chirurgie expérimentales
Université de Montréal, Montreal (Canada)

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UREA INHIBITION OF LACTATE DEHYDROGENASE

A CONVENIENT ROUTINE PROCEDURE

By

G. DISCOMBE and S. LUND

DIVISION OF LABORATORIES, CENTRAL MIDDLESEX HOSPITAL, LONDON, N.W. 10

A trustworthy and convenient method for estimating total and urea-stable lactate dehydrogenase is described, and the reasons which led to its adoption are explained. It uses NAD which is cheaper and more reliable than $NADH_2$, and it is thought produce more useful information than does the estimation of hydroxybutyric dehydrogenase.

Kinetic enzyme assays, in which the reaction is monitored continuously or at short intervals by a recording spectrophotometer, are better than colorimetric or Autoanalyser methods because (i) the reaction velocity is shown to be constant, (ii) no standards or calibration curves are needed, (iii) colour and turbidity are of little importance, (iv) the buffer-substrate can usually be frozen or freeze-dried and (v) the apparatus is always available within a few minutes of demand, and hence urgent requests are easily satisfied. Since colorimetric and Autoanalyser methods usually require special calibration, or use commercial enzyme standards of uncertain reliability (BOWERS et al. 1967), and cannot show constancy of reaction velocity, they are inherently less reliable than kinetic analyses.

The 5 lactate-dehydrogenase isoenzymes (LD 1 to 5, in order of decreasing electrophoretic mobility at pH 7—8.6) can be separated by electrophoresis and quantitated, though laboriously, by coupling the reduction of NAD with that of a tetrazolium salt (MARKERT and MØLLER 1959, VAN DER HELM 1961, 1962, BARNETT 1962). The fast and slow isoenzymes are inhibited to different degrees by a variety of treatments; the slow enzyme being inhibited more than the fast by heating to 53—55°C (PLAGEMAN et al. 1961) or 60°C (BELL 1963), acetone (LATNER and TURNER 1963), chloroform (WARBURTON and SMITH 1963), excess pyruvate (THIERS and VALLEE 1958, ROSALKI and WILKINSON 1960) and urea and some of its derivatives (RICHTERICH et al. 1962, WITHYCOMBE et al. 1965, HARDY 1965). EMERY (1967) claims that under his conditions (lactate substrate, tris buffer pH 9) urea-inactivation can be used to give an estimation of the proportions of the two LDH sub-units in the preparation under test.

It is clear that after myocardial infarction there is an increase in the proportion of LD 1 and 2 in serum, and some rapid method of measuring these

would be of clinical convenience. LD 1 and 2 are more active than LD 4 and 5 when α-hydroxybutyric acid (or α-oxobutyric acid) is used as substrate (ROSALKI and WILKINSON 1960, ELLIOTT and WILKINSON 1961, 1962, ROSALKI 1962, 1963), and the "hydroxy-butyric dehydrogenase" has been widely used for the confirmation of myocardial infarction. Unfortunately if kinetic methods are used, preliminary removal of pyruvate is essential; this requires preincubation with $NADH_2$, which is slow and inconvenient; the substrate cannot be made from 2-oxobutyric acid, but only from its sodium salt, since the former polymerizes and decarboxylates to an inhibitor (WILKINSON et al. 1968). Recently the possibilities of urea-inactivation have been realised, and several methods of utilising it have been described (BABSON 1967, BOHN et al. 1966, KONTTINEN and LINDY 1967, LINDY and KONTTINEN 1967, BATSAKIS et al. 1967, WELSHMAN and RIXON 1968, ROSNOWSKA et al. 1968, EMERY et al. 1968). Those of EMERY et al. (1968) and BABSON (1967) appear to be most reliable, since they alone use lactate as substrate.

THIERS and VALLEE (1958) showed that no LDH assay depending on the reduction of pyruvate could be satisfactory because of the narrow optimum zone for the concentrations of substrate and $NADH_2$; HESS (1958) showed that the pH optimum depended on the proportions of the different isoenzymes. Their conclusions have been repeatedly confirmed (AMADOR et al. 1965, MARYMONT et al. 1968) and the reliability of commercial batches of $NADH_2$ has been impugned (MCCOMB and GAY 1968). It was decided to use a method depending on the oxidation of lactate by NAD, and, since the enzyme has a very broad pH optimum in the region of pH 10, to study the 2-amino-2--methyl-1-propanol (2A2M1P) buffer of pH 10.17 used in the p-nitrophenyl phosphate assay of alkaline phosphatase (BOWERS and MCCOMB 1966) which was in routine use, and was known to be stable at room temperature for several months.

Experimental

The reference method (KING 1965) is based on that of WACKER et al. (1956) but uses the glycine buffer of KING (1959) (L → P, glycine, pH 10.1). All measurements were made in a Unicam SP 800 recording spectrophotometer using a 30°C thermostatic jacket, and corrected to 25°C using the factor 0.67; since 0.1 ml serum was used in a volume of 3.1 ml, the result was calculated as Δ OD/min × 3333. Preliminary trials showed that a final concentration of 1.75 mM NAD was needed for optimum reaction velocity and this was obtained by adding 0.2 ml NAD (20 mg/ml).

Buffer and substrate. 18.763 g glycine and 14.625 g sodium chloride are dissolved in water, made up to 500 ml, and dispensed in 55 ml volumes into 4 oz. medical flats which are frozen lying on their largest side to avoid crack-

ing. 128.6 g 70% syrupy lactic acid are mixed with 500 ml water, 1 mol caustic soda added (VOLUCRON, MAY and BAKER), cooled, diluted to 1000 ml and adjusted to pH 10 with N NaOH. This keeps for 6 months at +4°C.

Working buffer-substrate. In two 200 ml flasks place

Total enzyme		Urea-stable
25 ml	glycine saline	25 ml
20 ml	lactate	20 ml
25 ml	0.3 N NaOH	25 ml
none	urea	24 g

Water to 200 ml.

This is dispensed in 20—22 ml volumes in Universal containers which are stored at —20°C, thawed, and warmed to 30°C as required. 2.8 ml buffer-substrate are placed in a cuvette, 0.1 ml serum added and mixed, and within 5 min 0.2 ml NAD (20 mg/ml) added. The reaction is monitored at 60 second intervals for 5 min and the initial reaction rate observed. The reaction velocity is constant for at least 5 min if the enzyme activity is below 250 I.U./l; at higher levels the reaction is slowed, so that smaller volumes of serum should be used if accurate results are needed. The 2A2M1P buffer was used at several different concentrations, and the urea concentration was also varied.

Material

Small (250 mg) pieces of heart and liver obtained at necropsy were ground in a Griffith homogeniser with 0.9% NaCl, diluted, centrifuged, passed through a Seitz clarifying filter, and diluted with 0.9% NaCl containing 3% bovine albumin (GRIEFF and KELLY 1966) to an activity of about 200 I.U./litre. The solutions were dispensed in small bottles stored at —20° C and thawed as required.

Results

The apparent activity of the tissue extracts in 2A2M1P buffers of different concentrations, with and without urea, are shown in Tables I and II. In the absence of urea, 1.4M 2A2M1P increases the activity of the heart extract 1.2 times, and reduces that of the liver extract to 0.52 of its value in glycine buffer, but no conditions were found which provided discriminatory power better than that obtained with the glycine buffer and 2M urea, in which urea enhanced the activity of heart extract 1.02 times, but reduced that of liver to 0.07 of its original. The glycine buffer was adopted for routine use and estimation of total and urea-stable LDH has replaced H.B.D. for routine use since September 1968. Heparinised plasma and serum give identical results; within the normal range results are reproducible to within 7 units. Local clinical opinion is that these assays are more useful than that of H.B.D.

Table I

Inhibition of LDH by 2M urea in 2A2M1P buffers

Molarity of 2A2M1P buffer	% Inhibition	
	Heart extract	Liver extract
Reference	nil	92
0.1	14	92
0.2	20	92
0.4	26	92
0.6	30	93
1.0	41	92
1.4	48	93

Table II

Inhibition of LDH by varying concentrations of urea in 0.4M 2A2M1P buffer

Urea (Molarity)	% residual activity	
	Heart extract	Liver extract
0	100	100
0.5	108	32
1.0	102	11
1.5	89	6.5
2.0	80.5	4.5

Serum levels

Normal range: 25 subjects, volunteers and hospital patients convalescent or with illnesses unlikely to affect the LDH.

Total: 72 ± 15 I.U./l range 42—102 I.U./l.

Urea-stable: 48 ± 11 I.U./l range 26—70 I.U./l.

Plots on arithmetic probability paper indicated a normal distribution with 1% limits 20 and 120 I.U./l for total and 15 and 72 I.U./l for urea-stable LDH. Direct comparison with colorimetric H.B.D. assays (Rosalki 1962) (49 comparisons) indicate an upper limit for urea-stable LDH of 74 I.U./l, the correlation coefficient for the two being +0.934. Results above 120 I.U./l total or 73 I.U./l urea-stable are considered abnormal.

The results in sick patients are shown in Table III and agree well, as would be expected, with those of Rosalki (1963) using H.B.D. assays. During the first 7 days after infarction estimation of the urea-stable enzyme was slightly more sensitive than that of total L.D.H., and no patients with proved infarction had normal levels throughout this period; raised levels

Table III

Lactate dehydrogenase in 103 patients with various diseases

	Number of patients	Number with total above 120 I.U./l	Number with urea-stable above 73 I.U./l	Number with total above 400 I.U./l urea-stable above 350 I.U./l
Diseases of chest				
1. Proved myocardial infarct	48	41	48	1
2. Ischaemic disease, no detectable infarct	21	1	2	0
3. Pulmonary infarct	2	2	1	1
4. Pleurisy	1	0	0	0
5. Fibrositis	1	0	0	0
Anaemia				
Megaloblastic untreated	4	4	4	4
Megaloblastic treated	2	0	0	0
Liver disease				
Active infective hepatitis	6	0	0	0
Cholelithiasis	3	0	0	0
Carcinoma				
1. Advanced, metastases	7	7	7	0
2. Other	3	0	0	0
Miscellaneous disease	6	0	0	0

were often apparent within 24 hours of thrombosis. A few patients, especially those with initially high levels, had raised levels for much longer, up to 18 days. Most infarctions can be confirmed by estimations taken during the first 4 days after thrombosis. A few patients with coronary disease present with pain, ventricular ectopic beats and absent electrocardiographic changes; such "infarcts" do not usually show an increased enzyme level and have been grouped with ischaemic change. Among the "miscellaneous" group are included 1 patient with mesenteric artery thrombosis and two with steatorrhoea. One patient with severe acute bronchitis showed a rise of total and urea-stable enzyme to 173/120 during clinical improvement, there being no evidence of myocardial infarction or malignant disease. Elderly patients with angina and bronchitis present difficult problems, but when L.D.H. levels of over 400 I.U./l are found megaloblastic anaemia may be suspected, levels of up to 1500 I.U./l being sometimes found; such levels can be estimated accurately only by using small quantities of serum 0.02 or 0.01 ml.

The test is not particularly useful in liver disease: even in acute infective hepatitis and cholelithiasis with jaundice the total LDH is usually normal,

though the urea-stable component is often low: a high urea-labile component is often seen in acute myocardial failure and suggests some damage to liver cells.

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Dr. George DISCOMBE, Dr. Shewakram LUND — Division of Laboratories, Central Middlesex Hospital, London, N. W. 10

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ANNOUNCEMENT

The Toxicology Information Program at the National Library of Medicine, directed by Dr. Charles N. Rice, was created in January 1967 in accordance with recommendations of the President's Science Advisory Committee (PSAC) and a directive of the President. Two recommendations made by the Committee in the PSAC report, *Handling of Toxicological Information*,* were that a "computer-based system for handling toxicological information . . . be established and given high priority within the Department of Health, Education, and Welfare," and that the information system "be established with a speed commensurate with the urgency of the need".

Among its activities, the Toxicology Information Program has contracted with the University of Pittsburgh to conduct a comprehensive study to identify sources of toxicological information. Cooperation is being sought from world-wide sources in identifying (a) journals containing toxicological information which may not be abstracted or indexed by any of the major services, and (b) new abstracting and indexing services relating to toxicology that may not be generally available in the United States.

It is suggested that individuals who are aware of pertinent journals and/or abstracting and indexing services communicate in writing with the:

University of Pittsburgh
Toxicology Information Project
4616 Henry Street
Pittsburgh, Pennsylvania 15213

* Report of the President's Science Abvisory Committee, June 1966. Obtainable from the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402 (price 15 cents).

ACTA MEDICA

ТОМ 27 — ВЫП. 1

РЕЗЮМЕ

ПЕРИОДИЧЕСКИЕ ПАРОКСИМАЛЬНЫЕ РАЗРЯДЫ ПОСЛЕ РАЗМЯГЧЕНИЯ В ОБЛАСТИ СРЕДНЕЙ АРТЕРИИ ГОЛОВНОГО МОЗГА

П. ХАЛАС, Ш. ГЁДЕНЬ и Й. ХИДАШИ

У больного с размягчением головного мозга, вследствие односторонней закупорки средней артерии мозга, наблюдалась чередующаяся ЭЭГ-активность, содержащая отчасти отдельные периодические пароксизмальные разряды и отчасти серийные разряды, связанные плоскими участками. На основе функциональных особенностей электрических явлений и электропатологических связей два чередующиеся типа активности, наблюдавшиеся у больного, можно привести в аналогию с двумя главными типами периодических пароксизмальных явлений: с активностью, показывающей периодические комплексы [панэнцефалитического характера], и с активностью suppression-burst, развивающейся подобно ЭЭГ при кортикальной денервации. В этих ЭЭГ-явлениях можно полагать наличие общего патомеханизма, при котором, наряду с гипоксией, решающую роль играет своеобразное состояние обмена веществ денервированной коры головного мозга.

РЕГУЛЯЦИЯ КРОВООБРАЩЕНИЯ В КОНЕЧНОСТЯХ ПРИ ЛИМФАТИЧЕСКОМ ОТЕКЕ В КОНЕЧНОСТЯХ

Ф. ШОЛЬТИ, Й. НАДЬ, Й. ГЕРГЕЙ, М. ИШКУМ, И. КРАСНАИ, А. ХАРТАИ и М. ЧИЛЛАГ

У 8 больных с лимфатическим отеком в конечностях авторы изучали методом разведения красителя изменения кровотока, сосудистого сопротивления, потребления кислорода и фракций минутного объема кровообращения в конечностях. Соответствующие параметры 44 лиц с нормальной циркуляцией служили в качестве контроля.

Под влиянием длительного застоя лимфы кровообращение в конечностях нарастает в значительной мере, сосудистое сопротивление в конечностях понижается и фракция минутного объема кровообращения в конечностях увеличивается. Потребление кислорода в конечностях не показывает достоверного отклонения от величин, измеряемых в норме.

Заслуживает внимания факт, что при застое лимфы регуляция кровообращения конечностей изменяется в направлении, противоположном направлению ее изменения при препятственности венозного оттека.

Полагается, что при лимфатическом отеке значительная часть крови протекает через непитательные — короткие — сосудистые пути. Причиной этого явления, по всей вероятности, является прежде всего изменение регуляция микроциркуляции в конечностях.

ДЕЙСТВИЕ ХОЛЕСТЕРИНА ПИЩИ НА ОБМЕН ВИТАМИНА С У ПОДОПЫТНЫХ ЖИВОТНЫХ

Э. ГИНТЕР

Добавление холестерина к пище морской свинки повышает потребление витамина С. Это видно из уменьшения концентрации витамина С в тканях и из повышения количества витамина С, выделяемого с мочой. У кроликов и белых крыс, сами синтезирующих аскорбиновую кислоту, пища с холестерином повышает биосинтез авкорбиновой кислоты, вследствие чего витамин С накопляется в тканях и в повышенной мере выделяется с мочой.

Реакция животных, организм которых синтезирует аскорбиновую кислоту, так же как и реакция животных, неспособных синтезировать ее, равным образом обуславливаются обстоятельствами, сопровождающимися повышением потребности организма в аскорбиновой кислоте. Остается невыясненным вопрос, является ли основой этого механизма какая-то неспецифическая стрессорная ситуация или близкое родство между обменом аскорбиновой кислоты и обменом холестерина.

АНОМАЛИИ КАРТИНЫ ЭЭГ У НОРМАЛЬНЫХ БЛИЗНЕЦОВ

Я. ЛИПТАК, О. ЖАДАНЬИ, Я. БАЖО и О. КЛЕЙНИНГЕР

Авторы исследовали 60 нормальных близнецов (6 пар монозиготных и 22 пары дизиготных) в возрасте от 5 до 17 лет. При рождении эти близнецы были зрелыми плодами, заболеваний нервной системы у них не наблюдалось. Кроме отведений ЭЭГ (без наркоза) были проведены также антропологические, нейропсихиатрические и психологические исследования.

В 38% случаев авторы обнаружили аномалии (патологическую синхронизацию в 13%, крупные волны при гипервентиляции в 19%, дисритмии в 9%, очаговую активность в 5%. По сравнению с данными нормальных плодов это означает достоверное повышение. Между возрастом, весом при рождении, перинатальными осложнениями, зиготностью и аномалиями ЭЭГ не было найдено корреляции. Наблюдалась, однако, очень тесная, почти достоверная связь с неврологическими субнормальностями.

Ответственным за большое число аномалий ЭЭГ авторы считают умеренные пренатальные поражения головного мозга, возникшие у близнецов в результате неблагоприятных внутриутробных условий.

ДЕЙСТВИЕ МОЧЕГОННЫХ И ПЕРОРАЛЬНЫХ АНТИДИАБЕТИЧЕСКИХ СРЕДСТВ ПРИ НЕСАХАРНОМ МОЧЕИЗНУРЕНИИ И ПРИ ПСИХИЧЕСКОЙ ПОЛИДИПСИИ

Ф. ЛАСЛО, Л. ЦАКО, И. СИЙ и К. КОВАЧ

Авторы давалл 10 больным, страдающим несахарным мочеизнурением и не обладающим запасом антидиуретического гормона, а также 4 больным со психической полидипсией, салуретические средства (Альдактон, Хлорурит, или Хлорурит + Альдактон), а также пероральные антидиабетические средства (Диабинез, Орадиан), и изучали возникающие изменения водного хозяйства и обмена электролитов.

Установлено, что у больных несахарным мочеизнурением под влиянием мочегонных средств уменьшается диурез и умеренно нарастает удельный вес мочи. Все три изучаемые методы лечения вызывают повышение выделения натрия. Дача хлорурита обуславливает кроме этого и значительный калиурез, что можно предотвратить одновременной дачей Альдактона. В то же время Альдактон, будучи антагонистом альдостерона, потенцирует антидиуретическое действие Хлорурита. Во время лечения наблюдается уменьшение эндогенного креатининового клиренса. Это уменьшение выраженнее всего под влиянием Хлорурита. У лиц со психической полидипсией дача салуретических средств не привела к выявляемым изменениям водного хозяйства.

При даче Хлорпропамида больные несахарным мочеизнурением выделяли существенно меньше мочи, и удельный вес мочи также повышался в большей мере, чем после дачи мочегонных средств. В противоположность этому выделение натрия и калия, а также креатининовый клиренс оставались неизменными, и у лиц со психической полидипсией количество мочи также показало явное уменьшение. Орадиан оказался безэффективным.

Авторы придерживаются того мнения, что в терапии больных несахарным мочеизнурением рекомендуется прежде всего применение хлорпропамида (Диабинез) и хлоротиазида + спиронолактона (Хлорурит + Альдактон). Для выяснения эффективности длительной дачи лекарств необходимы еще дальнейшие наблюдения.

ДЕЙСТВИЕ ПОВРЕЖДЕНИЯ ПЕЧЕНИ И ЭКСПЕРИМЕНТАЛЬНОГО ВОСПАЛЕНИЯ НА КОНЦЕНТРАЦИЮ ГЛИКОПРОТЕИНОВ В СЫВОРОТКЕ

Я. ФЕХЕР, Л. ЯКАБ и Л. ТАКАЧ

Авторы изучали у крыс изменения концентрации компонентов гликопротеинов сыворотки (гексоза, гексозамин, сиалиновая кислота и серомукоид) после дачи кротонового масла, CCl_4 и CCl_4 + кротонового масла. При воспалении, вызванном кротоновым маслом,

повышалась концентрация гексозамина, сиалиновой кислоты и серомукоида. При поражении печени, вызванном дачей CCl_4, также наблюдалось достоверное повышение концентрации этих углеводных компонентов. При поражении печени вызывание воспаления дачей кротонового масла привело к достоверному повышению концентрации гексоза, гексозамина и сиалиновой кислоты, по сравнению с величинами, полученными при повреждении печени, вызванном дачей CCl_4.

ДАННЫЕ К ПАТОГЕНЕЗУ АНЕМИИ ПРИ ПЕРЕСАЖЕННОЙ АКР-ЛЕЙКЕМИИ

Т. БУРГЕР и Л. Г. ЛАЙТА

Пересадкой клеток селезенки животных, страдающих спонтанной АКР-лейкемией, авторы вызывали у гибридов АКР/СЗН острую лимфоидную лейкемию и использовали их в качестве модели в своих опытах. При прогрессировании болезни инкорпорация Fe^{59} эритроцитами уменьшается, и параллельно с этим анемия усиливается. В течение болезни повышается поглощение радиожелеза печенью; это наблюдение можно привести в связь с повышающейся инфильтрацией печени лейкемическими клетками.

Повышенная смертность наблюдалась у животных, получавших радиотерапию и трансплантацию лейкемических костномозговых клеток. Это указывает на понижение защитного действия лейкемического костного мозга против облучения. Через 15 дней после облучения наблюдается достоверная разница в 72-часовой инкорпорации радиожелеза между нормальными животными с трансплантацией лейкемического костного мозга. Значительная инкорпорация радиожелеза печенью облученных животных указывает на сравнительно ненарушенную железосвязывающую способность печени. В группе животных с трансплантацией лейкемического костного мозга вес селезенки и число макроскопически наблюдаемых колоний селезенки превышали соответствующие данные группы животных с пересадкой нормального костного мозга. На основе этой разницы можно полагать, что лейкемические бластоматозные клетки способны к образованию колоний.

ВРЕДНЫЕ ПОБОЧНЫЕ ДЕЙСТВИЯ ПРОИЗВОДНЫХ ПИПЕРАЗИНА У ПОДОПЫТНЫХ ЖИВОТНЫХ

II. Биохимическое исследование хрусталика глаза и повреждение лимфоцитов

Г. Й. КЕТЕЛЕШ, А. ДЕНКЛЕН, П. МЕЗЕИ и А. ТАКАЧ

В опытах на крысах при длительном применении 1,4- bis- (фенилизопропил)-пиперазина (2HCl) в хрусталике не неблюдается изменения содержания белков, рибонуклеиновой кислоты и кислоторастворимой рибозы, и синтеза рибонуклеиновой кислоты. Средняя инкорпорация аминовой кислоты, меченной C^{14}, была выше, чем у контролей. У подопытных животных наблюдалось in vitro нарушение деления периферических лимфоцитов, вызванного фитогемагглютинином, сказывающееся в расплывчатости структуры хромосом.

Хотя вопрос патомеханизма еще остается неясным, авторы обсуждают две возможности: нарушение проницаемости вследствие поражения капсулы хрусталика, вернее оболочек клеток и волокон, или нарушение механизма образования волокон из эпителиальных клеток на уровне клеточного ядра.

ВРЕДНЫЕ ПОБОЧНЫЕ ДЕЙСТВИЯ ПРОИЗВОДНЫХ ПИПЕРЕЗИНА У ПОДОПЫТНЫХ ЖИВОТНЫХ

I. Офтальмологические наблюдения

П. МЕЗЕИ, Г. Й. КЕТЕЛЕШ, А. ТАКАЧ и П. ЙОББАДЬИ

1,4- bis- (фенилизопропил)-пиперазин (2HCl), оказавшийся у людей катарактогенным соединением, при длительном применении вызывал патологические офтальмологические изменения также у крыс и мышей. Данное соединение оказывало у животных также фотосенсибилизирующий эффект. У подопытных животных наблюдались изменения конъюнктивы, роговицы и волос. Хотя плотной катаракты, подобной человеческой, не развивалось, отмечались тонкие патологические морфологические изменения хрусталика, предположительно вследствие нарушения образования волокон.

МЕТАБОЛИЗМ 4—^{14}C-ДЕГИДРОЭПИАНДРОСТЕРОНА У ЧЕЛОВЕКА in vitro.

I. Превращение 4—^{14}C-дегидроэпанидростерона в андрост-4-тен-3,17-дион, тестостерон и андростерон в кожных срезах здоровых женщин и мужчин in vitro

И. ФАРЕДИН, К. КОКАИ, И. ТОТ, А. Г. ФАЗЕКАШ и М. ЮЛЕС

Срезы брюшной кожи здоровых женщин и мужчин были инкубированы с [4—^{14}C] дегидроэпиандростероном в целях изучения биосинтеза стероидов высокой андрогенной активности. Было установлено, что как кожа женщин, так и кожа мужчин равным образом способна синтезировать андрост-4-ен-3,17-дион, 50-андростен-3,17-дион, андростерон и тестостерон из [4—^{14}C] дегидроэпиандростерона. Обсуждается теоретическое и практическое значение полученных результатов.

ЭЛЕКТРОКАРДИОГРАФИЧЕСКИЕ ИЗМЕНЕНИЯ ПРИ МЕТАБОЛИЧЕСКОМ КАРДИАЛЬНОМ НЕКРОЗЕ И ВЛИЯНИЕ АМИЛОРИДА НА ЭТИ ИЗМЕНЕНИЯ

Ш. ВАРГА, Х. Г. КЛАССЕН и Б. ШОЛЬМОШШ

В течение развития неокклюзионного метаболического кардиального некроза (вызываемого у крысы путем одновременного введения флюорокортизола, фосфата динатрия и кукурузного масла) продолжительность интервалов PR, ORS и QU удлиняется амплитуды зубцов P, R и S повышаются и зубцы T изменяются. Зубцы U закономерно появляются. Эти явления предшествуют гистологическим и макроскопическим кардиальным поражениям, и между ними и предварительно наблюдаемыми нарушениями содержания электролитов и кислот-оснований существует определенная связь. Наиболее выраженные электрокардиографические изменения отмечаются, как правило, при наличии макроскопических поражений, и они иногда сопровождаются аритмией, блокадой и бигеминией. Амилорид, щадящее калий вещество, не предотвращает повышения амплитуды зубцов P, R и S, но оно нормализует другие изменения и задерживает развитие кардиального некроза.

МОЧЕВИННОЕ ТОРМОЖЕНИЕ ДЕГИДРОГЕНЕЗЫ МОЛОЧНОЙ КИСЛОТЫ, УДОБНЫЙ ПОВСЕДНЕВНЫЙ МЕТОД ИССЛЕДОВАНИЯ

Д. ДИСКУМБ и Ш. ЛУНД

Дается описание надежного и удобного метода для определения общей и стойкой к мочевине дегидрогенезы молочной кислоты, и объяснение причин, приведших к его применению.

При этом методе используется NAD, более дешевая и более надежная чем $NADH_2$, и он, повидимому, обеспечивает более полезные информации, чем определение дегидрогенезы оксимасляной кислоты.

ANNOUNCEMENT OF AN IAEA SYMPOSIUM

Title: Dynamic Studies with Radioisotopes in Clinical Medicine and Research

Date: 31 August—4 September 1970

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PLATELET LIFE-SPAN AND SITES OF PLATELET DESTRUCTION IN IDIOPATHIC THROMBOCYTOPENIC PURPURA

T. BURGER and E. RIHMER

FIRST DEPARTMENT OF MEDICINE (DIRECTOR: PROF. T. JÁVOR), UNIVERSITY MEDICAL SCHOOL, PÉCS

(Received March 6, 1969)

Measurement and evaluation of platelet life-span and sequestration have been studied in a healthy control group and in patients with idiopathic thrombocytopenic purpura. ACD is considered a coagulant more suitable than EDTA for the preparation of platelet suspension, since recovery is more frequent with the use of the former drug. Essential differences were found in the sites of sequestration with ACD and EDTA. The greater amount of radioactivity was detected over the spleen, in connection with the geometrical situation of measurements over the organs. As compared with normal subjects, in ITP patients considerable differences were found in platelet survival, recovery and sequestration. In the majority of cases radioactivity over the spleen was increased, but an increase in radioactivity over the liver was also observed in a few patients showing a considerable shortened platelet survival.

Idiopathic thrombocytopenic purpura (ITP) is a condition characterized by thrombocytopenia, a normal or increased number of megakaryocytes in the bone marrow, and, in most cases, by the absence of enlargement of the spleen. The disease is generally regarded as an autoimmune disorder in which the patient produces an antiplatelet factor, several methods for demonstrating this factor are known [13, 15, 17, 20].

The advances in methodology in the past decade have made it possible to study the mechanism and kinetics of the development of thrombocytopenia. AAS and GARDNER devised a method for measuring the life-span of ^{51}Cr-labelled platelets, later COHEN et al. classified the thrombocytopenias on the basis of increased destruction, shortened life-span and decreased production of the platelets [1, 13]. BARTA and BURGER, and NAJEAN et al. confirmed the fact of peripheral destruction and also established its site [6, 11, 25, 10, 9, 29, 4, 26, 8, 12].

What has, however, remained something of a mystery is the course of the disease and the cause of spontaneous remissions and relapses. The present paper will report on a study of the sites of platelet destruction.

Material and methods

A total of 17 patients with ITP and 25 healthy controls were studied.

One group of patients was examined a few days after starting steroid treatment, but before remission, while another group at a time when, as a result of the above therapy, the

platelet count was around 100,000 and the patients had proved free of clinical symptoms [6, 10, 11].

For measuring platelet life-span, AAS' and GARDNER's slightly modified method was used in all of the patients, in some of them the anticoagulant suggested by ASTER and JANDL was also applied. Organ surface radioactivity was measured with a Gamma type 60 mm scintillation detector equipped with a collimator. The end window of the collimator was 7 cm in diameter; the inside opening, 3.5 cm; and the distance of the crystal from the outside edge of the opening, 6 cm. Measurements *in vivo* were performed over the spleen in the anterior axillary fold, over the right pulmonary lobes between the 1st and 3rd ribs, and over the heart in the medioclavicular line, by means of a Gamma NK—108 type scaler, in differential position at the photo-peak of ^{51}Cr, and from the results obtained the background values were subtracted.

Details of the platelet suspension preparation and those of the measurement of radioactivity *in vivo* have been described before [9, 10, 11]. For the platelet suspension, ASTER and JANDL's (1964) acid-citrate-dextrose (ACD) solution pH 6.5—6.7 was applied, with citric acid 0.8 g, sodium citrate, 2.2 g; and dextrose, 2.45 g for every 100 ml. According to recent reports [2, 25] cooling of the platelets during their isolation damages them by making them spherical, therefore cooling has been omitted in the present experiment (MURPHY and GARDNER, 1969).

AAS and GARDNER used ethylenediamine tetraacetate (EDTA) for the preparation of platelet suspension, and so did we at the beginning. Our own studies as well as the observations by ASTER and JANDL have, however, shown that EDTA with a pH below 6 is injurious to platelets.

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

Results

The results have been tabulated. The control data were grouped separately, according to the anticoagulant used, while the data of the patients according to the coagulant used.

Among the data obtained with EDTA (Table I) it is remarkable that the proportion of labelled thrombocytes reappearing in the circulation was relatively low, ranging between 20 and 30%. Platelet life-span was 9—11 days in healthy controls. Comparison of the values obtained over the liver with those over the spleen showed, with the exception of two cases, a predominant accumulation of radioactivity over the liver.

Table II lists the data obtained with ACD solution. In this group, recovery of activity was generally twice that in the EDTA group. In 15 control subjects, the activity accumulated mostly in the spleen, while in the remaining three patients spleen radioactivity did not exceed liver radioactivity.

Radioactivity over the lungs was occasionally unusually high in the first 24 hours of examination, when there was a slight aggregation of labelled platelets in the suspension, or in one of the patients when he contracted pneumonia. In the majority of cases, activity was far less over the lungs than over the heart.

Thrombocyte life-span was normal in both control groups.

Tables III and IV contain the data of patients with chronic ITP in the active stage and in remission, respectively. Platelet count and half-survival time were lower than in the control groups. Platelet recovery after reinfusion was also lower. The radioactivity data obtained in the active stage showed

Table I

Normal subjects
(studied with EDTA)

Name	Platelets/cu. mm	Survival time of platelets, days	Activity recovery per cent	Final spleen: liver ratio*
P. S.	250,000	9	15	0.30
K. Gy.	220,000	10	29	0.25
B. Á.	280,000	9	34	1.0
V. P.	190,000	9	20	1.2
Cs. I.	240,000	11	20	0.5
B. A.	270,000	10	23	0.3
K. E.	230,000	9	29	0.6
V. Gy.	300,000	10	29	0.8
K. H.	280,000	9	27	0.4
B. I.	240,000	11	28	0.5

* At the time of 10% platelet survival.

Table II

Normal subjects
(studied with ACD)

Name	Platelets/cu. mm	Survival time of platelets, days	Activity recovery per cent	Final spleen: liver ratio*
F. I.	230,000	8	41	1.2
K. I.	250,000	10	61	2.0
B. H.	240,000	9	53	0.9
K. I.	230,000	9	49	0.8
M. S.	260,000	9	61	1.2
M. K.	350,000	8	49	3.0
F. K.	320,000	9	53	1.3
F. S.	250,000	8	41	2.1
B. T.	280,000	11	58	1.5
Gy. K.	270,000	10	44	2.0
H. B.	185,000	11	45	1.4
B. S.	240,000	8	41	1.4
D. I.	230,000	10	39	1.0
K. B.	250,000	9	57	0.7
M. E.	350,000	9	48	2.3

* At the time of 10% platelet survival.

the predominance of the spleen over the other organs, except in three cases. During improvement platelet count and half-survival time were normal, except in two patients. More platelets reappeared in the circulation and the activity data were also closer to those observed in the control groups: in seven cases radioactivity was higher over the liver.

Table III

Idiopathic thrombocytopenic purpura in relapse
(studied with EDTA)

Patient	Sex	Duration of thrombocytopenia prior to study, months	Platelet level at time of study (per cu. mm)	T ½ of injected platelets, days	Activity recovery per cent	Final spleen: liver ratio*
D. R.	F	18	40,000	3 ½	13	3.0
B. Z.	F	32	10,000	1	9	1.1
F. J.	F	25	80,000	2.5	17	0.8
P. G.	F	8	50,000	2	9	3.0
S. N.	F	14	20,000	2	11	0.75
B. T.	M	11	20,000	1.5	24	3.0
P. I.	F	19	30,000	2.5	13	2.2
I. I.	F	27	45,000	2	5	0.4
M. Zs.	F	21	30,000	10 hours	7	1.1
H. M.	M	19	15,000	20 hours	3	1.1

* At the time of 10% platelet survival.

Table IV

Idiopathic thrombocytopenic purpura in remission
(studied with EDTA)

Patient	Sex	Duration of thrombocytopenia prior to study, months	Platelet level at time of study, per cu. mm	T ½ of injected platelets, days	Activity recovery per cent	Final spleen: liver ratio*
D. R.	F	18	80,000	4	11	2.0
B. Z.	F	32	110,000	4	16	1.3
F. J.	F	25	130,000	3.5	17	0.4
P. G.	F	8	125,000	4	19	0.6
S. N.	F	14	140,000	5	17	0.5
B. T.	M	11	300,000	3	21	1.3
P. I.	F	19	120,000	4	13	0.6
I. I.	F	27	100,000	4	15	0.3
M. Zs.	F	21	120,000	4	19	0.7
H. M.	M	19	130,000	3	26	0.4

* At the time of 10% platelet survival.

Tables V and VI show the results obtained with ACD in the active stage and in remission. In this group, too, the platelet count was uniformly low, platelet half-life-span short, and the proportion of labelled platelets reappearing in the circulation was below normal. The highest values were measured over the spleen in seven patients, and over the liver in three patients.

Table V

Idiopathic thrombocytopenic purpura in relapse
(studied with ACD)

Patient	Sex	Age	Duration of thrombocytopenia prior to study, year	Platelet level at time of study; per cu. mm	No. of previous transfusions	Activity recovery per cent	T ½ of injected platelets, days	Final spleen: liver ratio*
R. I.	F	38	5	40,000	—	12	1.5	13.0
Tu. I.	F		?	50,000	1	11	1.5	0.7
G. M.	F	48	½	30,000	1	9	2.0	1.2
T. M.	F	48	1	30,000	—	8	20 hours	3.5
H. M.	F	52	10 months	20,000	—	10	16 hours	0.5
T. R.	F	15	3 months	9,000	2	6	1 hour	0.7
Gy. M.	M	22	2	30,000	—	7	2	3.0
K. I.	F	67	14	30,000	—	12	3	1.3
T. M.	F	62	40	30,000	2	6	1 hour	3.5
M. L.	M	48	½	30,000	—	9	1	5.0

* At the time of 10% platelet survival.

Table VI

Idiopathic thrombocytopenic purpura in remission
(studied with ACD)

Patient	Sex	Age	Duration of thrombocytopenia prior to study, year	Platelet level at time of study per cu. mm	No: of previous transfusions	Activity recovery per cent	T ½ of injected platelets, days	Final spleen: liver ratio*
V. Z.	M	35	1.5	130,000	—	40	2.5	1.5
Ri. I.	F	65	2	100,000	—	39	3.5	2.2
T. M.	F	48	1	180,000	—	47	3.0	2.0
K. I.	F	39	32	90,000	9	41	3.0	2.4
R. M.	F	21	2	120,000	—	47	2.5	1.8
H. M.	F	52	10 months	180,000	—	63	4.0	6.0
T. I.	F	20	10 months	110,000	—	49	4.0	1.1

* At the time of 10% platelet survival.

In seven patients in remission the platelet count was at the lower limit of normal, and platelet half-life-span 1—2 days shorter than in the controls. Recovery of activity was nearly normal, and so was its distribution: in a single patient that activity was higher over the spleen, and in one patient that activity was identical over the liver and over the spleen.

Statistical analysis of the data revealed a significant difference ($p > 0.05$) in activity values and their recovery between the two control groups, i.e. between the two different anticoagulants.

On the other hand, there was no significant difference in the activity data between the controls and the patients with ACD treatment (Tables V and VI), but the EDTA group showed a significant difference in radioactivity over the liver between the active and inactive stages of the disease (Tables III and IV). In the groups with chronic ITP the differences in the other parameters, furthermore between the active and inactive stages were significant when the anticoagulants ACD and EDTA were used (Tables III, IV, V and VI).

Discussion

In our first studies of platelet life-span it soon became obvious that the recovery value was low. This pointed to damage of the platelets and was regarded as a phenomenon inherent in their natural function. A toxic effect of ^{51}Cr with its high specific activity (1.19 μCurie/ml/0.04 mg) did not come into question. When, using the anticoagulant EDTA, the platelets obtained from the thrombocyte suspension were spread on a siliconized slide it could be observed that the thrombocytes had assumed a spherical form. Similar observations were made by other authors [2]. KISSMEYER-NIELSEN and MADSEN demonstrated that the affinity of EDTA for Mg-ion had changed the ion transport through the platelet membrane and thereby impaired its metabolism [16]. ASTER and JANDL attached importance to the low pH characteristic of EDTA, the anticoagulant used in their experiments.

The acidifying effect was regarded by them as the decisive factor in the damage, to which they attributed the increased sequestration of platelets in the liver [2]. The pH of the EDTA used in our experiments was 5.5. It was in the liver that we, too, found the greater radioactivity indicative of increased accumulation. Probably as a result of the differences in the measuring apparatuses used, our results obtained with ACD did not support the spleen-liver ratio of 4 : 1 as much as those of ASTER and JANDL [2], SOLOMON and CLATANOFF [30] and others [4, 8, 3] who also used ACD and measured the greater radioactivity over the spleen.

Having observed only a general toxic effect from EDTA we do not doubt its specific effect causing platelet accumulation in the liver. In view

of the larger number of reticulo-endothelial cells in it, the liver is, logically, the site of platelet destruction. Considering that the difference in radioactivity between the liver and the spleen was not proportional to the difference in mass between the two organs [liver/spleen: 6—8/1], in a mass unit of the spleen more platelets are destroyed and therefore its radioactivity is only relatively greater. ASTER and JANDL [2] believe that the liver and the spleen are equally implicated in the accumulation of platelets and they accounted for the difference in surface activity by the fact that in the spleen 1 μC of ^{51}Cr produces about four times as many cpm over the surface as does 1 μC of ^{51}Cr in the liver.

The data obtained in the patients are listed in Tables III, IV, V and VI. Platelet life-span is pathologically shortened with EDTA as well as with ACD. The ratio between the radioactivities over the organs showing signs of sequestration changed insofar as sequestration increases in the spleen, but in a few cases, especially in those with a low number and greatly shortened platelet life-span, in the liver. Similar observations have been made by several workers [3, 8, 27]. The finding was not significantly influenced by the use of EDTA or ACD. In remission the results were closer to the data of the healthy controls. An essential difference in the recovery of activity, did, however, exist between the active and the inactive phases.

The data obtained by us earlier differ partly from what has been said above, as we had ascribed to the lungs a role in platelet sequestration. Since then we have improved our method of investigation and according to our more recent findings it is only in certain pulmonary conditions that in the lungs sequestration of platelets is increased [12].

It is an old observation that in ITP the thrombocytes are not of full value and easily vulnerable. This explains why only a small part of the thrombocytes reappears in the circulation. The increased destruction of platelets, and as a result their shortened life-span, can be attributed to the presence and toxic effect of the antiplatelet factor. The presence of this factor can be proved by giving the plasma of an ITP patient to a healthy acceptor, in whom it will produce thrombopenia. The phenomenon appears in about 60% of the cases and cannot be caused by an isoantibody, because it occurs also in the absence of a previous pregnancy or transfusion. The factor is 7 S IgG, which is fairly stable. As it can pass through the placenta, the antiplatelet factor will cause thrombocytopenia also in the foetus. If ITP plasma is given to a splenectomized subject, only a larger amount of it will produce the above effect [19, 23, 32, 31, 21, 28].

Activity measurements in the patients give valuable information as to the advisability of splenectomy. It is known that there are steroid-resistant subjects, in whom splenectomy fails to produce the desired result. The prospects of splenectomy are poor if, during the study of sequestration, radioactivity

accumulates mostly in the liver. BARTA et al. and NAJEAN et al. were the first to report this observation [6, 25]. As proved by our own findings, their statement is not of absolute validity, since in spite of the poor prognosis one of our patients had recovered after splenectomy and has been free of complaints for three years now [12]. In the majority of ITP patients, however, the spleen, especially if it is somewhat enlarged — is the site of pathological destruction [4, 20]. In a model experiment HARRINGTON et al. observed that platelets bound by antibodies were destroyed in the spleen [17, 7, 15]. This holds true also in ITP. Clinical observations show that the factor responsible for thrombocytopenia is present also after splenectomy and that it is effective also in the case of passive transmission i.e. the symptoms can be elicited with an intact reticuloendothelial system. This has been confirmed in experiments with reticuloendothelial system blockade [29]. BALDINI et al. demonstrated in dogs that after moderate sensitization the platelets are destroyed in the spleen, while, in severe cases, in the liver [5]. As compared with the data in the literature it was in a few cases only that we had to resort to splenectomy; these cases have not been shown in the tables. In addition to eliminating one of the sites of platelet destruction, splenectomy decreases the amount of tissue producing antiplatelet antibody. Besides steroid therapy and splenectomy, treatment of ITP with immunosuppressive has been attempted with favourable results. BALDINI achieved remarkable results with 6-mercaptopurine [5]. The results of splenectomy and of other kinds of treatment in patients with ITP will be described in another paper.

According to our findings, platelets sequestration takes place in the liver and the spleen. The toxic effect of antiplatelet antibodies may change the rate of sequestration at the various sites of platelet destruction.

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Dr. Tibor Burger, E. Rihmer
First Department of Medicine, Ifjuság u. 31. Pécs, Hungary

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EFFECT OF GLUCOSAMINE AND OF ITS DERIVATIVES ON $^{35}SO_4$-INCORPORATION INTO DOG AORTA

By

L. JAKAB and ÉVA SEREGÉLYI

THIRD DEPARTMENT OF MEDICINE, MEDICAL UNIVERSITY, BUDAPEST

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Incorporation of $^{35}SO_4$ by aortic tissue of 2 to 5 months old untreated dogs and of adult dogs with croton oil-induced inflammation was examined in vitro. Neither of the two glucosamine derivatives, N-acetyl-4-6-benzylidene-glucosamine and N-acetyl--4-6-p-oxy-benzene-glucosamine had any appreciable influence on the incorporation of $^{35}SO_4$. No stimulating effect of glucosamine on $^{35}SO_4$ incorporation was demonstrable either. $^{35}SO_4$ incorporation by the adventitial surface was found to exceed that of the intimal surface. The difference in uptake between the two layers increased with the length of incubation. Croton oil inflammation failed to affect significantly $^{35}SO_4$ incorporation by aortic tissue.

Incorporation of $^{35}SO_4$ into all three layers of the rabbit aorta was demonstrated by ODEBLAD and BOSTRÖM (1953). BELLMAN et al. (1956) found $^{35}SO_4$ incorporation to be identical all along the individual sections of the dog aorta and to vary parallel with tissue respiration. The aorta of the dog is known to contain chondroitin-4-SO_4, heparane-SO_4 and traces of hyaluronic acid and of dermatane-SO_4 (SIREK et al. 1964).

The glycoprotein (GP)- and glycosaminoglycane (GG)-contents of the aortic adventitia have received little attention, though it has been pointed out that the GG-content of the adventitia is far from negligible (MURATA et al. 1964, JÓZSA et al. 1964).

In an earlier study we examined the effect of glucosamine derivatives on $^{35}SO_4$-incorporation into calf cartilage and rabbit aorta (JAKAB et al. 1969; JAKAB et al. 1969).

The present study has been concerned with the effects of two glucosamine derivatives (Antimetabolite I: N-acetyl-4-6-benzylidene-glucosamine; Antimetabolite II: N-acetyl-4-6-p-oxy-benzene-glucosamine) on $^{35}SO_4$ incorporation by the dog aorta. The intima and adventitia were separately studied for possible antimetabolite effect of the substances.

By the study of the possible influence of the antimetabolites on GG-metabolism it was sought to gain evidence which might be relevant to human pathology as well. There are obvious relationships between the metabolism of the aortic layers and certain pathologic processes.

Material and method

Mongrel dogs of either sex were used. For the study of the effect of N-acetyl-4-6--benzylidene-glucosamine, the aortas were obtained from five dogs aged between 2 and 5 months, and those for the study of N-acetyl-4-6-p-oxy-benzene-glucosamine, from four dogs of similar age. Four young adult dogs were injected with a mixture of 0.1 ml croton oil + 0.4 ml sunflower oil subcutaneously into the left hind leg and killed five days later under chloralose anaesthesia. The aorta was removed and immediately freed from the adventitial fatty tissue. The thoracic segment of the aorta (and in some instances the aortic arch) was cut up into disks 6 mm in diameter and placed by threes into 25 ml Erlenmayer flasks containing a sulphate-free Krebs—Ringer-bicarbonate solution previously oxygenated at 37 °C for 30 minutes. It was to this system that the substances to be studied for their effects on SO_4-incorporation were added. The antimetabolites were invariably used at concentrations of 0.2 mM, glucosamine was used at a concentration of 0.1 mM. For the study of the combined effects of glucosamine and antimetabolite, glucosamine was added to the system after pre-incubation with the antimetabolite for 1 hour. After further incubation for another hour, 25 μC Na_2 $^{35}SO_4$was measured into each flask in which the total fluid volume was invariably 5 ml. Into the flasks serving for negative control, 1 ml of 10% trichloroacetic acid was placed together with the first active material. In the flasks serving for positive control, instead of the active substances Krebs—Ringer-bicarbonate solution was placed in the flasks. Incubation was continued for 4 and 8 hours. Subsequently, 1 ml of 10% trichloroacetic acid was measured into each system. After stirring, the entire fluid was sucked off. Then, after washing three times in cold tap water, 10 ml saturated Na_2SO_4 was added to each system and the mixture was left to stand for 12 hours. This was followed by shaking in a shaker for 15 minutes, and successive washing in cold tap water, physiological saline, alcohol and ether. After this procedure, radioactivity of the slices serving for negative control scarcely exceeded the background activity.

Radioactivity of three slices at a time was measured by an end-windowed GM tube of 1.54 mg/cm² wall thickness; activity of the intimal and adventitial surfaces was measured separately. The results were evaluated by Student's *t*-test.

Results

As compared to the controls, glucosamine at the given concentrations had no stimulating, and Antimetabolite I had no inhibitory effect on $^{35}SO_4$-incorporation whether used alone or in combination with glucosamine.

The combined effect of Antimetabolite I and glucosamine was compared to the effect of glucosamine alone. Although in some instances the changes attained the significance level, they were unsuited for evaluation owing to the discordance between the 4 and 8-hour results. This applies to the intimal and adventitial surfaces alike (Table I).

Antimetabolite II also failed to induce change in $^{35}SO_4$ incorporation, as compared to the controls, whether applied alone or in combination with glucosamine (Table II).

Nor did aortic tissue derived from dogs after previous administration of croton oil reveal any change attributable to the effect of glucosamine — or/and N-acetyl-4-6-benzylidene-glucosamine (Table III).

Comparison of the figures reflecting the incorporation by the adventitial and intimal surfaces yielded the following results.

In the N-acetyl-4-6-benzylidene-glucosamine group, incorporation by the adventitia exceeded that by the intima in all instances with the exception

Table I

Effect of N-acetyl-4-6-benzylidene-glucosamine on $^{35}SO_4$ incorporation (c. p. m.)

	Glucosamine (0 1 mM)				Antimetabolite I. (0.2 mM)			
	4h		8h		4h		8h	
	I	A	I	A	I	A	I	A
n	10	10	10	10	10	10	10	10
$\bar{x}$	71.0	122.6	485.3	1441.8	115.3	204.3	528.3	1816.9
SE	10.59	21.01	86.47	609.00	25.93	42.13	99.91	552.62

	Antimetabolite I (0 2 mM) + Glucosamine (0 1 mM)				Control			
	4		8h		4h		8h	
	I	A	I	A	I	A	I	A
n	9	9	10	10	10	10	10	10
$\bar{x}$	193.7	265.0	365.4	847.0	123.0	174.9	448.8	727. 3
SE	59.00	58.72	66.69	100.63	25.57	40.32	113.71	144.05

Table II

Effect of N-acetyl-4-6-p-oxy-benzene-glucosamine on $^{35}SO_4$ incorporation (c. p. m.)

	Glucosamine (0 1 mM)				Antimetabolite II. (0 2 mM)			
	4h		8h		4h		8h	
	I	A	I	A	I	A	I	A
n	8	8	8	8	8	8	8	8
$\bar{x}$	154.88	321.62	523.75	1096.75	126.62	173.88	515.88	959.62
SE	57.00	93.13	186.73	427.28	40.91	51.97	187.67	372.90

	Antimetabolite II (0.2 mM) + Glucosamine (0 1 mM)				Control			
	4h		8h		4h		8h	
	I	A	I	A	I	A	I	A
n	7	7	3	3	8	8	8	8
$\bar{x}$	317.86	473.29	1440.67	2130	188.00	361.62	567.75	896.50
SE	176.60	186.25	762.03	1037.02	60.93	141.50	211.84	316.14

of 8-hour incubation with glucosamine. (After combined administration of Antimetabolite I and glucosamine, the results after 4-hour incubation were at the borderline of significance) (Table IV).

In the Antimetabolite II group ,$^{35}SO_4$ incorporation of the adventitial surface also exceeded that of the intimal surface. The differences attained the borderline of significance in the majority of cases. The values for the glucos-

Table III

Effect of N-acetyl-4-6-benzylidene-glucosamine on $^{35}SO_4$ incorporation into aortic tissue of croton oil-treated dogs (c. p. m.)

	Glucosamine (0.1 mM)				Antimetabolite I (0.2 mM)			
	4h		8h		4h		8h	
	I	A	I	A	I	A	I	A
n	8	8	8	8	7	7	8	8
$\bar{x}$	155.38	625.00	550.38	1982.50	131.29	740.00	835.50	2385.38
SE	49.99	303.86	171.68	700.23	39.27	443.04	328.10	943.76

	Antimetabolite I (0.2 mM) + Glucosamine (0.1 mM)				Control			
	4h		8h		4h		8h	
	I	A	I	A	I	A	I	A
n	8	8	8	8	8	8	8	8
$\bar{x}$	217.125	586.875	405.875	1575.750	144.25	457.50	785.00	1458.38
SE	70.44	278.57	69.13	586.17	38.77	214.30	305.92	406.00

Table IV

Differences in activity between adventitial and intimal surfaces with and without N-acetyl-4-6-benzylidene-glucosamine

	Glucosamine		Antimetabolite		Control		Antimetabolite (0.2) + Glucosamine	
	4h	8h	4h	8h	4h	8h	4h	8h
n	10	10	10	10	10	10	9	10
diff.	51.6	956.5	89	1288.6	51.9	278.5	71.3	481.6
p%	<1	>10	<5	<5	<5	<5	>5	<0.01

amine effect after 8-hour incubation and for the effect of Antimetabolite II — glucosamine did not reach the level of significance (Table V).

Incorporation by the adventitia exceeded that by the intima in every instance. Even the adventitia of the aorta of animals of the croton oil group was found to take up more $^{35}SO_4$ than the intima after 8-hour incubation. Increased adventitial incorporation was demonstrable after 4-hour incubation, without, however, attaining the level of significance (Table VI).

The difference in activity between the adventitial and intimal surfaces showed a further increase after 8-hour incubation in comparison to the 4-hour values. The differences did not reach significance in the Antimetabolite I group for glucosamine and in the Antimetabolite II group, for glucosamine, antimetabolite, and antimetabolite + glucosamine (Table VII).

No difference was found between adult croton oil-treated and young untreated dogs in respect of aortic $^{35}SO_4$ incorporation (Table VIII).

Table V

Differences in activity between adventitial and intimal surfaces with and without N-acetyl-4-6-p-oxy-benzene-glucosamine

	Glucosamine		Antimetabolite		Control		Antimetabolite (0.2) + Glucosamine	
	4h	8h	4h	8h	4h	8h	4h	8h
n	8	8	8	8	8	8	7	3
diff.	166.75	573.00	47.25	443.75	173.62	328.75	155.43	689.33
p%	>5	>10	>5	>5	>5	>5	>10	>20

Table VI

Differences in activity between adventitial and intimal surfaces of croton oil-treated dog aorta with and without glucosamine derivatives

	Control	Glucosamine	Antimetabolite I	Antimetabolite I Glucosamine	Control	Glucosamine	Antimetabolite II	Antimetabolite II Glucosamine
n	8	8	7	8	8	8	8	8
diff.	313.25	469.62	608.71	369.75	673.38	1432.12	1549.88	1169.88
p%	>10	>10	>10	>10	<1	<5	<5	>5

Table VII

Differences in activity between adventitial and intimal surfaces, after 8-hour vs. 4-hour incubation

	Control	Glucosamine	Antimetabolite I	Antimetabolite I Glucosamine	Control	Glucosamine	Antimetabolite II	Antimetabolite II Glucosamine
n	10	10	10	9	8	8	8	8
diff.	266.6	904.9	1199.6	433.0	155.12	406.25	396.50	361.67
p%	<5	>10	<5	<0.1	>5	> 10	>10	>30

Antimetabolite I = N-acetyl-4-6-benzylidene-glucosamine
Antimetabolite II = N-acetyl-4-6-p-oxy-benzene-glucosamine

Table VIII

$^{35}SO_4$ incorporation by aortic tissue of croton oil-treated dogs and of controls

	4 h				8 h			
	I		A		I		A	
	Control	Croton	Control	Croton	Control	Croton	Control	Croton
n	18	8	18	8	18	8	18	8
$\bar{x}$	151.89	144.25	257.89	457.50	501.67	785.00	802.50	1458.38
$\bar{x}-\bar{y}$	7.64		199.61		283.33		655.88	
p%	>80		>30		>40		>10	

Discussion

There is no agreement in the literature concerning the GG composition and concentration of aortic tissue. It is, however, generally accepted that chondroitin-SO_4-es, dermatane-SO_4, hyaluronic acid and heparane SO_4 are invariably present in the aorta. According to MEYER et al. (1956) chondroitin-4-SO_4 and dermatane-SO_4 make up the two main GG-components of bovine aorta. According to BERENSON (1958) chondroitin-4-SO_4 accounts for 75% of the sulphated GG constituents of bovine aorta.

KAPLAN and MEYER (1960) regard chondroitin-6-SO_4 and dermatane-SO_4 as the two principal GG-components of human aorta. BUDDECKE (1960) in addition to the two last-named GG-components demonstrated keratane-SO_4 and chondroitin in human aorta.

BOSTRŐM et al. (1963), JACOBSON and BOSTRŐM (1964) studied the GG metabolism of bovine, TORII et al. (1954), that of human heart valves. Evidence concerning the GG-metabolism of aortic adventitia is, however, scanty.

BERENSON (1959) studied the bovine aorta by separating it into two layers. The inner layer contained the intima with the inner layer of the media, while the outer layer, the adventitia with the outer layer of the media. The two layers revealed no qualitative difference in respect of GG, but GG concentration in the inner layer exceeded that in the outer layer, and chondroitin-4-SO_4 and dermatane-SO_4 were isolated in greater amounts from the inner than from the outer layer. MURATA et al. (1964) found the GG contents of the aortic intima and media to be consistent with that of the adventitia, 1.6 mg GG per g wet tissue having been obtained from the adventitia. The results of JÓZSA et al. (1964) are consistent with these observations.

Aortic tissue contains considerable amounts of glycoproteins. BERTELSEN (1960) studied the intima and media of the human aorta for GP-concentration. In the young organism the GP contains half of the amount of hexosamine present in GG. RADHAKRISHNAMURTHY et al. (1965) identified a glucose-containing GP in bovine aorta. According to FISHKIN and SPANGLER (1968) the GP pattern of the bovine aorta changes in the course of foetal life and in adult bovine aorta it varies with age and sex; they could separate 10 to 12 GP fractions by polyacrylamide-electrophoresis.

GOLDSTEIN et al. (1968) showed immunologic cross-reactions between the structural GP of human or bovine heart valves and the parietal polysaccharides of Streptococcus haemolyticus A. The significance of this observation in human pathology is obvious.

Clearly, the $^{35}SO_4$ technique does not lend itself to the study of GP metabolism of tissues.

In the present study it has been demonstrated by the aid of a procedure differing in certain respects from that of other workers that the adventitia

of the dog aorta has a highly active GG-metabolism, it being able to incorporate more $^{35}SO_4$ than the intima. The difference in uptake between the two layers increases parallel with the length of incubation. The data thus suggest that the rate of GG metabolism is higher in the adventitia than in the intima. The results give no indication of the absolute values, since catabolism has not been studied.

As to the question whether or how far the data obtained by the present method allow an objective assessment, a possible adsorption in tissue and absorption of radiation involve much the same degree of distortion for either surface. This would, therefore, not affect the reliability of the results. This is particularly true if the counting-rates are invariably higher on one surface than on the other, as it was in fact the case here.

None of the two glucosamine derivatives under study caused any appreciable change in $^{35}SO_4$ incorporation. Interpretation of this observation from the point of view of a possible antimetabolite effect does not seem, however, entirely clear, since glucosamine also failed to stimulate $^{35}SO_4$ incorporation though it did exert an effect of this kind according to other evidence including our earlier studies of $^{35}SO_4$ uptake by calf cartilage. Apart from the various types of tissue, the species and age of the animals as well as the concentrations in which the agents were used, may well account for this incongruence.

Local inflammation induced by croton oil may affect the metabolism of remote organs or tissues. This is suggested by the studies of HOUCK (1963) who found that in the skin of areas quite remote from the site of necrotizing inflammation the amount of insoluble collagen was reduced while that of noncollagen protein increased. The phenomenon might be connected with a possible increase in the concentration of stress hormones.

In the present study croton oil-induced inflammation in adult dogs failed to affect significantly the $^{35}SO_4$ incorporation by aortic tissue. The observations have, however, been confined to the 5th day of inflammation and a study of aortic tissue at other intervals might be more informative.

*

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Dr. Lajos JAKAB
Dr. Éva SEREGÉLYI
University Medical School, Third Department of Medicine, Budapest VIII., Mező Imre út 17.

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SUPPRESSIVE EFFECT OF LYNESTRENOL ON LACTATION

By

M. KURCZ, I. NAGY, CS. KISS and L. HALMY

NATIONAL INSTITUT Y OF PUBLIC HEALTH, HEIM PÁL CHILDREN'S HOSPITAL, AND POSTGRADUATE MEDICAL SCHOOL, BUDAPEST

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The lactation inhibiting property of lynestrenol has been reproduced in the rat, Complete suppression of milk secretion required doses exceeding many times the human ones. Intensity of the effect was related to the magnitude of the dose and to the time when treatment had begun. Treatment was more successful if started immediately than on the second day after delivery. Lactation remained absent if treatment had begun in the last days of pregnancy and was continued after delivery. On the other hand, treatment discontinued immediately after delivery did not interfere with lactation. The conflicting results of earlier animal experiments are pointed out. The significance of the sucking stimulus in animal experiments as opposed to clinical conditions is emphasized. The possible mechanism of action of lynestrenol is discussed.

The influence of oral contraceptives on the mammary gland and lactation is a subject of controversy. Norethynodrel and its combination with mestranol have been found to induce lobuloalveolar transformation and secretory activity of the mammary gland in rats (KAHN, BAKER 1964, KAHN et al. 1965, MINAGUCHI, MEITES 1967). On the other hand, JOSHI and RAO (1966, 1968) found the combination to suppress lactation in rats and rabbits when its administration had been started on the 2nd day after delivery. This treatment had no such effect in mice. Neither was such an inhibition found by SAUNDERS (1967) in spite of the rats having been treated from the first day of pregnancy until the 21st day postpartum, although with low doses. The combination lynestrenol + mestranol was found inefficient by IJZERMAN (1966).

Clinical evidence is likewise inconsistent. Certain authors reported on slight disorders of lactation during the use of oral contraceptives (RICE-WRAY 1963, SATTERTHWAITE 1965, FERIN 1965, HEFNAWI 1966) while the observations of others were variable or contradictory (CHINNATAMBY 1963, GOMEZ-ROGERS et al. 1965), again others found lynestrenol + mestranol inefficient whether its administration had been started one or ten days after delivery (SEMM 1966, BORGLIN 1966). The occurrence of galactorrhoea was reported by GREGG (1966) and SCHACHNER (1966) under the effect of norethynodrel + mestranol, whereas KISS et al. (1967) are prescribing lynestrenol against extrapuerperal galactorrhoea. While HASKINS et al. (1964) achieved a permanent suppression of lactation by the use of medroxyprogesterone in

cases of the Chiari-Frommel-syndrome, HARRECKER et al. (1967) failed to note any effect of depot medroxyprogesterone on lactation.

The situation is rendered still more confusing by the following circumstances.

1. A reliable comparison of the different oral contraceptives is not possible in view of their different progesterone-oestrogen content and proportion.

2. If suppression of lactation ensued, the stimulus of sucking was invariably eliminated.

3. If, however, the compounds were given with the purpose of contraception during lactation, the stimulus of sucking obviously persisted.

4. The clinical doses are greatly different from those applied in animal experiments.

5. The suppressive effect on lactation of the progestogen factor has yet to be clarified on the grounds of animal experiments.

Recently GYŐRY and KISS (1968a, b) have attained a significant inhibition of lactation in 100% of 91 cases with lynestrenol without any side or after-effect. On the other hand, lynestrenol + mestranol administered to lactating women for contraceptive purposes had no effect on lactation. These results indicate, in addition to the significance of the stimulus of sucking, also the importance of the amount of the progestogen component (lynestrenol), as the suppression of lactation required 6 to 12 fold of the contraceptive dose.

The aim of the present study was to reproduce the effect of lynestrenol on lactation in the laboratory animal in order to throw light on the suppressive mechanism.

Material and method

Pregnant and lactating albino rats of "R-Amsterdam, Hungary" strain, kept on a standard diet and under identical conditions, were used. Each pregnant animal was kept in a separate cage and six young of each litter were preserved. If any newborn rat died it was replaced by a 4 to 6-day-old strong rat from mothers kept in reserve for this purpose. Milk secretion was measured by the weight curves of the young. The litters were weighed daily at identical times and the presence of milk in the stomach was checked. It must be mentioned that in opposition to the view of GLANCY and EDGREN (1968) daily weighings had no adverse influence whatsoever on the development of the litter (lethality, cannibalism). One ml of an aqueous suspension prepared from lynestrenol pills (Orgametril; N. V. Organon, Oss, Netherland) was administered by a stomach tube once or twice daily (at 8h and 20h). The controls received the same volume of tap water by the same route at identical times.

The test groups and administered doses are shown in Table I.

After treatment had come to an end, the mothers were not observed any longer. Weighing of the litters was continued and the surviving young were observed for a possible virilisation syndrome due to the treatment (BARRACLOUGH 1967, FLERKÓ 1967). Our observations in this respect will be published separately (NAGY et al.).

The lynestrenol-treated mothers were put together with males 30 days after weaning, and the course of the following pregnancy, the number of newborns, the intensity of lactation, and the condition of the offspring were registered so as to ascertain whether the massive doses of progestogen had any permanent adverse influence on the later course of reproduction. The results were evaluated mathematically by Student's *t*-test.

Table I

Test group	Number of animals	Treatment beginning day	End day	Lynestrenol* dose, mg/day
1. Control	10	—	—	—
2. Control with stomach tube	7	lactation 2nd day	lactation 10th day	—
3. Lynestrenol	8	18th day	28th day	2× 5
4. Lynestrenol	8	2nd day	10th day	2× 5
5. Lynestrenol	9	immediately after delivery	10th day	1× 5
6. Lynestrenol	8	2nd day	10th day	2×10
7. Lynestrenol	6	3 days before delivery	day of delivery	1×10
8. Lynestrenol	7	3 days before delivery	10th day	1×10

* Lynestrenol, 1 pill = 5 mg.

Results

The intensity of lactation was judged on the basis of the litter's weight curve. It can be seen from Fig. 1 that introduction of the tube into the stomach of the lactating rat twice daily was in itself sufficient for interfering with the development of the litter (probably in consequence of a stress effect). This was clearly shown by the controls which received water through the tube.

The effect of lynestrenol varied in intensity depending on the interval between delivery and the beginning of treatment as well as on the magnitude of the doses. A slight reduction of lactation was noted if treatment had been started on the 18th day after delivery, i.e. when lactation is at its maximum in the rat, by two pills administered on two occasions daily (GROSVENOR and TURNER 1958). The young which had their eyes open at this time were taken from these mothers and replaced by two-day-old rats.

Inhibition of lactation was more marked if the first dose had been given on the 2nd day postdelivery. However, these doses (10 mg lynestrenol) proved insufficient for a complete suppression of lactation. To attain this, two tablets had to be administered twice daily from the 2nd day onward. The mothers thus treated continued to feed, to protect their young and to keep them warm; in other words, their maternal instinct was unaffected. If milk secretion had stopped under the effect of four pills daily, inhibition of lactation could be maintained by two pills administered once daily.

If treatment had begun the first six hours postdelivery, even one pill daily was sufficient for reducing lactation. This indicates that lactation is more readily inhibited in the early postdelivery period (Fig. 2).

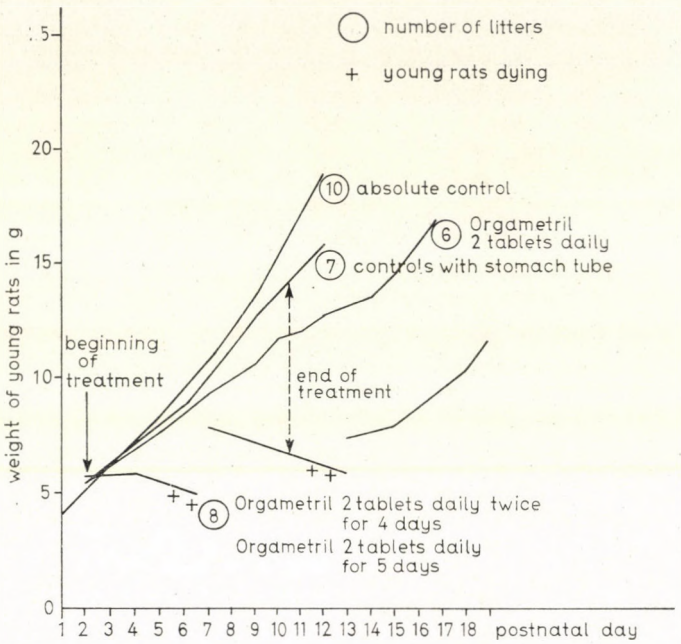

Fig. 1. Reduction in lactation intensity due to introduction of stomach tube and to lynestreno treatment

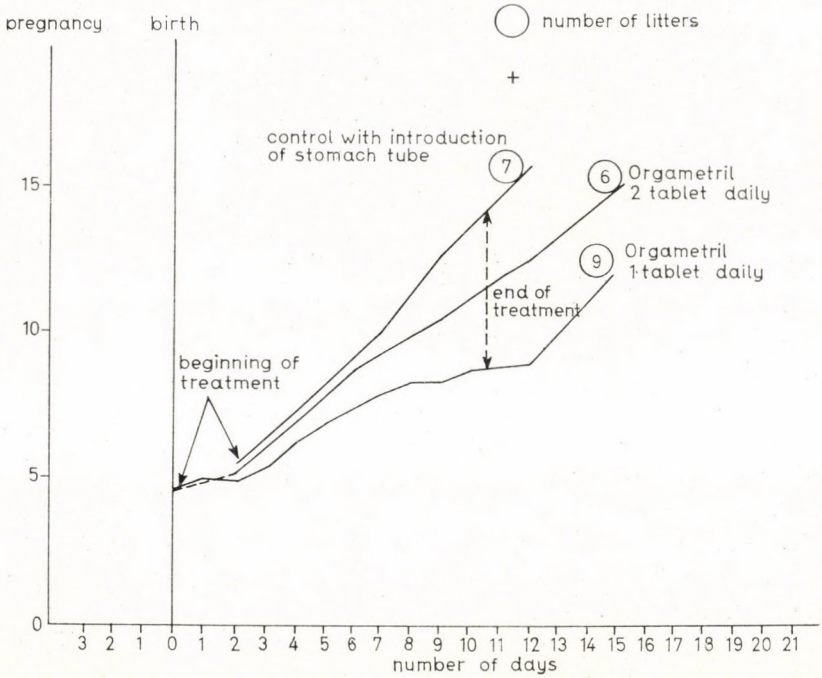

Fig. 2. Relationship between the time of the start of treatment and depression of lactation. Start immediately after delivery is more successful

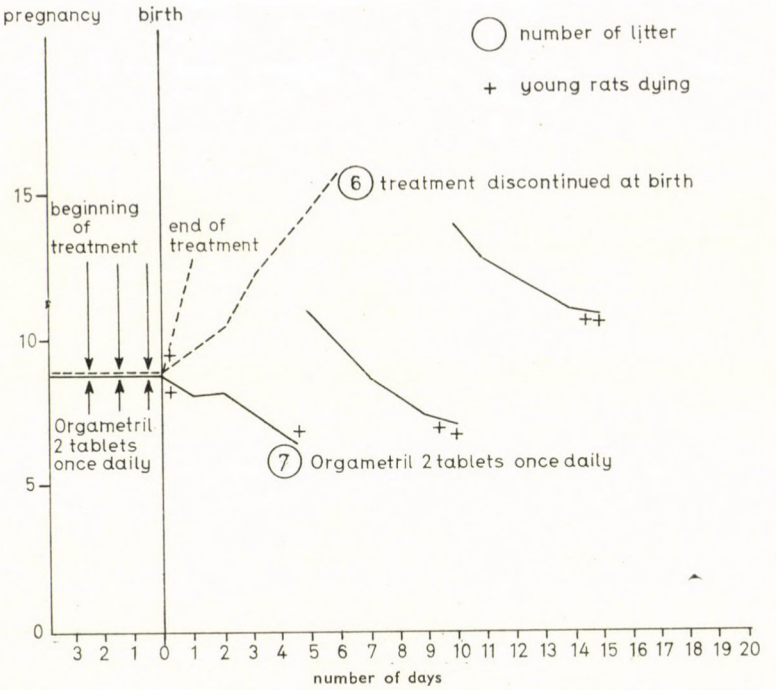

Fig. 3. Beginning of treatment during the last days of pregnancy. Cessation of treatment on the day of delivery is followed by lactation, its continuation results in complete inhibition of milk secretion

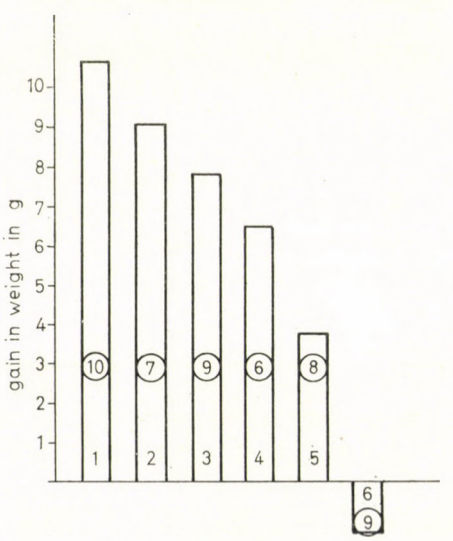

Fig. 4. Gain in weight per young rat during lynestrenol treatment. *1* Control; *2* Control with stomach tube; *3* 1 pill twice daily from 18th day onward; *4* 1 pill once daily from 2nd day onward; *5* 1 pill once daily immediately after delivery; *6* 2 pills twice daily

In the animals of the other series, lynestrenol treatment was started 2 to 6 days before delivery. Two out of 15 mothers died. The possibility of an injury caused by the introduction of the gastric tube was ruled out at autopsy. The 13 mothers delivered either dead foetuses or live ones the majority of which died within the first 24 hours. The mothers which had been treated during pregnancy and displayed a poor parturition activity were given intact strong baby rats instead of their dead or debile foetuses which they accepted and reared with apparently normal maternal instinct. In 50% of the mothers treatment was discontinued immediately after delivery which was followed by lactation of nearly the same intensity as in the normal controls. In contrast, milk secretion failed to appear in those mothers which had been treated until the 5th to 8th postdelivery day (Fig. 3).

Fig. 4 shows the gain in weight per young rat during treatment. The difference between the weight increases was highly significant: (1—2: $p < 0.05$; 2—3: $p < 0.01$; 2—4: $p < 0.01$; 2—5: $p < 0.001$).

Lynestrenol treatment, even with massive doses had no adverse consequence on subsequent fertility of the mother animals which were brought together with males 30 days after weaning. Conception, gestation, delivery, number and sex of offspring and lactation were found normal in all the animals.

Discussion

The lactation suppressive effect of lynestrenol was successfully reproduced in the rat, though with doses many times higher than those used in humans. The cause of this might be partly the species differences; the efficient doses of contraceptives in the rat are known to exceed many times the human doses. Another cause of the divergence between the human and the rat doses lies in the experimental conditions which were essentially different from the human ones, in view of the persistence of the vigorous sucking stimulus throughout the entire course of the experiment. It is known (SELYE 1934, BRUCE 1958, KURCZ 1967) that the sucking stimulus is capable of maintaining lactation even for a year. The continuous sucking stimulus must therefore exert a negative influence on the responsiveness to lynestrenol.

The present results clearly show that the magnitude of the doses and early treatment are essential factors. Suppression of lactation by lynestrenol was found to increase parallel with the dose. On the other hand, even smaller doses produced a better response if treatment had begun early. We saw no point in experimenting with 5 or even 25-fold doses of those applied in humans since on the evidence of other studies (CLANCY and EDGREN 1968, SAUNDERS 1967) low-dose contraceptive treatment had no effect on lactation even when started during pregnancy (EDGREN and CLANCY 1968).

The inhibitory effect of lynestrenol on lactation, the sucking stimulus being maintained, is reversible. When treatment is discontinued, milk secretion increases and soon attains the control level. If, however, treatment is started during pregnancy and continued for 5 to 8 days postdelivery, milk secretion remains absent. This suggests that lynestrenol interferes with the process of lactogenesis. The blood steroid level of mothers kept on maintenance doses of lynestrenol, remains high even after delivery which may well inhibit the release of lactogenic hormones by the pituitary gland. The finding that suspension of the treatment on the day of delivery is followed by the onset of lactation, is consistent with this interpretation.

Why milk secretion fails to appear if treatment is started prenatally and continued until the 8th day post partum, is not clear. Despite the continuous sucking stimulus, the long-term treatment may well affect the mammary gland together with the pituitary secretion of lactogenic hormone in a manner which may be adverse to milk production. Inhibition of milk secretion by lynestrenol does not seem to be related to the compound's slight oestrogenic effect. The clinical use of oestrogens for the suppression of lactation has its theoretical justification and in rats even massive doses are inefficient.

In order to throw more light on the problem, we have to gain insight into the effects of lynestrenol on the structure of the secreting and non-secreting mammary gland as well as on the pituitary secretion of trophic hormones. Such studies are in progress.

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Dr. Mihály KURCZ; National Institute of Public Health, Budapest IX., Gyáli út 4, Hungary

Dr. Iván NAGY; Children's Hospital Heim Pál, Budapest VIII., Üllői út 86, Hungary

Dr. Csaba KISS, Dr. László HALMY } Postgraduate Medical School, Budapest XIII., Szabolcs u. 34, Hungary

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CRITICAL FLICKER FREQUENCY SERIES EFFECT IN EPILEPTIC AND IN NEUROTIC PATIENTS

I. DIFFERENCES IN THE DAILY LEVEL VARIATION OF THE CFF SERIES

By

J. Szák, M. Ákos and K. Ákos

NINTH DISTRICT HOSPITAL GYÁLI ÚT AND EÖTVÖS L. UNIVERSITY LIBRARY, BUDAPEST

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A group of 17 epileptic and another of 18 neurotic patients, including both males and females of 17 to 35 years of age were studied for a period of 3 days to record three times daily a continuous and an intermittent series of 50 CFF data each. The test material of a total of 31200 CFF data was computer processed. The individual series were made comparable by means of 10 maxima and 10 minima given by the data groups of 5, and the averages of the respective values were studied for both groups in accordance with their variations through the three days and the three daily registrations. From the first to the third day the differentiating capacity in the epileptic group decreased, whereas in the group of neurotic patients it increased. For the time being no definite answer can be given to the question whether the difference thus found is due to learning, fatigue, or some other process.

Introduction

The neurological verification of epileptic symptoms is often difficult, due, among others, to the diversity of symptomatology and the possibility of transient interruptions in consciousness without seizures, as well as of various symptoms which would appear to be either "functional" or closely resemble neurotic states. If the physician has no occasion to observe attack, he has to rely upon information rendered by the patient or witnesses. In certain cases PEG, EEG, or AG cannot eliminate all doubts, either, as for instance during the free intervals an EEG typical of epilepsy can be obtained only in about 50% of the patients (Hill 1958). Thus, additional diagnostic methods are still to be sought for, the available methods ought to be refined and, perhaps, experimental investigations using new techniques should be conducted.

Such a new method is the application of the critical flicker frequency series effect (hereafter CFFSE), essentially a psychophysical process (Ákos and Ákos 1966). In the investigations to be described, this method has been employed in epileptics and in a control group consisting of neurotic patients with consideration to the differentiation of the two groups often presenting the most important problems. The CFFSE method differs radically from the techniques and procedures employed in epilepsy research. Its application in cases of the two disease types referred to above intended to determine whether it could prove suitable to distinguish the two groups.

Within the epileptic and neurotic patient groups no further differentiation will be effected below. Both groups include males and females in the age category of 17 to 35. Due to reasons impossible to explain here in detail, the test results of younger and older patients were omitted from the present report. The number of epileptic patients was 17 while that of the neurotics 18.

Method

Light source for the CFFSE examinations was an EM 80 type cathode ray tube, with a sheet of colourless transparent paper located in front thereof. This light source is watched by the patient with both eyes, through a funnel providing for a 40 cm distance of view, without any pre-adaptation. Light is flashed by the square pulses of the Solartron OS. 103 2A generator in equal light and dark intervals, at a frequency read off with one-tenth cps accuracy.

Always descending CFF data are recorded, that is, signals turning from steady to flicker type are seen. Each series consists of 50 CFF data rendered by the same subject within one test. The value fluctuations of the CFF data in the same series are called CFFSE. In each case first a continuously registered uninterrupted series is recorded then, after about 10 min, an intermittent series consisting of 50 further CFF data where every 10 CFF information is followed by an interval of 30 sec.

On the first day, one continuous and one intermittent series are rendered by each subject but these data are not evaluated. The data to be processed are obtained through

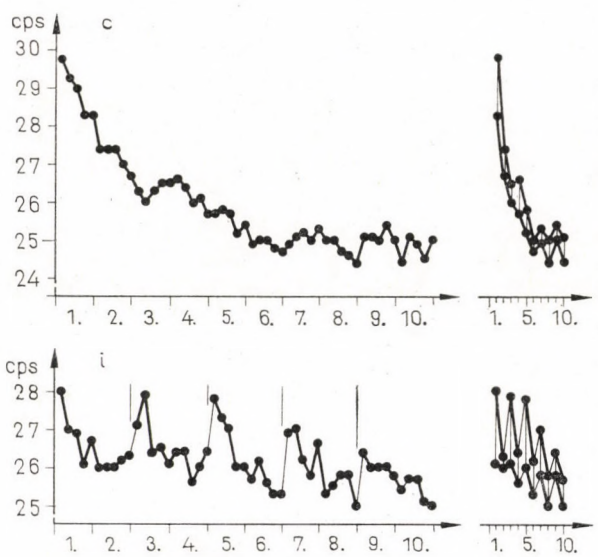

Fig. 1. CFFSE curves. The horizontal axis indicates from the left to the right the in-time sequence of recordings. The numbering represents the sequence of groups of five, from 1 to 10. The vertical axis reflects frequency in cps. Top curve continuous, bottom one intermittent, with the latter having the 4 interruption points indicated. Next to each of the two series curves their envelopes are presented. The abscissa of the envelope curves corresponds, from the left to the right, to the first, second, etc., five data of the series, while the points one above the other represent the maximum and minimum CFF frequency values, respectively, as selected by the groups of five. Connecting the maxima and minima has rendered the two envelope curves

the tests carried out during the next three days, when one continuous and one intermittent series are recorded three-times daily (early morning, late morning, and noon). Accordingly, 9 continuous and 9 intermittent series are obtained per subject, with a total of 900 CFF data.

Results

For the series consisting of 50 CFF data, the CFFSE can be demonstrated in the simplest manner as a curve (Fig. 1). This curve is plotted in a coordinate system, the horizontal axis of which indicates the data registration sequence

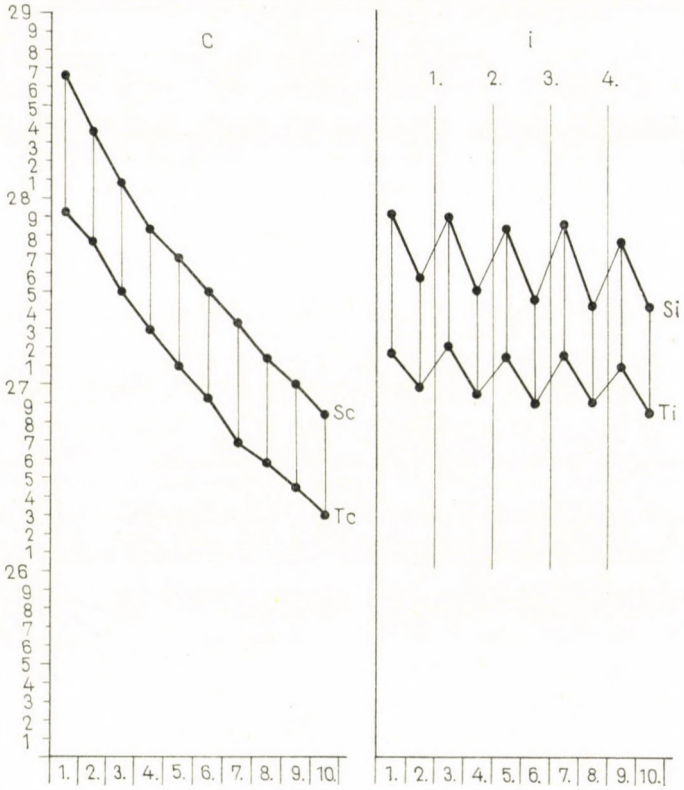

Fig. 2. Average envelope curves of the epileptic patient group of 17 persons, on the left for continuous and on the right for intermittent series. Each point represents the average of the corresponding 153 data. Sc indicates the curve of continuous maxima, Tc that of the continuous minima, Si the curve of intermittent maxima, and Ti the curve of intermittent minima. The co-ordinates correspond to those given for the envelopes in Fig. 1. By 1, 2, 3, 4 the interruptions are indicated

from the left to the right, whereas the vertical axis displays the frequency values in a one-tenth cps scale. Thus each CFF information is represented by a point, and connecting these points will render the curve of the series in question. Fig. 1 presents a continuous (top) and an intermittent (bottom) curve. At the right-hand side their envelope curves are seen.

The series can be compared by means of their envelope curves. These are obtained by selecting from every five CFF data of the series both the

maximum and minimum values, and by plotting them one above the other in a co-ordinate system, the ordinate of which presents the respective frequency values. The abscissa indicates, from the left to the right, the first, second,

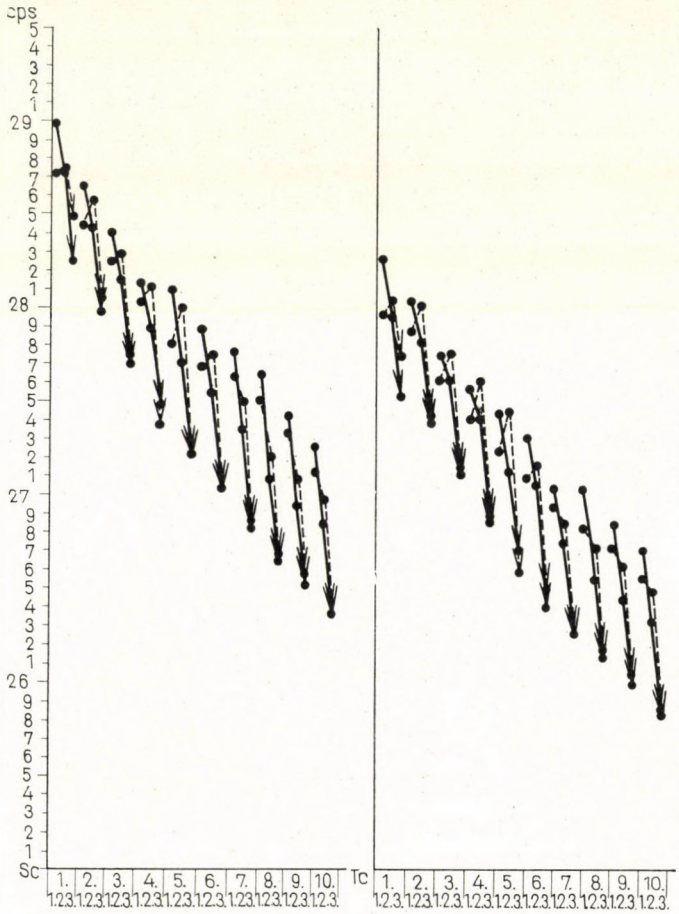

Fig. 3. Development of the continuous average maxima (Sc) and minima (Tc), respectively of the epileptic group, per day (dash line arrow) and per daily test time (full line arrow). On the horizontal axis the top numbers indicate the sequence of average groups of five, while the lower figures show the daily and morning to noon sequence, respectively. The vertical axis represents the frequency value in hundredth cps scale. Each point represents the average of the corresponding 51 data

third, etc. group of five data in the sequence of registration. The maximum and minimum of the first group of five data represent the first two points one above the other, the maximum and minimum of the second five CFF data give the second pair of points, and so on. Obviously, the values of each group of five will be located between the two extremes thus indicated. Connecting

the maxima and minima separately will define the two envelope curves of the series, with its full variability therebetween.

Averaging the values of the proper envelope curve points by series will render the mean values wherefrom the average envelope curves can be plotted.

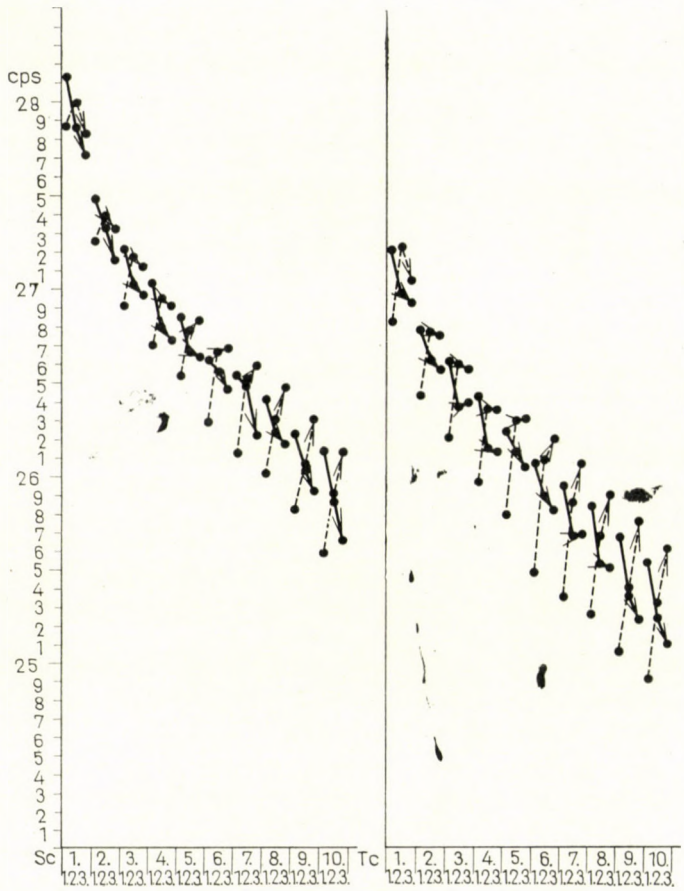

Fig. 4. Diagram of the neurotic group of 18 persons according to Fig. 3. Each point represents the average of the corresponding 53 data

As an example (Fig. 2), the average envelope curves of the whole epileptic group are shown, separately by continuous and intermittent series, respectively.

As regards the group difference under discussion, the average values were calculated in two different triple resolutions for both groups, by using a computer. In the first case, the averages were formed for the first, second, and third day, whereas in the second case in conformity with the first, second, and third occasions selected daily. Thus 6 point series were obtained with the

maxima (left) and minima (right), for the continuous series of the epileptic group (Fig. 3), where each point represented the average of 51 values. The daily variation of the respective values is indicated by a connecting dash line arrow, so the 10 dash line arrows show the daily variation of the maxima and minima, respectively, according to the average envelopes corresponding to

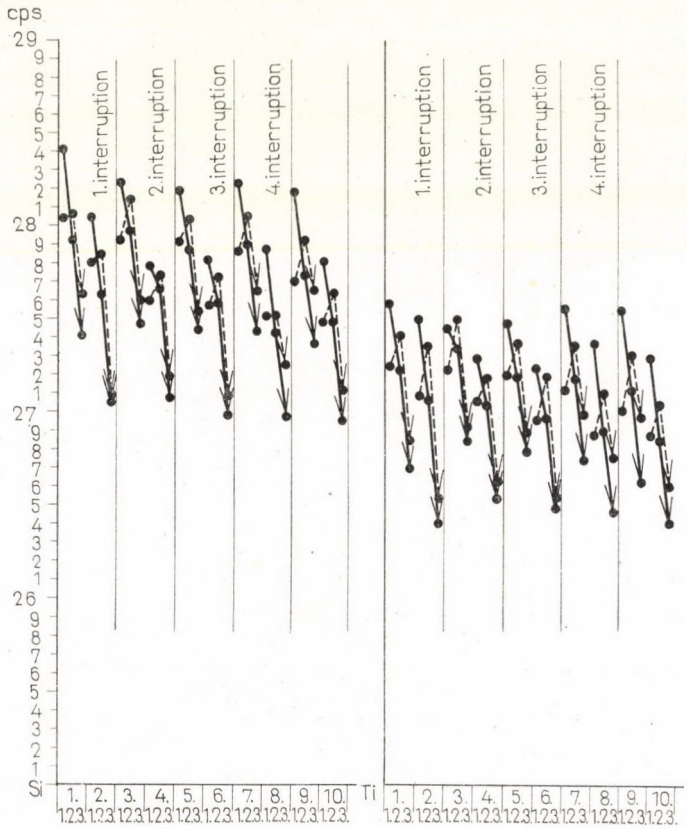

Fig. 5. Diagram of the epileptic group according to Fig. 3 but for intermittent series

the continuous series. The early morning, late morning, and noon averages of the continuous series envelope values are connected by full line arrows. Thus the diagram presents the variations day by day and from morning to noon alike, with the average values forming the continuous series envelopes.

Fig. 4 presents the same diagram for the group of neurotic patients. Here each point represents the average of 53 values.

Figures 5 and 6 show the variations of the average envelopes plotted on the basis of the intermittent series rendered by the epileptic and neurotic group, respectively, in day by day and from morning to noon versions.

Thus 40 arrows show for each disease group, that is, a total of 160 arrows, the variation of 3 averages in each case. Accordingly, the development of a total of 480 independent averages are taken into consideration for the two categories.

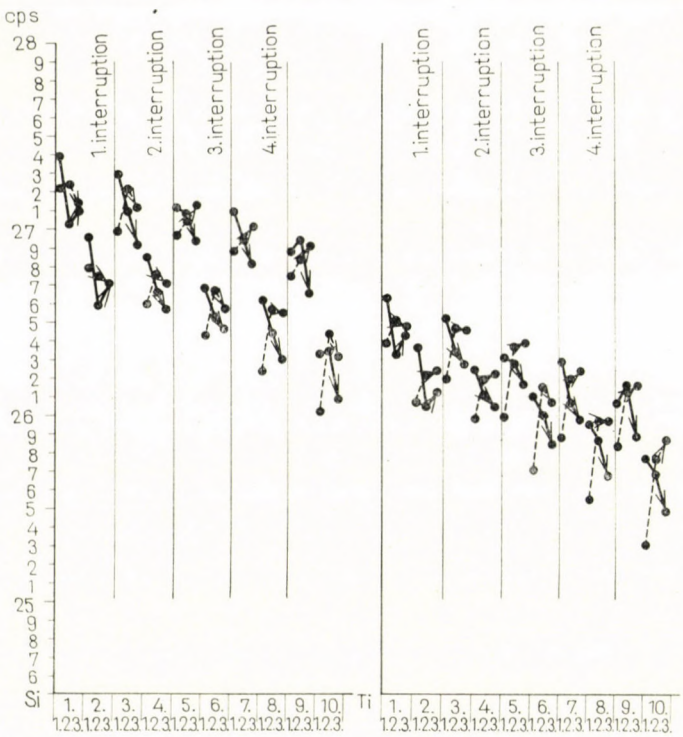

Fig. 6. Diagram of the neurotic patient group according to Fig. 5

Discussion

Fig. 2 reveals that the average envelope curves of the epileptic group exhibit a slope of negative exponential character for both the continuous and intermittent series. This general behaviour, characteristic of the entire series, is regarded as a stressed-habituational feature, verified by the appearance of ascents along the average envelopes of the intermittent series, following each interval (Ákos and Ákos 1968).

The slope of negative exponential character, typical of the complete series averages, can be discovered along each point sequence representing a series in both groups. This indicates that the CFFSE may be considered as a stress produced by successive data registration, or as a habituational process corresponding to the application of the monotonous series of stimuli.

From another aspect, however, the CFFSE test method described above may be interpreted as a repeated problem solution. Each subject must determine whether the given light source is steady or flickering. This decision must be made 50-times during each test, that is, 900-times during the 3-day period (actually 1000-times but the very first series pair, as referred to above, was omitted from the data processing).

Studying the variations exhibited by the averages of the individual values from morning to noon, in a separate manner, reveals that the individual average values show a consistent level decrease in both disease groups, that is, the ability to differentiate flicker will be reduced not only within the same series but, as far as the individual values are concerned, it will be impaired during the period from morning to noon. Thus the light and dark intervals must be gradually increased to make the subject observe the flicker. Further investigations are needed to decide whether this morning to noon variation reflects a stress accumulation corresponding to the series registrations repeated during this period, or the manifestation of a circadiem character stress resistance reduction.

If, on the other hand, the variations of the individual averages constituting the envelope curves are studied from the first to the third day, these reveal in the epileptic group a descending, and in the neurotic group an ascending character. The two disease categories are definitely different from this aspect. The average daily output of the epileptic group will decrease from the first to the third day with respect to differentiation while that of the neurotic category improves.

This peculiar characteristic exhibited by the neurotic group might be explained as a positive learning effect. This, however, would require the assumption of an opposite negative learning effect for the group of epileptic patients.

It must be emphasized that the investigations described here represent, above all, only an attempt to differentiate the two disease categories tested. Beyond the peculiar finding discussed above, the CFFSE methodology takes many other aspects into account as reported on by the monography referred to. These results will be presented in a subsequent paper. For the time being it is not our intention to suggest the application of the results published so far as a differential diagnostic method. The various age groups respond differently to which, as stressed earlier, particular attention should be paid. Another difficulty is that, although the young epileptic and neurotic patients as separate groups exhibit markedly different CFF series level variations, this difference is much less consistent individually (Table I). It can be observed that, among the total of 8 EEG positive cases, there was only one with an increasing daily average, whereas of the 9 EEG negative cases as many as 5 have displayed a level decrease characteristic of the group average.

Table I

Daily CFFSE level variation of epileptic and neurotic patients from the first to the third day as calculated on the basis of continuous series minima (Tc)

Epileptic group				Neurotic group		
Age (years)	Sex	EEG	Level variation	Age (years)	Sex	Level variation
31	f	+	desc	33	m	asc
24	f	+	desc	32	f	asc
20	m	—	desc	18	m	asc
31	f	+	desc	19	f	asc
27	m	—	desc	34	m	asc
18	f	+	desc	32	f	desc
27	m	—	desc	21	f	asc
27	f	+	asc	21	m	desc
18	f	—	asc	29	f	asc
30	m	+	desc	30	f	desc
22	f	+	desc	24	f	asc
22	m	+	desc	18	m	desc
32	f	—	asc	23	f	asc
30	m	—	asc	32	f	asc
28	f	—	asc	31	f	asc
18	f	—	desc	27	m	asc
25	m	—	desc	21	f	asc
				25	f	asc

Note: For each patient in both groups the following data are indicated: age at the test time, sex, symbol + for EEG results characteristic of epilepsy, symbol — for those indicating no such disease. The abbreviations "desc" and/or "asc" represent the variation of the right-hand side (Tc) value of the respective group in Figures 3 and 4, as observed during the period from the first to the third day, on the average per individuals.

Similarly, further research can only decide to what an extent the difference described here might be considered as specifically characteristic of epilepsy or neurosis.

No convincing statement may be made, either, on to what a degree the results obtained refer to the cerebrum as a whole, or to its certain parts, to the optic tract or the visual receptor itself, etc.

Finally, it should be noted that the patients of both groups received during the experiment their usual therapy.

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Dr. János Szák Budapest XII., Márvány u. 48, Hungary

Magda Ákos
Dr. Károly Ákos } Budapest II., Vöröshadsereg útja 35, Hungary

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SERUM DEHYDROEPIANDROSTERONE, ANDROSTERONE AND CORTISOL LEVEL IN PRIMARY POSTMENOPAUSAL AND OTHER TYPE OSTEOPOROSIS

By

I. HOLLÓ, T. FEHÉR and J. SZÜCS

FIRST DEPARTMENT OF MEDICINE, AND RESEARCH UNIT OF GERONTOLOGY, MEDICAL UNIVERSITY, BUDAPEST

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In patients with postmenopausal osteoporosis the plasma dehydroandrosterone level was found to be significantly lower than in controls of the same age with no evidence of osteoporosis. The plasma cortisol level was also reduced in osteoporosis, though to a lesser degree. The results are suggestive of a possible connection between postmenopausal osteoporosis and a reduction in adrenocortical androgen secretion.

In earlier studies we found a lower urinary output of androgen metabolites in patients with postmenopausal osteoporosis than in healthy subjects of similar age. Urinary excretion of androsterone was particularly reduced [1, 2, 3]. The results of different tolerance tests [2, 4] and of studies of the urinary excretion of 17-ketosteroids substituted on carbon-11 pointed to the possibility that a reduced androgen secretion by the adrenal cortex might be responsible for the low urinary output of androsterone in postmenopausal osteoporosis. It seemed of interest to continue these investigations by the study of the plasma level of the essential androgens and of cortisol in patients with primary osteoporosis.

Material and methods

Blood plasma was studied for dehydroepiandrosterone (DEA), androsterone (A) and cortisol (Hc) level by the method of FEHÉR et al. [5] which is suitable for the estimation of 17-ketosteroid esters (particularly of sulphate esters). The plasma was obtained from 15 subjects with postmenopausal osteoporosis (poromalacia), 7 with other types of primary osteoporosis, and 4 with secondary osteoporosis. The criteria of osteoporosis were X-ray of the dorsal or lumbal spine indicative of calcipenic osteopathy; a normal serum calcium, phosphor level and alkaline phosphatase activity; normal or slightly increased urinary excretion of calcium in response to a calcioprivic diet; after intravenous loading with 180 mg ionized calcium, at least 40% of the administered dose had to be eliminated in the urine in addition to the usual daily calcium output. The cases displaying all the criteria except that the 24-hour urinary excretion of calcium was less than 40% of the load, were classified in conformity with the literature as osteoporomalacia and tabulated under this diagnosis.

The patients with osteoporosis were otherwise healthy apart from an abnormal repolarization in the ECG or a blood pressure over 180 Hg MM occasionally. Their renal function was normal, even in the hypertensive cases. Liver function was normal in every case.

Since the normal blood androgen level varies with age and sex, the figures obtained in the group of postmenopausal osteoporosis were correlated to the plasma level of 19 healthy women of similar age; they were subjects revealing no abnormality at the Research Unit of Gerontology and healthy volunteers recruited from the staff of our department and partly patients with minor diseases which could not possibly affect the endocrine system. In this latter group no estimation of cortisol was done and their data were already utilized in an

other study [5] previously. In the table summing up the results obtained in "other types" of osteoporosis, in view of the small number of cases the extreme values of the hormones under study found at our laboratory in sex-and-age-matched control subjects are shown [5]. The results were evaluated mathematically by the t and χ^2 tests.

Results

The plasma hormone values of the subjects with postmenopausal osteopathy are set out in Table I, those of the age-matched control group, in Table II. The essential data of mathematical analysis are presented in Table III in which the quotients of the hormonal values are also shown.

In Case 1 with postmenopausal osteoporosis the blood androgen level was high, that of DEA being in the neighbourhood, and that of A even in excess, of the values found in young healthy males. Since the patient exhibited no sign of virilisation and the plasma androgen level was incongruent with those of the other, osteoporotic or healthy, subjects, they were not included

Table I

Plasma hormone levels in postmenopausal osteoporosis and poromalacia

No.	Age	Diagnosis	Hc μg per 100 ml	DEA μg per 100 ml	A μg per 100 ml
1	47	pm	13.1	68.7	69.3
2	67	op	15.1	8.2	8.5
3	62	op	13.2	18.2	20.0
4	59	op	8.6	13.6	14.6
5	58	op	7.8	12.5	5.8
6	57	op	3.8	15.5	5.5
7	54	op	11.7	7.8	19.4
8	54	op	6.2	18.0	11.4
9	50	op	9.0	9.6	6.4
10	51	op	4.7	5.2	2.6
11	49	op	7.5	20.7	7.1
12	47	op	7.5	13.0	10.0
13	46	op	8.3	12.9	10.3
14	59	pm	9.5	12.0	5.3
15	65	op	6.9	12.8	10.4

op = osteoporosis; pm = osteoporomalacia; Hc = Cortisol; DEA = Dehydroepiandrosterone; A = Androsterone

Table II

Plasma hormone levels in postmenopausal healthy controls

No.	Age	Hc μg per 100 ml	DEA μg per 100 ml	A μg per 100 ml %
1	57	14.0	8.0	8.0
2	53	22.3	41.5	22.0
3	58	15.2	30.5	29.4
4	56	14.1	22.9	2.9
5	55	6.4	14.1	5.8
6	52	21.0	41.3	10.3
7	63	4.5	20.0	6.6
8	57	11.0	13.0	6.0
9	49	15.8	47.0	21.0
10	63	9.1	30.6	9.3
11	56	16.2	31.0	7.0
12	59	12.0	35.5	15.4
13	60	10.2	44.4	14.8
14	50		38.5	22.0
15	52		20.0	23.5
16	53		23.0	6.4
17	56		26.0	14.0
18	58		24.0	16.0
19	61		18.0	9.5

For abbreviations, see Table I

Table III

Mathematical analysis

	DEA		A		Hc		A/DEA		Hc/DEA		Hc/A	
	C	P	C	P	C	P	C	P	C	P	C	P
n	19	14	19	14	13	14	19	14	13	14	13	14
$\bar{x}$	27.86	12.86	13.15	9.81	13.22	8.56	0.50	0.82	0.55	0.76	1.49	0.92
s	11.27	4.31	7.47	5.19	5.14	3.09	0.28	0.55	0.40	0.44	1.17	0.49

C = Controls; P = patients with osteoporosis; DEA = Dehydroepiandrosterone; A = Androsterone; Hc = Cortisol

in the evaluation as being due in all probability to some technical error. From the other figures it emerges that in the subjects with postmenopausal osteoporosis the plasma DEA level was considerably lower than that of normal subjects of similar age (mean: 12.9 μg per 100 ml, as against 27.9 μg per 100 ml). The difference between the two groups was highly significant, $p < 0.1\%$.

Although the mean plasma A level was slightly lower in the osteopathy group than in the control group, the difference was not significant. The plasma Hc-level was in the normal range in both groups but the mean values were significantly lower in the osteoporosis group than in the control group.

The greatest divergence between the two groups was displayed by the plasma DEA level. This was not only shown by the figures and their mathematical analysis, but also reflected by the fact that the mean values found in healthy menopausal women were never attained by those of any member of the osteoporosis group. Also, when comparing the frequency of attaining the arbitrary level of 18 μg per 100 ml, there was a significant ($p < 1\%$) difference between the two groups.

When the data presented in Tables I and II are considered for each subject separately, it is seen that (with the exception of Case 1 which whas disregarded for reasons outlined earlier) the plasma DEA level was invariably reduced in the osteoporosis group. This was, however, also the case in three patients of the control group. Now, it is common knowledge that the diagnosis of osteoporosis rests on positive X-rays. In their lack, biopsy would be the only conclusive diagnostic means. However, to be accessible to X-ray diagnosis, absorption of at least 40% of the bone substance must have taken place, in other words, the process must be fairly advanced to be detected radiologic-

Table IV

Plasma hormone levels in primary and secondary osteoporosis of various types

	Age sex	Type of osteoporosis	Actual values μg per 100 ml			Normal range μg per 100 ml		
			DEA	A	Hc	DEA	A	Hc
primary	38 female	castration	11.0	13.0	8.8			
	37 ,,	pregnancy	10.7	7.6	5.7	21.3—53.0	14.1—26.0	
	30 ,,	pregnancy, lactation	46.1	6.2	8.4			
	28 ,,	pregnancy	10.3	8.4	9.6			
	54 male	presenile	9.1	6.3		20.0—55.0	8.5—30.0	
	36 male	idiopathic	15.6	12.4	21.0			4.0—22.0
	33 ,,	idiopathic	85.6	8.0	12.0			
secondary	38 male	gastric resection	53.0	15.2	17.0	17.0—79.0	11.4—45.6	
	47 male	inactivity	36.0	21.0	8.8			
	41 ,,	malabsorption	24.2	0.0	3.6			
	46 female	acromegaly	100.8	45.0	7.8	18.0—38.5	9.5—22.0	

ally. We know on the other hand that osteoporosis affects approximately 20% of women in the menopause. The control group may therefore well have included seemingly healthy women, in whom the process would, however, sooner or later have become manifest and where the relatively low DEA level might have represented the earliest sign of osteoporosis.

Table IV sums up the data of 7 subjects with primary osteoporosis of miscellaneous types and of 4 with secondary osteoporosis. It can be seen that in 5 out of 7 cases of primary osteoporosis of miscellaneous types the plasma DEA level was reduced in relation to age and sex, whereas in the group of secondary osteoporosis the plasma DEA level was in the normal range or higher

Discussion

The present results have confirmed our view formed on the ground of earlier studies, that postmenopausal osteoporosis is associated with a poor androgen supply of the organism, and the slight, but none the less significant reduction in the plasma Hc-level has lent new support to our claim attributing the androgen deficiency in this condition to a functional impairment of the adrenal cortex. A certain contradiction between our earlier and the present results must, however, be pointed out. The finding of a reduced urinary A-output is seemingly at variance with the practically normal A and reduced plasma DEA level, but it might be interpreted as being due to the efficiency of the body's regulatory functions in respect of androgen homeostasis. An increase in tubular absorption of A when the plasma concentration of this substance is reduced would explain why the urinary output of A is reduced when its plasma level is practically normal. At his stage, the only indication of androgen-deficiency is the reduction in the plasma DEA level. Androgen clearance will provide a suitable test for this working hypothesis which, incidentally, also explains why, of all the factors examined, it was A which showed the greatest scatter.

Whether reduced DEA production plays a part in the pathomechanism of osteoporosis is a question to which the present study failed to provide an answer. Though DEA is an androgen of minimal virilizing effect, it non the less seems to have a marked influence on the bone metabolism and on epiphyseal cartilaginous ossification. On the evidence of recent studies [6] DEA inhibits the absorption of cranial bone in vitro and its effect on the epiphyseal cartilage of mice is nearly of the same potency as of identical doses of the strongly virilizing testosterone [7]. The present results as well as the data quoted above and numerous clinical observations make it justified to study the relationship between androgen deficiency and osteoporosis.

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Dr. István Holló
Dr. Tibor Fehér
Dr. János Szücs

BOTE I. Belklinika,
Budapest VIII., Korányi S. u. 2/a, Hungary

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CALCITONIN-EFFECT OF HUMAN BLOOD PLASMA

I. CALCITONIN-CHARACTER OF THE CALCIUM LOWERING PLASMA EXTRACT

By

I. HOLLÓ and J. ZOMBORY

RESEARCH UNIT OF GERONTOLOGY AND FIRST DEPARTMENT OF MEDICINE, MEDICAL UNIVERSITY, BUDAPEST

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Human plasma extract prepared by the method of STUTRIDGE et al. has been studied for its calcitonin-effect. In respect of the parameters considered characteristic of calcitonin, the extract was found to behave similarly to calcitonin. Its calcium-lowering effect was enhanced by anorganic phosphate, it depended on the age of the laboratory animal, and the dose-response curve was consistent with that of calcitonin. Further evidence has thus been furnished that the active factor of the plasma extract in question is identical with calcitonin. The results allowed to establish the optimum timing of the calcitonin bioassay in case of its sensitization with NaH_2PO_4.

STUTRIDGE and KUMAR in 1968 published a procedure for the isolation from human blood plasma of a factor which had a calcium-lowering effect in laboratory animals [1] and showed in two human subjects that experimental hypercalcaemia was accompanied by an increased calcium-lowering effect of their plasma. A parallel reduction of the serum phosphate level by the plasma extract was demonstrable in animals. The active factor was considered identical with calcitonin. Another research team [2] also demonstrated a human plasma factor exerting a calcitonin-effect. The inhibitory effect of the extract on bone resorption as a sign of the calcitonin character was shown on surviving bone tissue as well. On the other hand, the mammalian organism is known to possess some other hormones capable of inducing hypocalcaemia (glucagon, parotins) or hypophosphataemia (glucagon). It is furthermore known that dehydroepiandrosterone [3], hydrocortisone and stilboestrol [4] are inhibiting the absorption of surviving bone tissue. Considering these facts, the identity of the calcium-lowering plasma factor with calcitonin does not seem sufficiently established.

It is known from the literature that calcitonin activity is enhanced by slight amount of anorganic phosphate [5], that the calcium-lowering effect of calcitonin depends on the age of the laboratory animal [6], and that this effect shows the linear correlation with the logarithm of the dose. The aim of the present study was to ascertain whether the plasma extract possessed the above properties so as to establish its identity with calcitonin. The investigation was reduced to the question whether the foregoing actions were demonstrable in the plasma extract.

Material and method

An extract was prepared by the method of STUTRIDGE et al. [1] from the plasma of patients of either sex, between 20 and 40 years of age, in a good general condition, with no abnormality of calcium metabolism, bone disease, or with any evidence of endocrine, hepatic or renal disturbance. The calcium-lowering effect of the extract was examined in home-bred female Wistar rats of our own breed, weighing between 100 and 130 g, fed on an artificial diet and starved on the last day. The aliquot of the extract per rat was suspended in 0.5 ml physiological saline and a 0.3 M solution of NaH_2PO_4 (= 150 μM NaH_2PO_4) and injected into the tail vein. The test animals were killed by decapitation at determined times. The blood of two animals was pooled, therefore each figure in the tables represents the calcium values in the pooled sera of two animals. In part of the study, individual plasma extracts derived from individual subjects were used. These were utilized immediately. The calcium-lowering effect of each extract was tested against the values for the control animals at identical times. In this manner each group treated with a given extract had its control group, and the tables show paired data derived from the test group receiving an individual extract and the corresponding control group, the respective figures being set out next to or below each other. Since the extract was found to maintain its activity for a few weeks, another series was set up in which pooled extracts from a number of patients were used. The experiments made with individual plasma extracts are marked in the tables by the sample number. The doses administered are presented in the tables. The serum calcium level of the animals was estimated by flame photometry (Zeiss Model III) against artificial serum.

1. Calcium-lowering effect of the plasma extract

A total of 48 test rats was injected with extract in physiological saline, the controls with saline. The animals were decapitated 20, 40 and 60 minutes later. The doses per rat administered in the individual experiments and the results are shown in Table I. It can be seen that in each experiment the extract induced a reduction of the serum calcium level as compared to the control and the 40-minute values were below the lowest limit of the normal range. Thus, the extract had a calcium-lowering effect, a finding confirmed by the subsequent assays. It is also seen from Table I that, in conformity with published evidence relating to porcine calcium, elevation of the doses of extract within the order of magnitude scarcely affected the change in the calcium level.

2. Is the calcium-lowering effect of the plasma extract affected by anorganic phosphate ?

It was shown by HIRSCH in the rat that small intraperitoneal doses of phosphate, too slight to affect the serum calcium level, enhanced the effect of calcitonin administered simultaneously by the intravenous route [5]. The observation has then been confirmed and even utilized for the sensitization of calcitonin titration [8]. One of the aims of the present study was to ascertain whether anorganic phosphate would similarly enhance the calcium-lowering effect of our extract. Since in our experience this forms a more homogeneous suspension in the NaH_2PO_4-containing solvent than in physiological saline, the phosphate solution was administered intravenously as the solvent of the

Table I

Effect on the serum calcium levels of rats of plasma extract suspended in saline

	Sample No.	Plasma extract mg/rat	Serum Ca level, mg per 100 ml		
			20'	40'	60'
Control			10.0	10.0	10.0
			10.0	10.0	—
Treated	2	4	9.2	8.6	9.6
			9.8	8.8	—
Control			10.0	10.1	—
			10.0	9.9	—
Treated	4	5	9.5	8.5	—
			9.2	8.7	—
Control			10.0	10.0	10.0
			10.0	9.8	—
Treated	3	6	9.1	8.4	9.2
			8.9	8.5	—
Control			9.9	10.0	9.9
			10.0	9.8	—
Treated	1	7	8.9	8.5	9.1
			8.7	8.3	—
Control			10.0	10.1	10.0
			9.8	10.0	—
Treated	5	8	8.8	8.2	9.2
			8.4	8.0	—

extract instead of the intraperitoneal route. It was therefore necessary to examine whether under the given experimental conditions the phosphate itself had any influence on the serum calcium level.

Half of each extract obtained from the same person was administered in saline and the other half as a suspension in a 0.3 M NaH_2PO_4 solution, each to a different group of 20 animals, injected into the tail vein. Two further groups of 20 animals each were given the respective solvents. The animals were killed by bleeding 30 minutes after injection in conformity with the procedure described by HIRSCH [5]. The results are shown in Table II. It can be seen that on intravenous administration the phosphate by itself induced a lowering of the calcium level, though generally not below the lowest normal

Table II

Effect on the serum calcium level of rats of saline and of phosphate alone and of plasma extract suspended in saline and in phosphate

Sample No.	Plasma extract mg/rat	Serum Ca level mg per 100 ml			
		Saline		0.3 M NaH_2PO_4	
		Control	Treated	Control	Treated
10	4	10.0	8.8	9.1	7.8
		10.0	9.2	9.2	7.9
8	4	10.0	9.1	8.9	8.0
		9.8	9.3	9.1	8.1
7	5	10.1	8.7	9.0	7.6
		10.0	9.0	9.2	7.8
6	6	9.9	8.8	9.3	7.4
		10.0	8.6	9.4	7.8
9	7	10.0	8.5	8.8	6.4
		9.8	8.7	8.9	6.5

limit. Though the extract suspended in phosphate solution induced lower serum calcium levels than the phosphate alone or the saline suspension of the extract, yet the fact that the solvent alone was capable of producing a fall has made the potentiating effect of phosphate inconclusive.

These findings have prompted us to compare the time course of the calcium-lowering effect of the phosphate, of calcitonin in phosphate solution, and of calcitonin in saline solution. A moderate enhancement of the calcitonin effect is known to increase the duration rather than the degree of hypocalcaemia [5]. It was therefore to be expected that, if the calcium-lowering effect of the solvent ceased earlier than that of calcitonin, a possible potentiation would be revealed by the finding of a more prolonged activity of the extract suspended in phosphate, than of that suspended in saline. This proved to be the case indeed as shown by Table III. At 60 minutes after injection of the phosphate suspension, the serum calcium level was still reduced while after administration of phosphate alone the level had returned to its normal value. On comparing the effect of extract in phosphate and extract in saline 60 min. after injection (Table I), it was found that the phosphate enhanced and prolonged the effect of the extract. Since, however, the plasma extracts in saline figuring in Table I and those in phosphate figuring in Table III had been derived from different persons, moreover, the tests were not done simultaneously, another series of experiments was carried out in which the same extract

Table III

Effect of phosphate solvent and of plasma extract suspended in phosphate solvent on serum calcium level of rats, at different intervals

	Sample number	Serum Ca level, mg per 100 ml			
		Minutes after administrati on			
		20'	40'	60'	120'
Control		10.0	9.0	9.9	10.0
		9.8	9.1	9.9	9.9
Treated	15	8.0	8.5	9.0	9.2
		7.8	8.7	8.9	9.1
Control		9.6	8.9	10.0	9.9
		9.9	9.0	10.0	10.1
Treated	14	7.6	8.1	8.8	9.5
		8.0	8.4	9.1	9.3
Control		10.0	9.1	9.9	10.0
		10.0	9.1	9.8	10.0
Treated	16	7.4	8.0	8.6	8.8
		7.8	8.2	8.8	—
Control		9.5	8.8	10.0	9.9
		9.8	8.7	9.6	10.0
Treated	12	7.5	8.4	8.2	9.0
		7.5	8.0	8.6	—
Control		10.0	9.0	9.8	10.0
		9.9	8.7	10.0	10.0
Treated	17	7.8	8.1	8.6	9.1
		7.4	8.3	8.3	—

was administered in saline and also in phosphate to 5-week-old rats simultaneously, and the results were compared. Table IV shows that at 60 minutes when the effect of the extract suspended in saline and that of phosphate alone was over, the extract suspended in phosphate still maintained its calcium-lowering effect. Thus, anorganic phosphate prolonged and intensified the effect of the extract in the same manner as that of calcitonin. From this it followed that if the estimation of calcitonin in the plasma extract is sensitized with intravenous phosphate, the calcium level should be determined 60 minutes after injection of the extract. This is what we did in the further experiments.

Table IV

Effect of phosphate solvent, plasma extract suspended in saline and in phosphate, on the serum calcium level of rats, 60 min. after injection

Serum calcium level of rats aged 5 weeks and 14 months to plasma extract suspended in phosphate

Serum Ca level (mg per 100 ml)					
Physiological saline		Phosphate solvent			
5 week old rats		5 week old rats		14 month old rats	
Control	Treated	Control	Treated	Control	Treated
9.6	9.2	9.6	8.5	9.7	10.0
10.0	9.8	9.4	8.4	9.7	9.6
				9.9	9.8
				9.8	9.8
9.9	9.5	9.8	8.2	10.0	10.0
10.0	9.7	10.0	8.9	10.0	9.8
				9.6	9.7
				9.9	10.0
10.0	9.2	10.0	9.1	9.9	10.0
10.0	9.6	10.0	8.6	10.0	9.6
				9.7	10.0
				9.9	9.8
10.0	9.8	9.9	9.0	10.0	9.6
10.0	9.9	10.0	8.5	9.9	9.6
				9.9	10.0
				9.9	10.0

3. Effect of plasma extract on 5-week and 14-month-old rats

Calcitonin-hypocalcaemia greatly depends on the age of the laboratory animal. In fully developed normal animals calcitonin failed to affect the serum calcium level whereas in young animals it lowered it significantly. Therefore, only young animals can be used for the assay of calcitonin. Thus, we compared the calcium-lowering effect of mixed, pooled plasma extract in 16 animals aged 5 weeks and in 32 animals aged 14 months by administering 50 mg/kg of extract in phosphate solvent. The controls were given the same volume of phosphate. The animals were sacrificed by bleeding 60 minutes after injection. Results are shown in Table IV. In contrast to the distinct reduction in the calcium level in the five-week-old animals, in the 14-month-old animals

the level remained unchanged. Human plasma extract has thus proved age-dependent, similarly to calcitonin.

4. Dose-response curve of plasma extract and of calcitonin derived from porcine thyroid

The calcium-lowering effect of calcitonin shows a linear correlation with the logarithm of the dose [7]; on this relationship is based the biologic titration of calcitonin. It seemed pertinent to compare the plasma extract with calcitonin isolated from porcine thyroid.

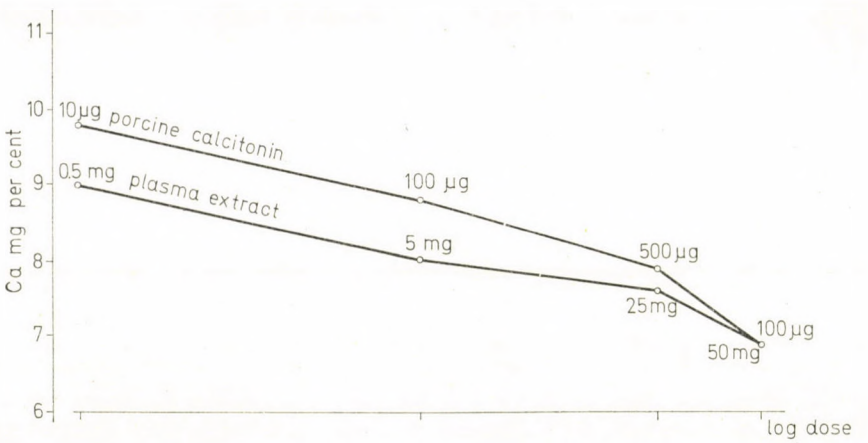

Fig 1. Log dose response of porcine calcitonin and of plasma extract

Porcine calcitonin was administered in doses of 10—100—500 and 1000 μg in three series, each dose being administered to a separate group of 12 rats. The plasma extract was administered in doses of 0.5, 5, 25 and 50 mg, each dose to 4 rats. The mean calcium values obtained in response to the various doses of the two substances are presented semi-logarithmically in Fig. 1. With both substances, the responses show a linear regression related to the logarithm of the dose. The linearity and parallelism between the doses of 10 and 500 μg and 0.5 and 25 mg, respectively, attained the degree required for establishing the identity with calcitonin of the active principle of the extract. A striking observation was the unusual intensity of the response to the maximal doses when, instead, a reduction should have occurred. However, the behaviour of the two substances was consistent also in this respect which was interpreted as a further proof of their identity, regardless of the fact that we have no explanation for the phenomenon itself except for a possible exhaustion of some compensatory mechanism.

Discussion and conclusions

In respect of the parameters examined in the present study the plasma extract prepared by the method of STUTRIDGE et al. behaved exactly in the same manner as calcitonin: its calcium-lowering effect was enhanced by anorganic phosphate, it depended on the age of the animal and the dose-response curve was also consistent with that of calcitonin. Further evidence has thus been furnished in support of the identity with calcitonin of the active principle of the plasma extract. Incidentally, the results also allowed to establish the optimum timing of the calcitonin bioassay in the case of its sensitization with NaH_2PO_4.

*

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Dr. István HOLLÓ; First Department of Medicine, University Medical School, Budapest VIII., Korányi Sándor u. 2/a.

Dr. József ZOMBORY; University Medicial School, Research Unit of Gerontology, Budapest VIII., Somogyi B. u. 3, Hungary

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CALCITONIN-EFFECT OF HUMAN BLOOD-PLASMA

II. PLASMA CALCITONIN ACTIVITY IN 18 SUBJECTS WITH NORMAL CALCIUM METABOLISM

By

I. HOLLÓ and J. ZOMBORY

RESEARCH UNIT OF GERONTOLOGY AND FIRST DEPARTMENT OF MEDICINE, MEDICAL UNIVERSITY, BUDAPEST

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Human plasma extracts prepared by the method of STUTRIDGE et al. were studied for calcitonin activity on the basis of their calcium level lowering effect in rats. All extracts prepared from 18 subjects displayed a calcitonin activity. The results emphasize the significance of calcitonin in the maintenance of normal calcium and bone metabolism.

Calcitonin was regarded as a hormone closely involved in the control of hypercalcaemia and it was assumed that calcitonin secretion depended on the stimulus of hypercalcaemia. STUTRIDGE and KUMAR in 1968 were the first to demonstrate calcitonin activity in normal plasma of two healthy subjects [1]. Another team [2] reported on five further observations in 1969. These data, though relating to individual cases, definitely suggested that the secretion of calcitonin was continuous and that the hormone had a role in the regulation of the serum calcium level and of bone metabolism. However, the above studies on plasma calcitonin carried out by two different methods in a total of seven subjects, did not seem conclusive enough. It was particularly in view of the possible existence of hypocalcitoninaemic diseases (osteoporosis [3]) that it seemed important to decide the question whether a calcitonin activity was present with any regularity in the plasma of subjects with normal calcium and bone metabolism. Therefore, the plasma calcitonin level has been studied in 18 subjects.

Method

The study involved subjects of either sex, between 20 and 40 years of age, in a good general condition, with no evidence of any abnormality of calcium and bone metabolism or of any disease of the endocrine organs, liver and kidney. The plasma extract was prepared from fasting plasma by the method of STUTRIDGE et al. [1]. The calcitonin effect of this extract has been demonstrated in the literature and confirmed by our previous study [4]. Activity of the extract was estimated by its calcium-lowering effect. Female Wistar rats of our own breed, between 100 and 130 g body weight, fed on an artificial diet, were used after a 24-hour fast. Groups of four animals were formed. Each group received plasma extract obtained from a different subject. The dose was 2.5 mg per animal in 0.5 ml of a 0.3 M NaH_2PO_4 solution, injected into the tail vein. The controls were given the same volume of the solvent. 60 minutes after the injection the animals were killed by decapitation. Blood from two animals was pooled; therefore, each value shown in Table I stands for two rats. Extracts prepared from the plasma of several patients were tested against a common control group. The activity of

Table I

Effect of human plasma extract on serum calcium level of rats

No.	Serum calcium level, mg per 100 ml		Difference in mean serum calcium level between treated and control animals, per cent
	Treated	Controls	
1.	7.9 8.3		— 17.4
2.	8.1 8.5		— 15.4
3.	8.1 8.5	10.0	— 15.4
4.	8.3 8.7	9.6	— 14.1
5.	7.6 8.0		— 21.3
6.	8.2 8.8	10.0 9.8	— 14.2
7.	8.2 8.4	9.8	— 15.4
8.	8.6 7.8	9.8	— 16.4
9.	8.2 8.6	9.6	— 11.6
10.	8.2 8.2	9.4	— 13.7
11.	8.1 8.5	9.6 10.0	— 15.8
12.	7.6 8.2	10.0	— 20.3
13.	8.6 8.0	9.8	— 16.2
14.	8.8 8.8	10.0 10.0	— 12.0
15.	9.0 8.6	9.8 10.0	— 11.2
16.	8.1 8.3	9.8 9.8	— 16.4
17.	7.8 8.4	9.4 9.6	— 14.8
18.	8.1 7.9	10.0 9.6	— 18.4

the plasma extract was expressed by comparing the calcium levels of the animals treated by plasma extract with the calcium level of untreated controls. Plasma calcium was estimated by flame photometry (Zeiss, Model III) against artificial serum.

Results, discussion

Results are shown in Table I. It can be seen that the plasma extracts of all subjects induced hypocalcaemia in the rats. The results supported the claim that calcitonin occurs normally in the plasma of individuals with a normal calcium metabolism and that its activity is closely involved in the regulation of calcium homeostasis and bone metabolism.

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Dr. István Holló; First Department of Medicine, University Medical School, Budapest VIII., Korányi Sándor u. 2/a.

Dr. József Zombory; University Medical School, Research Unit of Gerontology, Budapest VIII., Somogyi B. u. 3, Hungary

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PULMONARY LIPID METABOLISM IN EXPERIMENTAL FAT EMBOLISM

By

E. VÁNDOR and Erzsébet ANDA

NATIONAL INSTITUTE OF TRAUMATOLOGY, BUDAPEST

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Pulmonary lipid metabolism and its changes after fat embolisation have been studied in the dog. Lipoprotein lipase activity in outflowing arterial blood was higher than in inflowing venous blood. On embolisation, a tendency to a further increase in A—V difference could be established. In the controls, the serum lipase level did not show any change during the experimental period; triolein administration induced insignificant changes. The lungs could not have played any role in the insignificant increase in activity. The free fatty acid level rose continuously in both the control and the experimental groups. After triolein administration the consequent, but statistically not significant A—V difference of the controls disappeared. After ^{131}I-triolein administration, radioactivity of the lipid fraction in the blood revealed a V—A difference, indicating a triolein consumption by the lungs. The total fatty acid level was significantly higher in the embolised animals than in the controls. Since free fatty acid and triolein could not account for such a great increase, other lipids had to be taken into consideration as a source of the excess fatty acids. The experiments revealed that after a fat loading specific and aspecific changes occur in pulmonary fat metabolism. To the first category belong changes in endogenous lipoprotein lipase activity and free fatty acid metabolisation in the lungs, while to the second group, the elevation of the plasma total fatty acid level.

The important role of the lungs in experimental fat embolism has been established. In the present studies an attempt has been made to correlate the changes in the activity of some of the characteristic lipid enzymes of lung tissue and the events of embolisation. By this means the possible role of the lipid enzymes could be established. On the other hand, fat embolisation proved to be a good model for demonstrating the response pattern of the pulmonary lipid system to a given stimulus, i.e. to test the regulating capacity of this system.

Methods

The experiments were carried out on male mongrel dogs weighing 25 kg on the average, after a starvation period of 20 hours. Under pentobarbital anaesthesia (30 mg/kg) arterial samples *(A)* were drawn from the left carotid artery, while mixed venous blood *(V)* was taken by means of a catheter introduced into the right ventricle. Blood pressure was recorded by a mercury manometer in the right femoral artery. To prevent blood clotting the cannula was filled with isotonic citrate solution instead of using heparin, for the latter substance would have interfered with the determination of endogenous lipoprotein lipase. Samples were taken at the time of cannulation and then at 1 hour intervals through a 6 hour period.

After the first control samples having been taken, non-emulgeated triolein containing ^{131}I was administered in a dose of 0.5 ml/kg into the saphenous vein. The injection was accomplished within one minute. The dose caused an embolism of medium degree.

The control animals were subjected to all the above procedures except embolisation·

Table I

Activity of endogenous lipoprotein lipase, serum lipase, and plasma

	Hours	0	1	2
				Endogenous lipoprotein
I.	A $\bar{x} \pm$ S. E.	1.22 ± 0.14	1.39 ± 0.26	1.47 ± 0.15 P_1
	V $\bar{x} \pm$ S. E.	1.05 ± 0.13	1.02 ± 0.16	0.95 ± 0.15 P_1
II.	A $\bar{x} \pm$ S. E.	1.22 ± 0.14	1.32 ± 0.14	1.69 ± 0.17 P_1, P_2
	V $\bar{x} \pm$ S. E.	1.05 ± 0.13	1.08 ± 0.16	1.17 ± 0.14 P_1
				Serum lipase
I.	A $\bar{x} \pm$ S. E.	191 ± 45	194 ± 53	194 ± 65
	V $\bar{x} \pm$ S. E.	183 ± 45	196 ± 58	191 ± 53
II.	A $\bar{x} \pm$ S. E.	191 ± 45	250 ± 98	359 ± 105
	V $\bar{x} \pm$ S. E.	183 ± 45	252 ± 88	354 ± 110
				Percentual changes in free
I.	A $\bar{x} \pm$ S. E.	102.0 ± 3.9	123.9 ± 7.6 P_2	151.0 ± 11.8 P_2
	V $\bar{x} \pm$ S. E.	100.0 ± 2.9	145.4 ± 14.7 P_2	171.6 ± 26.7 P_2
II.	A $\bar{x} \pm$ S. E.	102.0 ± 3.9	153.3 ± 20.5 P_2	176.1 ± 20.2 P_2
	V $\bar{x} \pm$ S. E.	100.0 ± 2.9	159.5 ± 15.3 P_2	187.6 ± 18.7 P_2
				Percentual changes in radioactivity of
II.	A $\bar{x} \pm$ S. E.		90.2 ± 2.4 P_1	85.0 ± 3.0
	V $\bar{x} \pm$ S. E.		100.0 ± 2.9 P_1	87.5 ± 2.6 P_2
				Percentual changes in total fatty acid
I.	A $\bar{x} \pm$ S. E.	100.0 ± 20.1	137.0 ± 20.4	136.0 ± 29.6
	V $\bar{x} \pm$ S. E.	114.1 ± 14.6	126.5 ± 6.9	131.1 ± 12.7
II.	A $\bar{x} \pm$ S. E.	100.0 ± 20.1	117.0 ± 8.7	127.0 ± 19.0
	V $\bar{x} \pm$ S. E.	114.1 ± 14.6	126.3 ± 3.4	144.1 ± 15.8

I = control group; II = experimental group. A = arterial sample; V = venous sample.

free fatty acid levels, lipid bound radioactivity, and total fatty acids

3	4	5	6
lipase activity			
1.48 ± 0.22	1.35 ± 0.30	1.04 ± 0.27	1.41 ± 0.28
1.12 ± 0.23	1.14 ± 0.27	1.91 ± 0.20	1.27 ± 0.19
1.63 ± 0.19	1.67 ± 0.17 P_2	1.26 ± 0.14	1.22 ± 0.10
1.13 ± 0.18	1.31 ± 0.25	1.09 ± 0.13	0.97 ± 0.15
activity			
187 ± 53	184 ± 63	164 ± 52	180 ± 53
187 ± 54	180 ± 55	180 ± 66	188 ± 65
282 ± 114	275 ± 83	254 ± 70	243 ± 82
305 ± 98	243 ± 78	241 ± 81	241 ± 81
fatty acid content of plasma			
146.8 ± 11.7 P_2	185.1 ± 29.0 P_2	219.5 ± 31.3 P_2	206.1 ± 8.0 P_2
183.6 ± 34.0 P_2	221.0 ± 14.7 P_2, P_3	222.8 ± 46.9 P_2	242.3 ± 58.0 P_2
186.5 ± 12.5 P_2	155.4 ± 20.9 P_2	235.0 ± 47.4 P_2	205.1 ± 31.6 P_2
177.3 ± 21.2 P_2	160.8 ± 21.7 P_2, P_3	222.0 ± 25.0 P_2	199.0 ± 33.1 P_2
lipid fraction of plasma			
80.5 ± 2.2 P_2	80.1 ± 2.4 P_2	74.5 ± 3.1 P_2	72.6 ± 2.9 P_2
82.1 ± 2.1 P_2	82.3 ± 2.7 P_2	77.4 ± 1.8 P_2	75.0 ± 2.5 P_2
content of plasma			
146.0 ± 22.4	142.0 ± 21.3	152.0 ± 15.4	134.0 ± 21.5
140.1 ± 15.7	125.1 ± 18.3	130.1 ± 13.9	131.1 ± 14.8
164.0 ± 32.4	186.0 ± 32.7 P_2	166.0 ± 16.1 P_2	185.0 ± 16.8 P_2
165.1 ± 20.6 P_2	196.1 ± 31.4 P_2	187.1 ± 28.8 P_2	191.1 ± 22.5 P_2

For explanation, see methods and calculations.

Chemical analysis

Endogenous lipoprotein lipase. As reported by CSEH and SZABÓ (1963), this enzyme is activated by blood clotting; this allows to determine enzyme activity without previous administration of heparin.

The activity of lipoprotein lipase was determined in freshly clotted sera according to SZABÓ and CSEH (1963). The blood samples were defibrinated immediately and centrifuged. Enzymatic activity was tested by the manometric method of SAILER et al. (1961); activity was expressed in terms of μM CO_2 released from 1 ml serum at 37 °C and pH 8.2, during one hour.

Serum lipase activity was determined by the method of WEBER (1965), and expressed by μM NaOH equivalent to the quantity of fatty acids set free from 1 liter of serum during 1 min.

Free fatty acid content of heparinized plasma was determined by the method of DOLE (1956). In view of individual differences, the mM/l values were expressed in percentage of the initial V value for each dog. The average of the initial values amounted to 0.470 ± 0.013 mM/l in V plasmas, and to 0.480 ± 0.018 mM/l in A plasmas.

Total fatty acids were determined in heparin-treated plasma by the method of DOLE and MEINERTZ (1960). mM/l values were expressed in per cents of the initial A values. The average initial value was 12.20 ± 2.45 mM/l for A plasma, and 13.95 ± 2.04 mM/l for V plasma.

^{131}I-triolein. The administered 0.5 ml/kg of triolein contained 80—100 μC of ^{131}I-labelled triolein. Radioactivity was assayed in the lipid fraction of the plasma. In each dog cpm/ml values for the lipid fraction at 60 minutes were considered 100%, to which were related the values for the subsequent samples. Activity was tested by a thallium activated vacuum NaI crystal detector.

Calculations. Values in Table I represent the averages obtained by summing up the initial values for both the control and the experimental groups (0 values in Table I). Significance was calculated by Student's t test. The values were considered significant when $p < 5\%$.

Significance was tested between corresponding A and V values within one group, as well as the deviation of these values from their own 0 values. Significance was tested also between the corresponding values for the experimental and the control groups. If a significant difference occurred in the mean values in these three relations, it was denoted by P_1, P_2 and P_3, respectively, in the column of corresponding mean values in Table I.

^{131}I triolein and serum lipase were determined in control and embolised groups, each containing 8 animals, while groups of 5 animals served for free fatty acid, lipoprotein lipase and total fatty acid determination.

Results

Experimental data are summarized in Table I. The changes in the parameters studied were as follows.

Endogenous lipoprotein lipase activity in both the control and the experimental groups showed in the A samples an increase as compared to the initial value (Fig. 1). In each case the A values were higher than the corresponding V values. The increase of A values became more pronounced after triolein administration. The A—V difference in enzyme activity may be indicative of the active role of the lungs in activation and/or release of the enzyme.

Serum lipase. The corresponding A and V values of the control group did not deviate from each other (Fig. 2). The triolein did not induce any difference between arterial and venous values, although an insignificant increase occurred in both values. Thus the lungs do not have any specific influence on the regulation of serum lipase activity.

Free fatty acids. In the control groups there was no significant difference in free fatty acid content between arterial and venous blood, though the venous values were consequently higher (Fig. 3). During the course of the

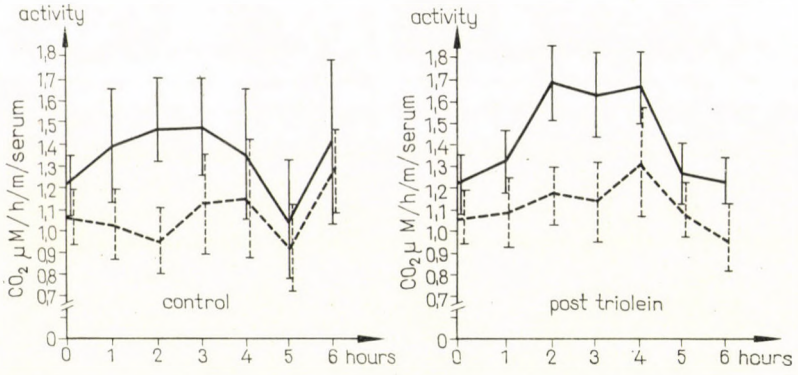

Fig. 1. Endogenous lipoprotein lipase activity. Broken lines: activity in venous serum. Solid lines: activity in arterial serum

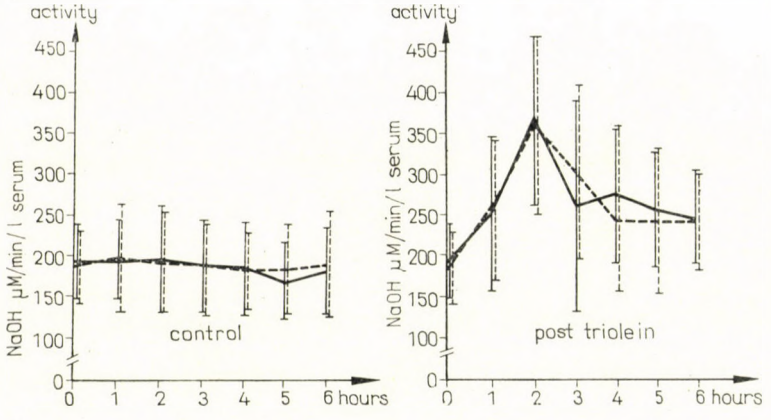

Fig. 2. Serum lipase activity. Broken lines: activity in venous serum. Solid lines: activity in arterial serum

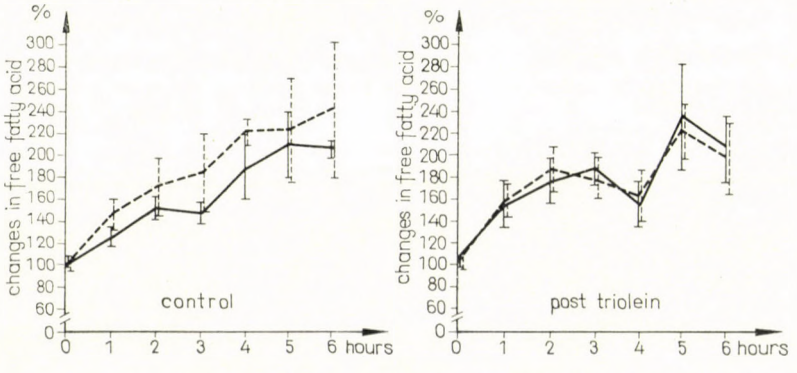

Fig. 3. Plasma free fatty acid level. 0 hour values for venous plasma were considered 100%, to which all the subsequent values were related. Broken lines: venous plasma. Solid lines: arterial plasma

experiment, arterial as well as venous free fatty acid levels were rising continuously. This rise was significant statistically as compared to the initial values; triolein administration did not alter this trend.

The A—V difference found in the control group diminished after triolein administration. Thus, the continuous fatty acid consumption by the lungs seems to be blocked by embolisation.

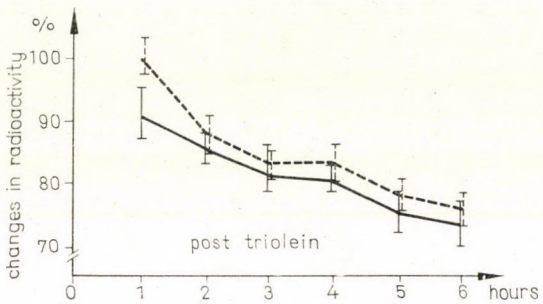

Fig. 4. Radioactivity in arterial and venous plasma lipid fractions after ^{131}I-triolein administration. Broken line: venous plasma. Solid line: arterial plasma

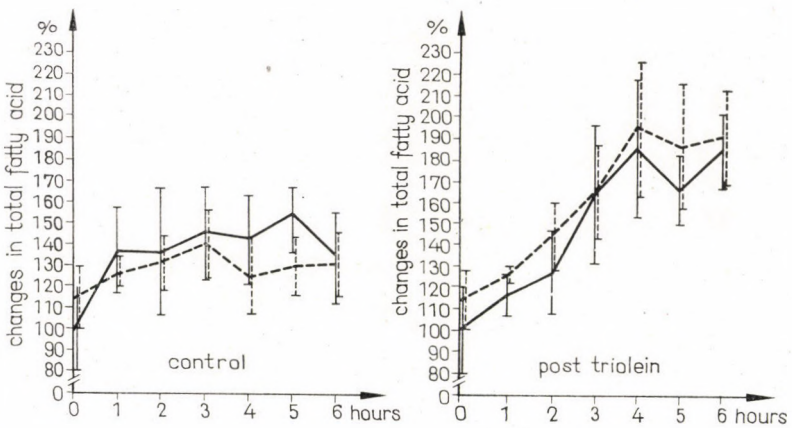

Fig. 5. Plasma total fatty acid content. The 0 hour values for arterial plasma were considered 100%, to which all the subsequent values were related. Broken lines: total fatty acid content in venous plasma. Solid lines: total fatty acid content in arterial plasma

^{131}I-triolein. The radioactivity of the lipid fraction was always higher in the venous than in the arterial samples (Fig. 4). This V—A difference proved to be significant in the first hour. Activity in both the arterial and the venous samples decreased gradually during the experiment. The V—A difference indicated an active metabolisation of triolein by the lungs. The continuous decrease in activity during the experiment might have been connected with

the constant elimination of ^{131}I-triolein by organs other than the lungs. If considering plasma volume to amount to 41 ml/kg, 1.8% of the labelled triolein could be detected in the first hour in the lipid fraction of A as compared to 1.6% for V.

Total fatty acids displayed no significant A—V difference either in the controls or in the experimental group (Fig. 5). While in the control group no significant increase in blood total fatty acid level could be demonstrated, in the experimental group the increase became significant by the 3rd hour. This latter change could not be due to the elevated free fatty acid level, neither to the administered triolein itself.

Discussion

The role of the lungs in fat embolism has been studied by a number of authors. In triolein embolised rats PARADES et al. (1965) established a filtering action of the lungs. While of ingested triolein the lungs contained 67—70%, the share in other organs never surpassed 1%. The simultaneous accumulation of free ^{131}I in the thyroid allowed to conclude to an active metabolisation of triolein by the lungs. HOLCZABEK (1964) observed in fat embolism an increase in the total extractable lipids of pulmonary tissue; this was chiefly due to an increase in the triglyceride fraction. SZABÓ et al. (1967) found a rapid uptake by the lungs of 54—75% of the triolein injected. HÜLSMANN et al. (1964) demonstrated a lipolytic activity in the lungs of rats. This lipolysis must have been due to at least two types of enzyme as inferred from the action of phenothiazine derivatives. ARMSTRONG et al. (1967) observed an increase in pulmonary lipase activity in rats with fat embolism. This was confirmed by SZABÓ et al. (1967) in the rabbit. According to KORN (1959), the lungs belong to lipoprotein lipase containing tissues. HEINEMANN (1960) found in the lungs of rabbits free fatty acids, indicative of lipolytic activity. MALLOW and CERA (1967) demonstrated lipoprotein lipase activity in the myocardium of rats.

In the present experiments, lipid metabolism and its changes after fat embolisation have been studied in vivo. The A—V difference of several lipid parameters in systemic blood was indicative of an active role of the lungs in lipid metabolism. As to endogenous lipoprotein lipase, its activity was higher in arterial than in venous blood, indicating a constant release of the enzyme by the lungs. The lungs may thus be considered one of the chief sources of bloodborne lipoprotein lipase activity. Embolisation seemed to elicit a further release of the enzyme or of one of its activators from the lungs, as suggested by the significantly increased enzyme activity in arterial blood in the 2nd and 4th hours. The fact that the lungs overloaded by triolein exhibited an increased

enzyme activity may suggest a possible regulatory function of the lung in lipid metabolism.

In the present study, triolein administration failed to elicit any significant increase in the A—V difference of serum lipase. Thus, in contrast with their effect on lipoprotein lipase, the lungs play no active role in the regulation of serum lipase activity. The apparent increase in serum lipase activity 2 hours after embolisation was not significant statistically. Therefore, in contrast to other authors, we could not establish any decisive alteration of this enzyme in fat embolism.

PELTIER and SCOTT (1957), after administering human neutral fat intravenously to dogs, observed an elevation of the serum lipase level. However, a scrutiny of these data reveals no significant change in the serum lipase level. ARMSTRONG et al. (1967) found a significantly increased activity in the lungs 48 hours after triolein administration.

In the present studies there was no significant difference in the plasma free fatty acid level between the two groups. Thus, embolisation seems to have no effect on the free fatty acid level. As far as A—V differences are concerned, in the control group a consequent but statistically not significant difference could be established, which disappeared on embolisation. This may indicate a free fatty acid consumption of the lungs, which disappears after embolisation.

The significant elevation of the free fatty acid level during the plasma ment in both the control and the experimental groups should be ascribed to the surgical traumatization. This in itself leads to increased catecholamine levels (FRANKSON et al. 1954; BIRKE et al. 1958). On the other hand, catecholamines are known to induce a release of free fatty acids (DOLE and MEINERTZ 1960; GORDON and CHERKES 1956). WADSTRÖM (1958) actually demonstrated a free fatty acid release in the plasma of patients during surgical trauma, as well as in traumatized rats, CARLSON and LILJEDAHL (1963) found a similar increase in traumatized dogs.

The total fatty acids did not change significantly in the control animals, either in arterial or in venous blood and no A—V difference could be established. Though triolein administration failed to induce any A—V difference, both the arterial and the venous levels rose significantly as compared to the initial value. The elevation of the total fatty acid level may be ascribed to a mobilisation of lipids as a direct consequence of the triolein loading in the form of embolisation. A mobilisation of phospholipids and/or cholesterol esters may also be suspected. The rise in total fatty acids could not be ascribed to free fatty acid or triolein administration, since these alone could not have accounted for the increase. The increase in free fatty acids was similar in the two groups, amounting to 0.5 mM/l on the average. On the other hand, the circulating amount of triolein or its fatty acid fraction was not more than 0.8—0.9 mM/l,

as shown by the radioactivity assays. At the peak of total fatty acid increase, the level exceeded the control by 7.2 mM/l which was obviously in excess of the 0.9 mM/l possibly originating from triolein.

In the rabbit, elevated serum glyceride and phospholipid levels were observed to follow traumatisation (JOHNSON and WADSTRÖM 1965). While no change was found in the plasma phospholipid and glyceride levels in traumatized rats, a decrease of these values was characteristic of humans exposed to surgical trauma (WADSTRÖM 1958). On studying the elimination of artificial fat emulsion from the plasma, HALLBERG (1965) established a profound change in endogenous plasma triglycerides.

The present studies revealed an elimination of the administered triolein; by the end of the first hour, 1.8% of the dose administered could be detected in the circulation. This was in agreement with the data of SZABÓ et al. (1967) who detected in rabbits 1% of the administered amount of triolein. The V—A difference which existed as long as 6 hours together with the continuous decrease in the ^{131}I-triolein level throughout the experiment, were indicative of an active triolein consumption by pulmonary tissue.

The present results may suggest the following pattern of lipid metabolism regulation. Each load stimulus may elicit a double response of fat metabolism, a specific and an aspecific one. The high degree of total fatty acid mobilisation as demonstrated in the present experiments may belong to the latter category. On the other hand, a tendency to increase the output of endogenous lipoprotein lipase from the lungs may reflect an organotropic effect of the same stimulus. Although statistically not significant, the tendency to decrease the A—V difference of free fatty acids in the lungs may be considered another specific, i.e. local, effect. In the present experiments, serum lipase did not seem to participate in the regulatory pattern following fat embolism. Thus, experimental fat embolism is followed by both local and general changes of fat embolism.

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Dr. Ervin VÁNDOR, Dr. Erzsébet ANDA
Országos Traumatológiai Intézet
Budapest VIII., Mező I. út 17. Hungary

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EFFECT OF PINEAL BODY ON THYROID-THYMUS CORRELATIONS

^{131}I UPTAKE BY RAT THYMUS AND SALIVARY GLAND

By

G. CSABA, I. RÉTI and J. FISCHER

INSTITUTE OF HISTOLOGY AND EMBRYOLOGY, SEMMELWEIS MEDICAL UNIVERSITY, AND INSTITUTE OF MATHEMATICAL RESEARCH, HUNGARIAN ACADEMY OF SCIENCES, BUDAPEST

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The effects of pinealectomy, thyroidectomy and TSH-administration on iodine uptake by the thymus and the submandibular gland have been studied and the mutual relations of these organs have been analysed mathematically, using ^{131}I as indicator. Iodine uptake by the thymus was approximately one and a half times that by the submandibular gland, and showed variations depending on the intervention. Pinealectomy increased thyroid iodine uptake; the same intervention or thyroidectomy reduced thymic iodine uptake. TSH did not affect iodine uptake by the thymus or by the submandibular gland but enhanced it after pinealectomy and thyroidectomy. While TSH is regarded as the primary, and the pineal body as the secondary, regulator of thyroid iodine metabolism, for the thymus and the salivary glands the reverse is believed to be true. The possible mechanism of these effects is discussed and connected with the relationship between the pineal body and the branchiogenic organs as well as with the mutual relations of the individual branchiogenic organs.

It has been shown [2—5, 9] that the pineal body regulates the functions of the branchiogenic endocrine organs. In agreement with the results of other workers [7, 8, 12] we found after pinealectomy a significant elevation in the iodine uptake by the thyroid and succeeded in demonstrating that pinealectomy was followed by a decrease in parathyroid function [9]. In other studies [5] we found that the branchiogenous endocrine organs had the capacity of substituting one another's function, for instance the thymus proved to be a more important regulator of calcium metabolism than the parathyroid itself. The existence of close correlations between these branchiogenic organs is indicated also by the transformative capacity of thymic cells [13, 15] as acini of thyroidal character have been demonstrated in the thymus under normal conditions, and especially after thyroidectomy.

All these were considered to mean that, similarly to the thyroid, the thymus is also capable of iodine uptake and of taking over the functions of the thyroid after thyroidectomy. The present study was intended to clarify this problem, consideration being given to the regulatory role of the pineal body and to the possible influence of TSH on the thymus.

Material and method

Sixty male Wistar CB rats of a home-bred strain, weighing between 200 and 250 g were used. The animals were kept on standard compressed rat food (Lati-Gödöllő) containing 400 μg/kg KI. Water was allowed *ad libitum*.

The following eight groups of 7 or 8 animals each were set up.
Controls,
Thyroidectomy,
Pinealectomy,
Thyroidectomy + pinealectomy,
TSH-treatment,
Thyroidectomy + TSH-treatment,
Pinealectomy + TSH-treatment,
Thyroidectomy + pinealectomy + TSH treatment.

The operations were performed under ether anaesthesia. For haemostasis, fibrin foam was used. Success of the operation was checked after sacrifice of the animals and, if necessary, by histologic study.

Estimation of iodine uptake was undertaken by the isotope technique, two months after pinealectomy; 10 days before, thyroidectomy was performed. In the respective groups TSH (Ambinon-Organon) was administered in a single dose of 1 IU/100 g body weight 24 hours before isotope administration.

Isotope study

The animals were given 12.5 μCi/100 g body weight of $Na^{131}I$ intraperitoneally 2 hours prior to sacrifice. The carrier-free $Na^{131}I$ (Isotope Institute of the Hungarian Academy of Sciences) contained 100 μCi/ml of activity.

The animals were killed by an overdose of ether. The organs to be studied (both lobes of the thyroid, thymus, both submandibular glands, femoral muscle) were carefully cleaned, then dried to constant weight under an infrared lamp. After weighing on analytic balance,

Table I

Geometric means and confidential interval of 95% of standardised functional activity

	Thyroid	Thymus	Submandibular gland
Control	3442 2769—4293	3.79 3.36—4.29	2.73 2.19—3.41
Thyroidectomy		5.02 4.22—5.98	3.21 2.34—4.39
Pinealectomy	4624 3381—6324	2.83 2.38—3.37	1.83 1.34—2.51
Thyroidectomy + pinealectomy		2.62 2.12—3.25	2.98 2.03—4.37
TSH	4550 3327—6223	4.11 3.45—4.90	3.24 2.37—4.44
Pinealectomy + TSH	3034 2218—4150	3.94 3.35—4.62	3.19 2.39—4.27
Thyroidectomy + TSH		4.06 3.40—4.83	2.56 1.92—3.43
Pinealectomy + thyroidectomy + TSH		4.74 4.04—5.57	3.56 2.67—4.76

To Tables II, III and IV

Right upper half: relative values of the geometric means (column in percentage of row) and probability levels (p %)according to Student's test:

+ = $5\% < p < 10\%$ nearly significant
A = $p < 5\%$ significant
AA = $p < 1\%$ highly significant
AAA = $p < 0.1\%$ very highly significant

Left lower half: confidence limits of 95% of the relative values given above (row in percentage of column).

Table II

Mean standardized functional activity of thyroid, compared between the individual groups

	Control	Thyroidectomy	Pinealectomy	Thyroidectomy + pinealectomy	TSH	Pinealectomy + TSH	Thyroidectomy + TSH	Pinealectomy + thyroidectomy + TSH
Control		—	1.343 >10	—	1.321 >10	0.881 >50	—	—
Thyroidectomy	—			—			—	—
Pinealectomy	1.969 0.916			—		0.656 >5+		
Thyroidectomy + pinealectomy	—	—	—				—	— — —
TSH	1.938 0.901					0.667 >5+		—
Pinealectomy + TSH	1.292 0.601		1.021 0.422		1.037 0.428			
Thyroidectomy + TSH	—	—			—			—
Pinealectomy + thyroidectomy + TSH	—	—	—	—	—	—	—	

Table III

Mean standardized functional activity of thymus, compared between the individual groups

	Control	Thyroidectomy	Pinealectomy	Thyroidectomy + Pinealectomy	TSH	Pinealectomy + TSH	Thyroidectomy + TSH	Pinealectomy + thyroidectomy + TSH
Control		1.324 <5 A	0.746 <1 AA	0.692 <1 AA	1.084 >40	1.038 >70	1.069 >50	1.250 <5 A
Thyroidectomy	1.641 1.069			0.522 <0.1 AAA			0.807 >5+	0.944 >60
Pinealectomy	0.925 0.602			0.927 >50		1.390 <1 AA		1.675 <0.1 AAA
Thyroidectomy + pinealectomy	0.885 0.541	0.689 0.396	1.222 0.703					1.807 <0.1 AAA
TSH	1.343 0.875		1.762 1.096			0.957 70	0.986 90	1.153 >20
Pinealectomy + TSH	1.271 0.847				1.214 0.755			1.205 >10
Thyroidectomy + TSH	1.324 0.863	1.033 0.631			1.261 0.771			1.169 >10
Pinealectomy + thyroidectomy + TSH	1.531 1.021	1.197 0.745	2.123 1.321	2.360 1.384	1.462 0.910	1.514 0.959	1.514 0.923	

Table IV

Mean standardized functional activity of submandibular gland, compared between the individual groups

	Control	Thyroidectomy	Pinealectomy	Thyroidectomy + pinealectomy	TSH	Pinealectomy + TSH	Thyroidectomy + TSH	Pinealectomy + thyroidectomy + TSH
Control		1.175 >40	0.672 <5 A	1.091 >60	1.189 >30	1.169 >30	0.940 >70	1.306 >10
Thyroidectomy	1.722 0.802			0.929 >70			0.800 >20	1.112 >60
Pinealectomy	0.984 0.458			1.626 >5+		1.742 <5 A		1.945 <1 AA
Thyroidectomy + pinealectomy	1.698 0.701	1.524 0.566	2.667 0.991					1.197 >40
TSH	1.742 0.811					0.984 >90	0.791 >20	1.099 >60
Pinealectomy + TSH	1.683 0.813		2.667 1.138		1.506 0.643			1.117 >50
Thyroidectomy + TSH	1.351 0.653	1.224 0.522			1.211 0.517			1.390 >10
Pinealectomy + thyroidectomy + TSH	1.879 0.908	1.702 0.726	2.979 1.271	1.936 0.740	1.683 0.718	1.683 0.741	2.094 0.923	

the organs were liquefied in concentrated HNO_3 and the total activity was measured: for this purpose, the samples in all test tubes were made up to a uniform volume with ethanol. To secure a homogeneous distribution, a few drops of hyamine were added to each sample. Radioactivity was measured in a well-type scintillation detector, and the values were given in mg per dry organ weight.

The submandibular gland and the muscle served as controls. The salivary gland, possessing an iodine metabolism of its own [1, 6, 10, 11], provided a suitable means for the comparative study of the specific, controlled iodine uptake by the thymus; the muscle served as absolute control.

Mathematical analysis

The results were subjected to statistical analysis. The number of pulses referred to organ weight was divided by the corresponding value for the muscle (serving as absolute control). The geometrical means of these values and the confidence intervals are presented in Table I.

The comparable paired groups were correlated by expressing the geometrical means of one group as a percentage of the other. The significance tests were performed by taking the logarithms of relative values for muscle in each individual animal as a statistically comparable series. Tables II, III and IV show the correlations between the quotients of the paired groups, as also the probability levels characterizing the significance of the deviations, and the 95% confidence limits referred to the quotients between the two groups.

This method of analysis was chosen to refer the values for the individual control organs to the values for muscle and to ensure the homogeneous level for the control results while obtaining biologically interpretable data. This was performed by expressing the count number per organ weight for every organ in percentage of the corresponding value for muscle. The parameters obtained in this manner thus represent the standardized functional activity of the respective organ.

Evaluation of the various effects for the individual organs was based on the quotients of the group averages. In biological terms this means, how many times greater is standardized functional activity in one case than in another, in other words, the effects of two treatments are compared by relating the average of one standardized functional activity to that of another on a percentage basis. This was not only consistent with our method of working on a quotient basis in the comparison of organs and treatments and not only afforded the most reliable means for statistical analysis but also seemed more relevant than the differences between the organs.

Results

Thyroid (Table II)

Iodine content of the thyroid subsequent to pinealectomy or TSH-treatment showed an elevation of approximately 30 to 35% as compared to the controls; the results were not significant statistically. Combination of the two kinds of treatment was followed by a 13% reduction in relation to the controls, and one of approximately 50% in relation to the individual interventions.

Thymus (Table III)

Iodine uptake by the thymus rose significantly, by approximately 30% after thyroidectomy, in contrast to a highly significant, 30 to 35% reduction of its iodine uptake subsequent to pinealectomy and to the combined intervention. TSH did not affect iodine uptake. When given after the two operations, it increased iodine uptake to the control range, reducing the uptake by 25%

as compared to thyroidectomy and elevating it by 35 to 40% as compared to pinealectomy. Administered after the double intervention it produces a highly significant elevation by approximately 80% as compared to its own control equally subjected to the double intervention but in relation to TSH-treatment alone the elevation did not exceed 15% and failed to reach significance.

Submandibular gland (Table IV)

Iodine uptake after thyroidectomy rose by approximately 17% (not significant) and after pinealectomy it decreased by 30 to 35% (significant). After the double operation the value was in the control range. TSH had little influence on iodine uptake but associated with the double intervention it caused a 30% elevation. Thyroidectomy with pinealectomy or with TSH was inefficient. TSH, administered to thyroidectomized animals reduced iodine uptake by 25% as compared to thyroidectomized controls. Pinealectomy, in association with thyroidectomy resulted in a 60 to 65% elevation as compared to the group subjected to pinealectomy alone. TSH administered after the double intervention, caused a 90 to 95% elevation as compared to the group subjected to the same interventions without subsequent TSH treatment.

Discussion

The present results support the findings of VAN DYKE [14] which indicate that the thymus is capable of iodine uptake. This amounts to 3.8 times that of muscle tissue, which is reflecting the general distribution of iodine in the organism, and to 1.5 times that of the submandibular gland which has a specific iodine uptake of its own. Obviously, iodine uptake per mg thymus tissue in comparison to the uptake per mg of thyroid tissue is not more than a thousandth part of the latter, but if uptake by the entire gland is considered it may be as high as 1/100 to 1/50 of thyroid uptake, and under certain conditions, even higher. It would be erroneous to ascribe the variations in thymic iodine uptake simply to the presence or the absence of the thyroid, since, though the thymus increases its iodine uptake after thyroidectomy, it does so only in the presence of the pineal body. In pinealectomized and thyroidectomized animals, iodine uptake by the thymus is reduced despite the absence of the thyroid.

Iodine having been taken up by the organs under study and the individual interventions having been accompanied by variations in the iodine level in the various organs, the iodine content has been accepted as a suitable indicator serving as a basis for a tentative interpretation of the relationships.

Thyroid activity is inhibited by the pineal body, as shown by our earlier results [3] as well as by other data [7, 8, 12], therefore we must accept it

even though the relevant figures obtained in the present study were not significant statistically. Removal of the pineal body was found to represent a thyroid release of the same extent as did stimulation by TSH (Fig. 1). This suggests that the pineal body is capable of joining the primarily TSH-controlled system as an actual secondary regulator. The question which the present study has left unexplained is why the two stimulatory treatments, pinealectomy + TSH, have abolished each other's effect and reduced iodine uptake even in relation to the controls.

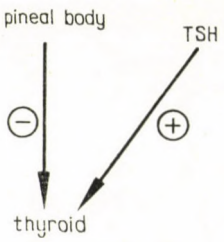

Fig. 1. Effect of pineal body and of TSH on thyroid iodine uptake

As concerns the thymus, the pineal body seems to stimulate iodine uptake by this organ in the same way as it stimulated mastocytogenesis [4] and $Na_2{}^{35}SO_4$ incorporation [2], pinealectomy being followed by a decrease in all these functions in the thymus. In contrast, the thyroid exerts an inhibitory influence of the same extent on thymic iodine uptake, in agreement with its negative influence on mastocytogenesis [4].

If either of the two glands is removed, it is the absence of the responsible regulator, the pineal body, which is decisive. Thus, iodine uptake will be reduced and a compensation of the reduction by the thyroprival state is not possible.

The effect of TSH on the thymus does not become manifest in the normal animal. If, however, both the pineal body and the thyroid are removed, TSH elevates iodine uptake by about 25% as compared to the controls, and by about 80% as compared to the joint effect of the two interventions. Since neither the pineal body nor the thyroid gland have produced separately an effect of this kind, it must be concluded that it is their joint influence which makes the thymus resistant to TSH: in fact, after removal of the two glands TSH increased iodine uptake by exerting a direct action on the thymus. This suggests that the thymus cells involved in iodine uptake are TSH-sensitive, similarly to the corresponding cells of the thyroid (Fig. 2).

The thymus has a quantitative prevalence over the submandibular gland in respect of iodine uptake. After removal of both the pineal body and the

thyroid this prevalence had ceased, and administration of TSH after the joint intervention — when the submandibular gland was far less responsive to TSH than the thymus — restored it again. This proves that

1. the prevalence of the thymus over the submandibular gland in respect of iodine uptake is ensured by hormonal regulation (pineal body, TSH, thyroid), and

2. TSH is capable of joining as a secondary regulator the system controlled primarily by the pineal body.

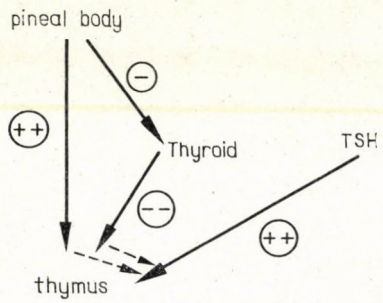

Fig. 2. Effect of pineal body, thyroid and TSH on thymic iodine uptake, and mutual relations

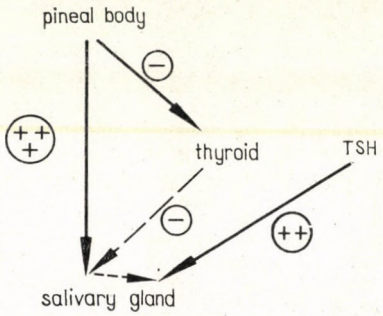

Fig. 3. Effect of pineal body, thyroid and TSH on iodine uptake by the submandibular gland, and mutual relations

The salivary gland takes up less iodine than does the thymus. Its hormonal regulation seems similar to that of the thymus, the prevalence of the individual active components is, however, different (Fig. 3).

It has long been known that the salivary glands are taking up iodine and that this uptake may amount to a multiple of the plasma iodine concentration [1, 6, 10, 11]. It is also known that the submandibular gland is highly sensitive to hormonal influences: removal of the pituitary or the thyroid or of the gonads results in its atrophy, while administration of the hormones of the two last named organs, in its hypertrophy. TSH has failed to affect iodine uptake by the salivary glands of normal animals which is consistent with the present results. BROWN-GRANT assumed [1] that although the mechanism of iodine uptake is the same in the thyroid and the salivary gland, the TSH-dependence of this mechanism is confined to the thyroid. On the evidence of the present results, after pinealectomy TSH exerts a marked influence on iodine uptake by the salivary gland. The mechanism of iodine uptake is thus similar in this respect, too, though being inhibited in the salivary gland and in the thymus.

According to the present findings, the two branchiogenic organs, the thyroid and the thymus are both capable of iodine uptake in a similar manner though to a different extent. This may well represent a common property of all organs derived from the branchial arches, which would account for the

capacity of the submandibular gland of taking up iodine. On the other hand, the higher control of the thyroid and of the two other organs is different in respect of the pineal body, i.e. inhibitory to the thyroid, and identical in respect of TSH, being necessary for iodine uptake by the thymus and the submandibular gland. TSH thus represents a stimulator of iodine uptake by the branchiogenic organs but its effect on the thymus and salivary gland does not become manifest unless the primary regulation has been eliminated.

The present study has thus furnished further evidence concerning the regulation of the branchiogenic organs by the pineal body and on the mutual relationship of these organs.

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Dr. György Csaba
Dr. István Réti
SOTE Szövet és Fejlődéstani Intézete
Budapest IX.,Tűzoltó u. 58, Hungary

János Fischer
MTA Matematikai Kutató Intézete
Budapest V., Reáltanoda u. 13/15, Hungary

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PERMANENT HYPERTENSION INDUCED BY LIGATION OF ONE RENAL ARTERY IN THE DOG

By

Ágnes FEKETE

INSTITUTE OF PHYSIOLOGY, MEDICAL UNIVERSITY, BUDAPEST

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Ligation of the left renal artery in dogs with an intact right kidney was followed by persisting hypertension with progressive renal changes and leading to death beyond the 420th day. When hypertension had persisted for a certain time, elimination of the ischaemic kidney no longer caused a normalization of blood pressure. Survival was adversely affected by the prolonged presence of the ischaemic kidney in the organism. After total ligation of one renal artery in the presence of an intact contralateral kidney the resulting hypertension is probably due to a renin mechanism. The hypertensive influence of the ischaemic kidney induces arteriolonecrosis in the intact kidney. As the site of progressive, irreversible vascular lesions it is the originally "unaffected" kidney which is in fact responsible for the persistence of hypertension and for deterioration of renal function. The changes in renal function observed in the present model might be brought in analogy with human malignant hypertension.

The connections between kidney and hypertensive disease, in the absence of any renal disturbance, were first pointed out by ALBUTT, in 1895. A few years later, Tigerstedt and Bergmann extracted a hypertensive substance from the kidney; the factor responsible for the pressor effect was termed renin. "Renovascular" hypertension is regarded today as a consequence of a disturbance of renal haemodynamics associated with the release of a pressor substance. Among the vascular lesions which may be responsible for hypertension, narrowing or obstruction of the renal artery or of its branches is a common condition and has attracted much interest.

IMBER and GYMER [36], POUTASSE [48], WINTER [55], KENNEDY et al. [37], HOLLEY et al. [32] reported ample clinical and statistical evidence concerning human renovascular hypertension. Normalization or improvement of the malignant hypertension after removal of the ischaemic kidney was reported by HOWARD et al. [34], PERRY [43], MORRIS et al. [39], etc. In these cases the narrowing of the renal artery had been diagnosed by aortography or radioactive diodrast renography. According to POUTASSE [48] nephrectomy would ensure the best results in recent hypertension of sudden onset. On the other hand, CONNOR et al. [8] who followed up a series of patients with renovascular hypertension for a period of three years, regard nephrectomy as useless beyond the 10th to 12th month after onset.

The experimental study of renovascular hypertension is linked with the names of HARTWICH [30] and GOLDBLATT et al. [24] who demonstrated that

unilateral restriction of renal blood flow (by an adjustable clamp of the Goldblatt-type) results in temporary hypertension. In the presence of an unaffected contralateral kidney, normalization of blood pressure occurred within 20 days, or immediately after removal of the ischaemic kidney. Successive clamping of both kidneys with a difference of two weeks or removal of an intact kidney after constriction of the renal artery on the other side resulted in permanent hypertension. PICKERING and PRINZMETALL [46] could induce hypertension under similar conditions in rabbits, but WILSON and BYROM [54] noted the development of persisting hypertension in rats after constriction of one renal artery even though the other kidney had been spared.

PICKERING [44] called attention to the observation that elimination of the ischaemic kidney beyond the 7th week after constriction of its artery failed to normalize blood pressure. HUBER [35] removed the affected kidney at various intervals after arterial clamping in rats and studied the vasculature of the unaffected contralateral kidney. Hypertension depending in severity on the degree of arterial constriction was demonstrable at the end of 14 days, parallel with an arteriolar damage in the "intact" kidney. In this stage of "fixed" hypertension, removal of the ischaemic kidney was no longer able to normalize blood pressure.

We have succeeded in creating an experimental model of human renovascular hypertension by complete ligation of one renal artery in the dog. Hypertension ensued in the presence of an intact contralateral kidney and proved fatal in 1 to 2 years. In the present studies we intended to gain a closer insight into the acute and chronic hypertension induced by ligation of the renal artery of one kidney and to explore the chances for the normalization of blood pressure by removal of the affected kidney.

Material and methods

Full-grown dogs were used in the experiments. Under pentobarbital anaesthesia under aseptic conditions a paracostal incision was made, complete ligation on the left renal artery (and of the eventual accessory renal branches of the aorta) was carried out near their origin and the wound was closed. Blood pressure was determined preoperatively and postoperatively at regular intervals, by direct reading of mean arterial pressure through a femoral cannula, with a mercury manometer, in the alert state, the animals having been habituated to the intervention.

Each animal was placed in a metabolic cage for 3 day periods both before and at 10 or 20 day intervals after the operation, and 24 hour urine was collected during these periods. In the female animals which had been perineotomized for this purpose, the collection periods ended with evacuation of the bladder with a catheter. On the 2nd day of these observation periods, blood pressure was checked and blood samples were obtained for the estimation in serum and urine of creatinine nitrogen, sodium, potassium and osmolarity; urinary protein and sediment. The observations were continued for one year, in some cases as long as two years, or until the animal's death.

In the other part of the study which was concerned with the possibilities of normalization of blood pressure, the left kidney with the ligated artery was removed under aseptic conditions from a midline incision 20 and 40 days after the first intervention in two, and 80, and 120

days after it, in three dogs. These animals with a single (unaffected) kidney were kept in metabolic cages and followed up as described above for one or two years.

During the 3 day observation periods, one or two members of each group were examined simultaneously with the controls so as to avoid the eventual influence on the results of season, temperature, and diet.

Calculations. Daily urinary output was referred to sq. m. body surface as calculated from body weight. In the Figures, the values for the same animal are connected with a line of the same type. Each mark on the curves represents the arithmetic mean of three 24 hour urinary values, i.e. of three measurements obtained in the three-day observation periods.

Results

Fig. 1 presents the blood pressure values obtained at 10 and 20 daily intervals in dogs with ligated left renal artery and one intact right kidney.

As compared to the normal range (K) of 135 $\pm$ 3 mmHg blood pressure began to rise on the 7th day after ligation, to remain high throughout the 420

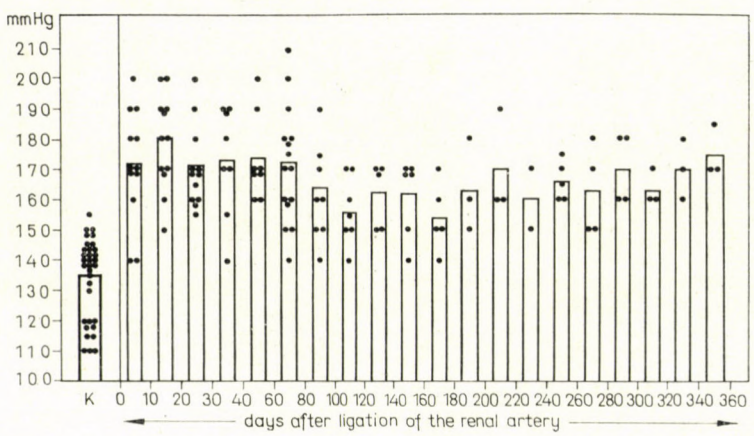

Fig. 1. Arterial blood pressure in dogs before and after ligation of the left renal artery (K), with an intact right kidney

days of observation. Mean blood pressure, its scatter ($\bar{x} \pm s_{\bar{x}}$), the numbers of tests and of the animals concerned are presented in Table I. All mean values were significantly higher than the controls ($p < 0.001$).

The changes in the parameters of renal function as compared to the preoperative control values are also presented in Table I. After ligation of the renal artery, certain changes manifested themselves. After 16 days, GFR as measured by endogenous creatinine clearance decreased while urinary output per minute increased, the concentration capacity diminished, isosthenuria

Table I

Blood pressure and parameters of renal function ($\bar{x} \pm s_{\bar{x}}$), on days 1

mm/Hg	K	10–20	21–30	31–40	41–60	61–80
Blood pressure	135±3 n = 32	180±4 n = 12	171±4 n = 12	173±5 n = 8	174±5 n = 8	172±5 n = 15
V ml/min	0.57±0.50 n = 61	65±0·08 n = 22	0.68±0·10 n = 15	0.73±0·08 n = 11	0.85±0.11 n = 8	0.42±0.10 n = 27
$U/P_{creat} \cdot V$ ml/min	51±2 n = 61	36±3 n = 22	36±1 n = 155	35±3 2. n = 11	31±4 n = 8	52±4 n = 27
U_{osm}/P_{osm}	2.96±0.20 n = 61	1.94±0.18 n = 22	1.70±0.25 n = 15	2.15±0.18 n = 11	1.87±0.24 n = 8	3.28±0.39 n = 27
$U_{na} \cdot V$ μEq/min	49±5 n = 61	45±7 n = 22	43±9 n = 15	100±25 n = 11	198±40 n = 8	81±35 n = 27
$U_k \cdot V$ μEq/min	25±2 n = 61	24±2 n = 22	26±2 n = 15	22±2 n = 11	37±9 n = 8	15±3 n = 27
S_{creat} mg/100 ml	0.7±0.05 n = 24	1.0±0.1 n = 12	1.0±0.1 n = 9	1.2±0.1 n = 7	1.0±0.1 n = 4	0.9±0.1 n = 9
NPN, mg/100 ml	30±1 n = 24	34±2 n = 12	30±2 n = 9	38±2 n = 7	34±2 n = 4	34±3 n = 9
S_{Na} mEq/l	152±1 n = 24	153±1 n = 12	151±1 n = 9	149±7 n = 7	153±0 n = 4	144±1 n = 9
S_K mEq/l	4.7±0.2 n = 24	3.8±0.1 n = 12	3.8±0.1 n = 9	4.5±0.2 n = 7	4.5±0.1 n = 4	4.6±0.2 n = 9
Number of dogs studied	22	12	12	8	4	9

developed. At the same time, the serum sodium, potassium and creatinine level was unchanged, and there was no increase in NPN neither was there proteinuria. Three hypertensive dogs (Nos 61, 62, 77) were followed up for 420 days; their essential data are presented separately in Fig. 2.

Is it possible to influence the chronic hypertension by removing the ischaemic kidney?

Table II shows mean values ($\bar{x} \pm s_{\bar{x}}$) (i) in the presence of the left kidney with constricted renal artery; (ii) in the case of nephrectomy performed 20 or 40 days after ligation; (iii) in the case of nephrectomy performed 80 or 120 days after ligation. It was found that by the 20th to 40th day a hyperten-

to 420 after ligation of left renal artery, the right kidney having been left intact

81–100	101–120	121–160	161–200	201–240	241–280	281–320	321–420
164±7 n = 8	156±6 n = 7	162±6 n = 11	160±6 n = 8	165±8 n = 6	165±6 n = 9	168±6 n = 7	175±5 n = 6
0.90±0.10 n = 13	0.56±0.13 n = 15	0.90±0.20 n = 10	1.10±0.14 n = 10	1.86±0.31 n = 9	1.51±0.34 n = 9	1.35±0.32 n = 9	1.32±0.17 n = 9
46±6 n = 13	44±13 n = 15	55±6 n = 10	28±5 n = 10	23±3 n = 9	23±3 = 9	26±3 n = 9	28±3 n = 9
2.74±0.43 n = 13	2.42±0.63 n = 15	2.54±0.33 n = 10	2.34±0.50 n = 10	1.16±0.33 n = 9	1.44±0.40 n = 9	1.25±0.18 n = 9	1.25±0.17 n = 9
188±34 n = 13	97±42 n = 15	152±51 n = 10	126±21 n = 10	56±15 n = 9	36±9 n = 9	57±3 n = 9	58±10 n = 9
30±6 n = 13	15±6 n = 15	15±3 n = 10	14±2 n = 10	24±3 n = 9	17±3 n = 9	40±6 n = 9	34±5 n = 9
0.9±0.1 n = 7	0.9±0.02 n = 5	0.9±0.1 n = 3	1.0±0.2 n = 3	1.0±0.2 n = 3	1.1±0 n = 3	1.4±0.1 n = 3	1.2±0.1 n = 3
42±4 n = 7	37±4 n = 5	49±15 n = 3	35±1 n = 3	31±3 n = 3	35±2 n = 3	33±1 n = 3	31±2 n = 3
150±0 n = 7	145±3 n = 5	144±1 n = 3	146±2 n = 3	146±1 n = 3	154±2 n = 3	153±3 n = 3	158±1 n = 3
3.9±0.1 n = 7	3.9±0.1 n = 5	4.4±0.2 n = 3	4.3±0.1 n = 3	4.3±0.3 n = 3	4.3±0.1 n = 3	4.2±0 n = 3	4.1±0.1 n = 3
7	5	3	3	3	3	3	3

sive state has formed which is no longer influenced by the removal of the affected kidney.

Fig. 3 shows the behaviour of blood pressure and renal function when the interval between ligation of the renal artery and nephrectomy was 20 or 40 days (Nos 20 and 52). It can be seen that hypertension remained unchanged while renal function was slightly impaired; polyuria and isosthenuria appeared at about the 8th month. Survival after ligation was limited to two years. On the other hand, the animals subjected to nephrectomy 20 or 25 days after ligation of the renal artery, though remaining hypertensive, preserved their normal kidney function through the entire period of observation of 2 to 3 years and survived.

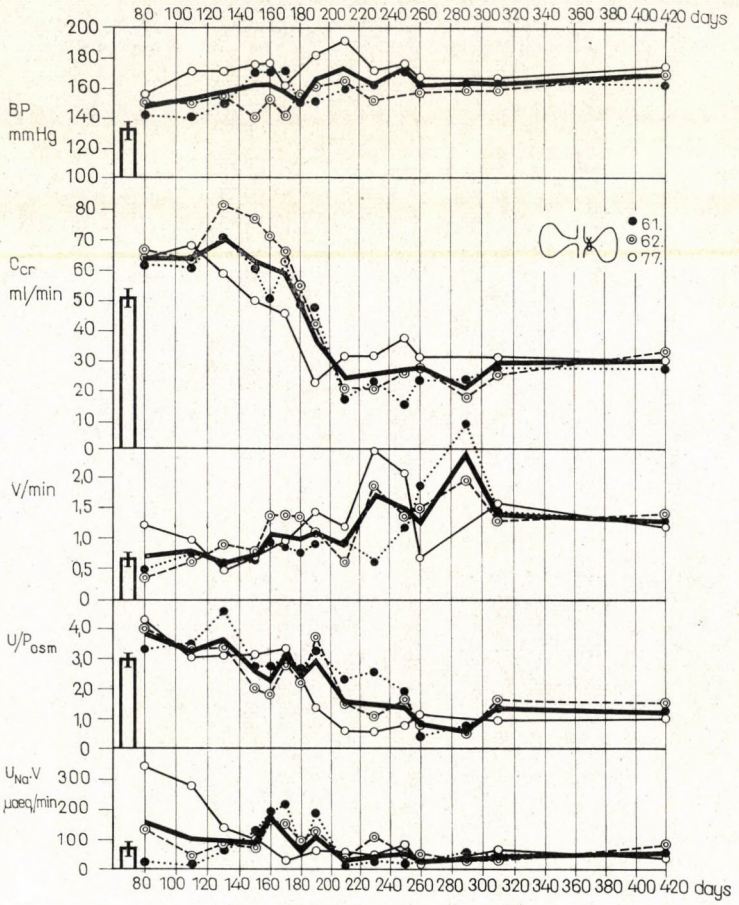

Fig. 2. Changes in renal function after ligation of left renal artery in the presence of unaffected right kidney, from the 80th post-ligation day until death. Thin lines mark the values for individual animals, heavy lines the mean values derived from three animals. The columns represent control values obtained in presence of both kidneys

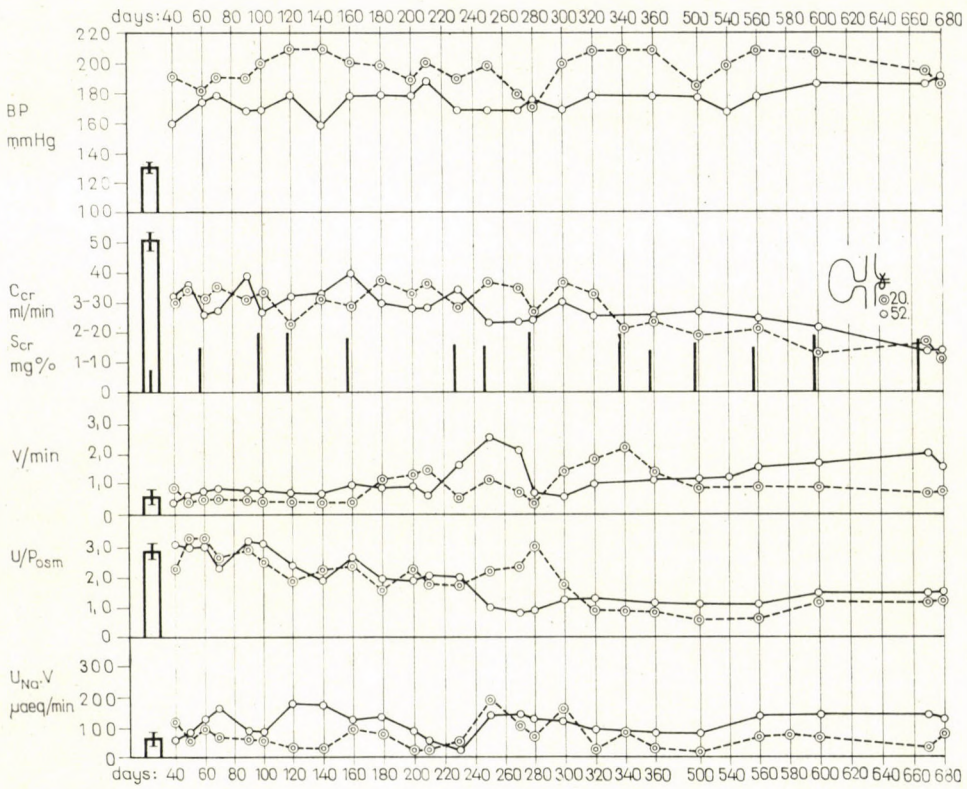

Fig. 3. Changes in function of right kidney following removal of ischaemic left kidney on the 40th day after ligation of left renal artery, until spontaneous death of the animals. Signs as in Fig. 2

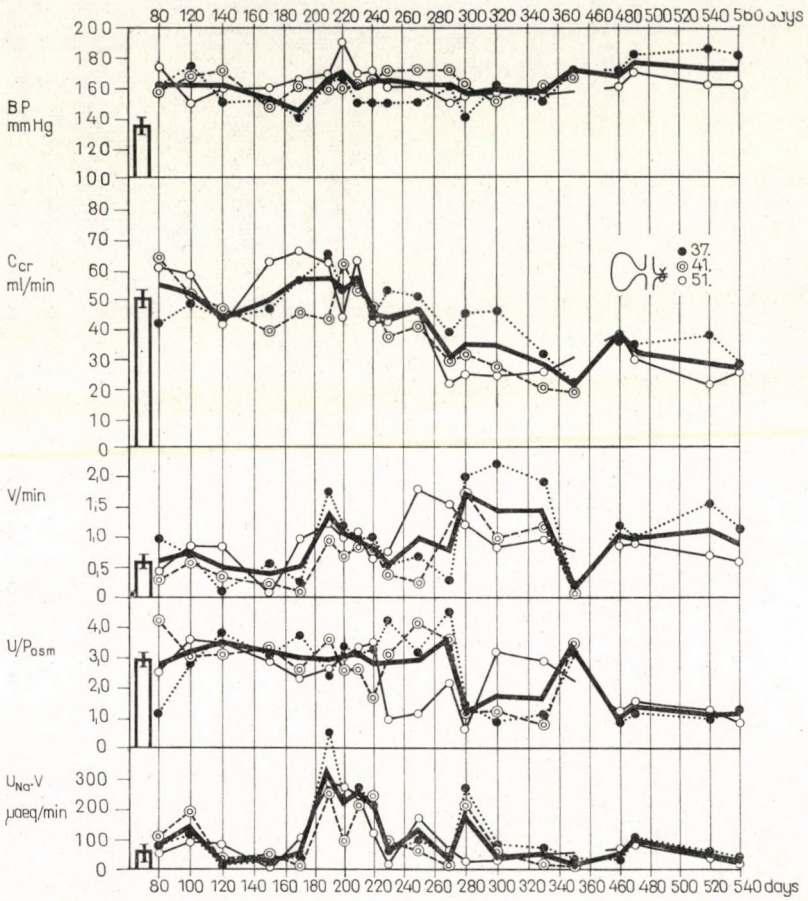

Fig. 4. Changes in function of right kidney following removal of ischaemic left kidney on the 80th day after ligation of left renal artery, until spontaneous death of the animals

Renal function was found to deteriorate faster in the hypertensive animals if the interval between ligation of the renal artery and nephrectomy was as long as 80 days (Nos 37, 41, and 51; Fig. 4). Deterioration was still faster and survival shorter if nephrectomy was performed 120 days after ligation (Nos 47 and 48, Fig. 5).

In the animals which succumbed to the hypertensive condition, and in which the ischaemic kidney had been left in place, the kidney of the unaffected side displayed hypertensive changes. Bowman's capsule was grossly, the tubular basement membrane slightly, thickened. The small vessels displayed proliferation of the intima, swelling and disorganization of the elastic layer, swelling

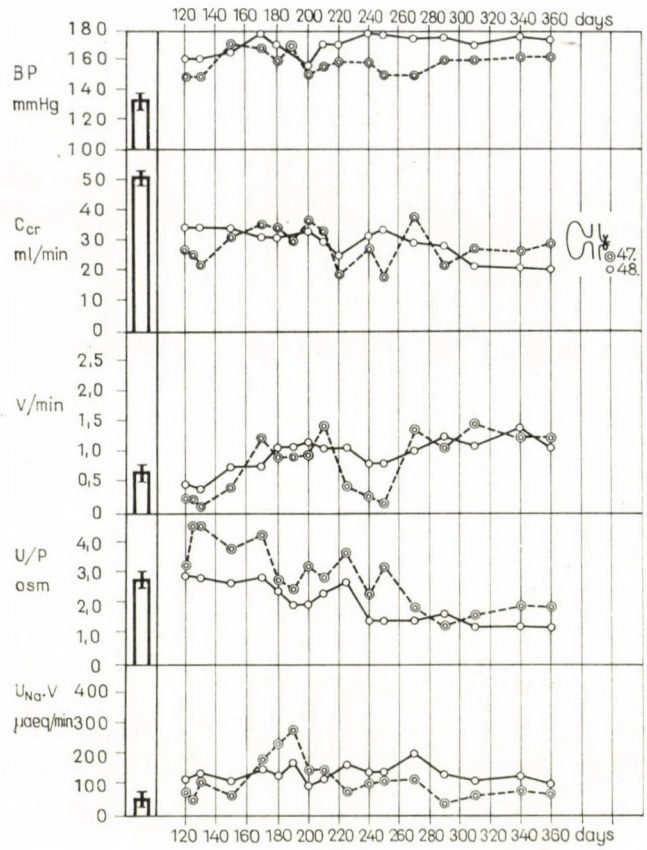

Fig 5. Changes in function of right kidney following removal of ischaemic left kidney on the 120th day after ligation of left renal artery until spontaneous death of the animals

and fibrinoid necrosis of the media. In the afferent arterioles gross hyaline degeneration and fibrosis of the glomerular loops and in the larger arteries myoelastofibrosis were present, as signs of an advanced hypertensive process (Fig. 6).

The kidneys whose arteries had been ligated showed all the characteristics of a contracted kidney, i.e. shrinking of the cortex, complete tubular hyalinization, necrosis of the medullary substance.

In those animals in which the ischaemic kidney had been removed a certain time after arterial ligation, the kidney of the unaffected side also exhibited vascular lesions characteristic of a hypertensive process, such as arteriolonecrosis and myoelastofibrosis (Fig. 7).

Table II

Effect of left nephrectomy performed 20—40, and 80—120, days after ligation of left renal

K	1—20	21—40	41—60	61—80	81—120	121—160
135±3	176±4	173±4	179±5	180±7	168±6	162±4
	$p < 0.001$					
135±3			163±2	166±5	166±3	180±5
			$p < 0.01$	$p > 0.05$	$p > 0.05$	$p < 0.01$
			$p < 0.001$			
135±3					168±4	164±2
					$p > 0.05$	$p > 0.05$
					$p < 0.001$	

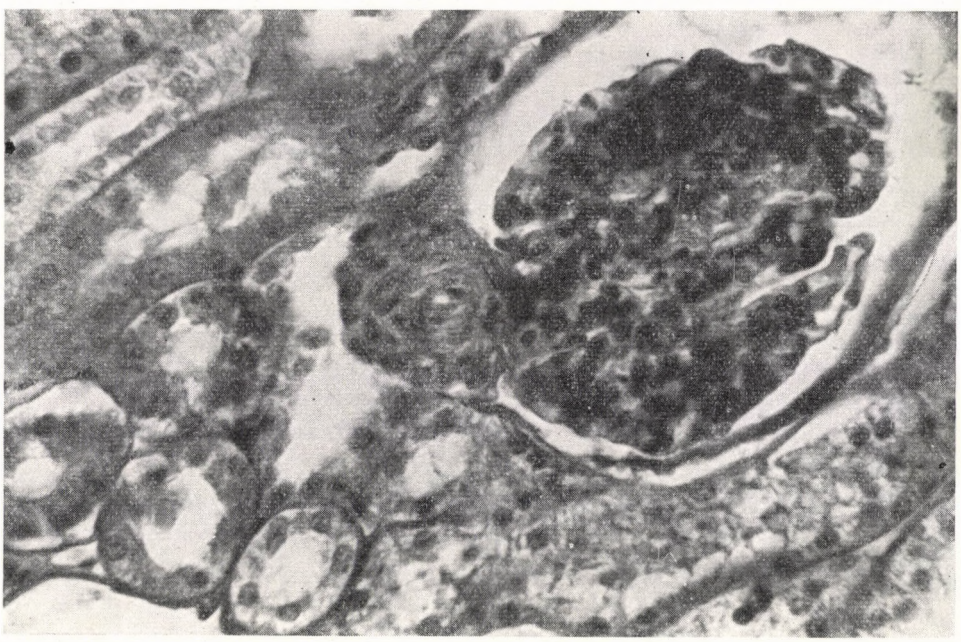

Fig. 6. Right kidney of animal No. 61, 420 days after ligation of left renal artery. Hyperplasia of smooth muscular elements and of connective tissue in the media, with narrowing of afferent arteriole. Mallory's triple dye, 440×

artery, on blood pressure, as compared to hypertensive model and to control values

161—200	200	Day after intervention
160±4	165±4	Mean blood pressure in group with ligated left renal artery and intact right kidney
		Correlation with control values
177±4	185±3	Mean blood pressure in group with left kidney removed 20—40 days after ligation of left renal artery
$p < 0.01$	$p < 0.001$	Correlation with hypertensive group with both kidneys left in place
		Correlation with control values
167±3	165±3	Mean pressure in group with left kidney removed 40—120 days after ligation of left renal artery
$p > 0.05$	$p > 0.05$	Correlation with hypertensive group with both kidneys left in place
		Correlation with control values

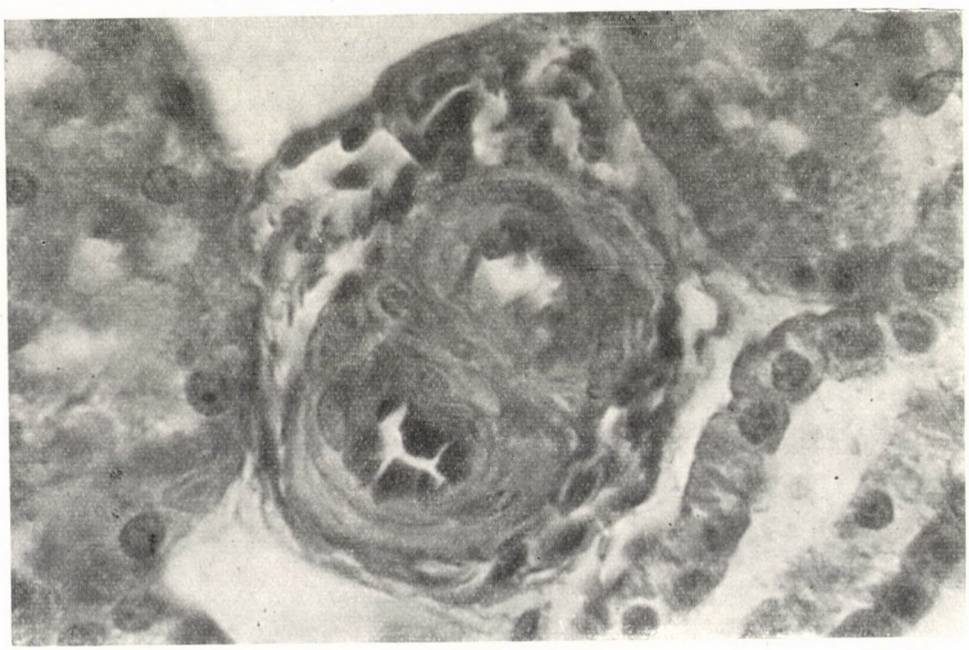

Fig. 7. Right kidney of animal No. 41, 356 days after ligation of left renal artery. Interlobular artery of 3rd order with thickening of media owing to increase in connective tissue. Mallory's triple dye, 880 ×

Discussion

It is common knowledge that every intervention affecting renal blood supply constitutes a source of hypertension. Renovascular hypertension is basically due to an impairment of renal blood supply in consequence of a decrease of pressure in the renal arterial system. The greater the narrowing of the arteriolar lumen, the more malignant the process, the more likely it results in complete renal failure.

It is an accepted fact that renal tissue poorly supplied with blood owing to arterial narrowing forms a pressor substance which then induces and maintains hypertension in the organism. Some authors attributed the transitory character of hypertension induced in dogs and rabbits by constriction of one renal artery to a "neutralizing" effect of the intact kidney. FLASHER [16] as well as SCHLEGEL et al. [49] claimed on these very grounds that induction of persistent hypertension requires constriction of both renal arteries. There is, however, consistent evidence to the contrary. It has been found, e.g. by WILSON and BYROM [54], FRIEDMANN et al. [20] in the rat, and by GROLLMANN [26] in the rabbit that hypertension induced by constriction of one renal artery becomes chronic even if the other kidney has been spared. BYROM and DODSON [7], and FLOYER [17] furthermore showed that when in the rat renal ischaemia has been present for a few weeks, elimination of the affected kidney is no longer effective in restoring normal blood pressure, and that the other, unaffected kidney also displays destructive vascular changes. Neither is a relief of arterial constriction effective in such cases, a fact which obviously proves that in that phase the hypertension is maintained by the seemingly "intact" contralateral kidney. The vascular lesions responsible for the hypertensive state have been demonstrated by numerous authors in one or both kidneys [27, 29, 48].

Unilateral obstruction of the renal artery, a common occurrence in man, poses major diagnostic and therapeutic problems [8, 21, 25]. The decisive point here is the condition of the other, the "intact" kidney, and whether it shows sequelae of the hypertension. Thus far the induction of unilateral renal artery obstruction and the studies of its consequences in the other kidney have been confined to rat experiments. The present model may claim the following advantages. 1. It permits to study the acute and chronic hypertensive changes of the unaffected kidney in the dog; 2. it provides for total obstruction of the renal artery, in contrast to its incomplete obstruction which involves incalculable individual differences in renal blood supply.

It has been demonstrated by the present studies that total ligation of one renal artery in dogs, the other kidney being left intact, results in acute, and subsequently in chronic, hypertension. A rise in blood pressure is demonstrable from the seventh day onward and blood pressure remains significantly

increased in relation to the controls throughout the entire period of observation (420 days survival). Complete obstruction of one renal artery in dogs is thus followed by malignant hypertension which then affects the function of the contralateral kidney and leads to death. In the dead animals with their left renal artery ligated the right kidney always displayed grave hypertensive vascular lesions.

It was only too obvious to attribute the malignant hypertension developing after ligation of one renal artery to the presence of the ischaemic kidney. The experiments in which the ischaemic kidney was removed at various intervals after ligation, made it evident that if hypertension had been present for 20 to 40 days, elimination of the ischaemic kidney was no longer able to normalize blood pressure; moreover, the longer the hypertensive state had persisted, the greater was the general severity of the process and the nearer the fatal outcome.

The present results might be of interest since they allow to study renovascular hypertension in the dog, i.e. in a species fairly related to man in respect of renal function, and support the clinical observations where the attempts to normalize the high blood pressure due to unilateral renal arterial obstruction and to improve the consequential malignant nephrosclerosis by removal of the nonfunctioning kidney were unsuccessful [2, 8, 9, 25].

*

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Dr. Ágnes FEKETE, Institute of Physiology, Medical University, Budapest, VIII., Puskin u. 9, Hungary

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RUBEOSIS IRIDIS

By

Gy. IMRE

SECOND DEPARTMENT OF OPHTHALMOLOGY, SEMMELWEIS MEDICAL UNIVERSITY, BUDAPEST

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Of 18 cases of rubeosis iridis in 15 the rate of aqueous flow was reduced. The average F value, normally 2.3 cu.mm/min., was as low as 0.66 cu.mm/min. Rubeosis of the iris is thus associated with an impaired ciliary body secretion and the consecutive reduction of the rate of aqueous flow may result in an increased concentration of the vasoproliferative factor in the anterior chamber. In cases of retinal vessel occlusion, the determination of the F value has a prognostic significance.

According to ASHTON (1957) and SMITH (1961), it is the diffusion of the vasoproliferative factor through the vitreous into the aqueous humour which accounts for neovascularization of the iris surface being in contact with the aqueous humour, particularly of the pupillary border and of the chamber angle, a process occurring after obliteration of the retinal vessels. SCHULZE (1967) attributes chronic ischaemia of the iris to rubeosis. RAITTA and VANNAS (1969) also believe that neovascularization of the iris is due to anoxia since by means of fluorescein angiography they could demonstrate slowing of the blood stream in the iris in processes of this kind.

The present report deals with tonographic studies on eyes with rubeosis of the iris; the results have allowed to reconcile the views outlined in the foregoing.

Methods

In eyes affected with rubeosis iridis the C value was determined by tonography and the rate of aqueous flow was calculated from the formula, $F = (P_0 - 10) \cdot C$. Tonographic examinations were performed in the morning hours. In the cases where P_0 was less than 10 mm Hg, the F value was considered 0 and no tonography was performed.

Results

The data of the patients and the results of the studies are shown in Table I.

Of 18 eyes with rubeosis iridis of various causes, 8 displayed a reduced (4 to 12 mm Hg), 1 a normal, and 9 an elevated intraocular pressure. The C value was normal in 3 cases and definitely reduced in 10, the average being 0.08 ± 0.07. The rate of aqueous flow was normal in 3, definitely reduced in

Table I

No.	Age	Sex	Primary disease	P_6	C	F
1	44	♂	Periphlebitis of the retina	11	0.19	0.19
2	45	♂	Occlusion of central retinal vein	64	0.04	2.16
3	50	♀	Occlusion of central retinal vein	43	0	0
4	61	♀	Occlusion of central retinal vein	47	0	0
5	62	♀	Diabetic retinitis	37	0.05	1.35
6	62	♀	Diabetic retinitis	75	0.03	1.95
7	51	♀	Diabetic retinitis	37	0	0
8	9	♂	Coats' disease	17	0.08	0.56
9	64	♂	Occlusion of internal carotid	26.5	0.09	1.48
10	39	♂	Chronic uveitis	4	—	0
11	42	♀	Chronic uveitis	12	0.20	0.40
12	36	♀	Chronic uveitis	6	—	0
13	68	♀	Chronic uveitis	8	—	0
14	60	♀	Chronic uveitis	47	0.03	1.11
15	64	♀	Chronic uveitis	28	0.14	2.52
16	19	♂	Chronic uveitis	7	—	0
17	19	♂	Chronic uveitis	9	—	0
18	38	♀	Chronic uveitis	11	0.18	0.18

15 cases, average 0.66 $\pm$ 0.85 cu. mm/min. BECKER, KESKEY and CHRISTENSEN (1956) consider 2.3 cu.mm/min. to represent the normal mean rate of aqueous flow.

Discussion

In 50 per cent of our patients with iris rubeosis, there has never been any evidence of increased intraocular pressure which rules out an aetiologic relationship between rubeosis and elevated intraocular pressure. The results of RAITTA, VANNAS and AUREKOSKI (1968) are also opposed to any possible causal role of elevated intraocular pressure in the pathomechanism of rubeosis. Vascular proliferations involving the iris and chamber angle constitute, however, a source of secondary glaucoma. Reduction in the C value is also merely secondary to neovascularization. On the other hand, the considerably diminished aqueous humour production in 15 out of 18 of the present cases, regardless of the intraocular pressure, seems to be connected with the aetiology of rubeosis. The vasoproliferative factor diffusing into the aqueous humour, a factor identifiable with lactic acid produced in the course of glycolysis (IMRE 1964, 1966, 1969) may have a role in neovascularization of the anterior surface of

the iris and of the chamber angle. The rate of aqueous flow must be obviously reduced so that this substance should attain a high concentration in the anterior chamber. This mechanism accounts for the production of rubeosis after obliteration of the retinal vessels when the rate of aqueous flow is reduced as it was indeed the case in 7 out of 9 eyes presented in Table I. In these instances ciliary circulation is also affected (SCHULZE 1967, RAITTA and VANNAS 1968). However, with the exception of rubeosis associated with uveitis, no isolated rubeosis of the iris without retinal lesions has ever occurred, which agrees with the claim of ASHTON (1957) and of SMITH (1961) in that the vasoproliferative factor diffuses forward from the retina. Reduced production of aqueous humour thus favours the development of rubeosis, therefore in the case of a central retinal vein occlusion or of diabetic retinopathy, the F value may be of prognostic significance. Reduction of the rate of aqueous flow in such cases makes it advisable to prescribe drugs stimulating aqueous humour production (pilocarpine, vasodilators) and local application of heat as well.

Reduced flow of aqueous humour plays an equally important part in the aetiology of rubeosis iridis if the primary condition is uveitis. This is confirmed by the fact that in 8 out of 9 cases shown in Table I the rate of aqueous flow was definitely reduced. A secretory disturbance of the ciliary body is common in uveitis or cyclitis. True rubeosis iridis is, however, rare, since the secretory disorders are mostly transient and, even if they persist, cell proliferation would be scarcely ever of an extent to account for the production of the vasoproliferative factor.

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Dr. György IMRE; Budapest VIII., Mária u. 39. Hungary

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РЕЗЮМЕ

ИЗУЧЕНИЕ ПРОДОЛЖИТЕЛЬНО СТИ ЖИЗНИ И МЕСТА ГИБЕЛИ КРОВЯНЫХ ПЛАСТИНОК ПРИ ИДИОПАТИЧЕСКОЙ ТРОМБОЦИТОПЕНИЧЕСКОЙ ПУРПУРЕ

Т. БУРГЕР и Е. РИХМЕР

Обсуждается проблема оценки результатов исследования продолжительности жизни и секвастрации кровяных пластинок у здоровых контрольных лиц и у больных с идиопатической тромбоцитопенической пурпурой. На основе полученных результатов авторы придерживаются того взгляда, что при приготовлении взвеси тромбоцитов ACD [раствор из цитрата натрия + лимонной кислоты + 2% декстрозы] является более подходящим антикоагулянтом, чем ЭДТА, так как при его применении получается «recovery» более значительного размера. Значительная разница наблюдается в отношении места секвестрации, в зависимости от применяемого вида антикоагулянта. Больщую активность измеряли, как правило, над селезенкой. Это явление связано с геометрическими условиями при надорганных измерениях.

При идиопатической тромбоцитопенической прурпуре авторы отмечали значительные отклонения в продолжительности жизни и секвестрации тромбоцитов. У большинства больных была найдена повышенная активность при измерении над селезенкой. Однако, наблюдалось также повышение активности над печенью при очень короткой продолжительности жизни кровяных пластинок.

ДЕЙСТВИЕ ГЛЮКОЗАМИНА И ЕГО ПРОИЗВОДНЫХ НА ИНКОРПОРАЦИЮ $^{35}SO_4$ В АОРТУ СОБАК

Л. ЯКАБ и Е. ШЕРЕГЕЙИ

Авторы изучали инкорпорацию $^{35}SO_4$ in vitro в аортальные ткани 2—5-месячных контрольных собак и молодых взрослых собак, получавших кротоновое масло. Исследуемые производные глюкозамина (N-ацетил-4-6-бензилиден-глюкозамин и N-ацетил-4-6-окси бензал-глюкозамин) не вызывали оцениваемых изменений в инкорпорации $^{35}SO_4$. При применении глюкозамина также не было отмечено повышения инкорпорации $^{35}SO_4$. На адвентициальной поверхности аорты инкорпорация $^{35}SO_4$ повышена по сравнению с поглощением, измеряемым на интимальной поверхности. При более длительном времени инкубации эта разница еще повышается. Воспаление, вызванное дачей кротонового масла, не приводит к оцениваемым изменениям инкорпорации $^{35}SO_4$ аортальной тканью.

ЭКСПЕРИМЕНТАЛЬНОЕ ИЗУЧЕНИЕ ЗАДЕРЖИВАЮЩЕГО ОТДЕЛЕНИЯ МОЛОКА ДЕЙСТВИЯ ЛИНЕСТРЕНОЛА (ОРГАМЕТРИЛ)

М. КУРЦ, И. НАДЬ, Ч. КИШШ и Л. ХАЛМИ

Авторы проверили в опытах на животных результаты, полученные Дьери и Кишш (1968а и б) относительно аблактировки дачей Оргаметрила.

У крысы при помощи линестренола можно задерживать лактацию. Однако, для аблактировки или торможения отделения молока следует применять многократное количество человеческой дозы. Интенсивность эффекта препарата зависит от величины применяемой дозы и от срока начала дачи оргаметрила. При применении немедленно после родов эффект линестренола намного интенсивнее, чем если введение препарата начинается на второй день лактации. В случае дачи линестренола уже в последние дни беременности и

продолжения его применения после родов, лактация не начинается. С другой стороны, если прекратить применение линестренола во время родов, то лактация не нарушается.

Авторы обращают внимание на разногласные результаты опытов на животных, на важность сосательного стимула, которая исключается при клиническом применении препарата, и обсуждают также возможный механизм действия Оргаметрила.

ИЗУЧЕНИЕ ЭФФЕКТА СЕРИИ КРИТИЧЕСКИХ ЧАСТОТ СЛИЯНИЯ У ЭПИЛЕПТИКОВ И НЕВРОТИКОВ

Й. САК, М. АКОШ и К. АКОШ

Исследования проводились на группе 17 эпилептиков и на группе 18 невротиков (женщины и мужчины в возрасте от 17 до 35 лет). У больных три дня подряд были по три раза в день сняты непрерывные серии из 50 данных критических частот слияния и интермиттирующие серии из 50 данных критических частот слияния. Затем полученные [31 200] данные были разработаны в компутере. Из отдельных серий были выделены 10 групп по 5 максимальных данных и 10 групп по 5 минимальных данных. Средние величины 20 выделенных групп были сопоставлены друг с другом, и изучались их изменения, наблюдаемые в двух группах больных в течение трех дней и трех частей суток. Оказалось, что в группе эпилептиков в отношении всех средних величин наблюдается уменьшение способности к дифференциации от первого до третьего дня, а в группе невротиков отмечается повышение этой способности.

Авторы еще не могут высказать мнения о том, следует ли приписать эту разницу процессу учения, утомления или прочим явлениям. Приходится еще продолжать исследования и в распределении по возрастным группам, в целях уяснения вопроса о том, можно ли рассматривать эту разницу реальной с точки зрения дифференциальной диагностики. Необходимо также принимать во внимание факторы, подробно обсуждаемые в цитированной монографии и относящиеся к анализу эффекта серии критических частот слияния.

СОДЕРЖАНИЕ ДЕГИДРОЭПИАНДРОСТЕРОНА, АНДРОСТЕРОНА И КОРТИЗОЛА В ПЛАЗМЕ КРОВИ БОЛЬНЫХ С ОСТЕОПОРОЗОМ ПОСЛЕ ПРЕКРАЩЕНИЯ МЕСЯЧНЫХ И С ПЕРВИЧНЫМ ОСТЕОПОРОЗОМ ДРУГОГО ТИПА

И. ХОЛЛО, Т. ФЕХЕР и Я. СЮЧ

В плазме больных с остеопорозом в период после менопаузы содержание дегидроэпиандростерона достоверно понижено по сравнению с женщинами подобного возраста без остеопороза. Содержание *Hc* также ниже в плазме больных с порозом, однако это понижение значительно меньше понижения содержания дегидроепиандростерона. На основе полученных результатов авторы выдвигают положение о возможности наличия причинной связи между остеопорозом после менопаузы и пониженной секреции андрогенов корой надпочечников.

ИССЛЕДОВАНИЕ КАЛЬЦИТОНИНО-ПОДОБНОГО ДЕЙСТВИЯ ЧЕЛОВЕЧЕСКОЙ ПЛАЗМЫ КРОВИ. I. НЕКОТОРЫЕ БИОЛОГИЧЕСКИЕ СВОЙСТВА КАЛЬЦИТОНИНА В ВЫТЯЖКЕ ПЛАЗМЫ, ВЫЗЫВАЮЩЕМ ГИПОКАЛЬЦИЕМИЮ

И. ХОЛЛО и Й. ЗОМБОРИ

Исследования авторов указывают на то, что вытяжка плазмы, полученная методом Стутриджа и сотрудников, в отношении изучаемых параметров оказывает кальцитонино-подобное действие. Анорганический фосфат усиливает действие вытяжки, и ее вызывающее гипокальциемию влияние зависит от возраста тест-животных. Форма кривой доза/действие двух веществ также очень подобна. Эксперименты авторов предоставляют новые доказательства того положения, что в вытяжке плазмы, полученной указанным методом, действующим веществом является кальцитонин. Авторы определяли также методику, при помощи которой можно оптимально использовать $Na\ H_2\ PO_2$ для повышения чувствительности биологического титрования вытяжки плазмы.

ИССЛЕДОВАНИЕ КАЛЬЦИТОНИНО-ПОДОБНОГО ДЕЙСТВИЯ ЧЕЛОВЕЧЕСКОЙ ПЛАЗМЫ КРОВИ. II. АКТИВНОСТЬ КАЛЬЦИТОНИНА ПЛАЗМЫ У 18 ЛИЦ С НОРМАЛЬНЫМ ОБМЕНОМ КАЛЬЦИЯ

И. ХОЛЛО и Й. ЗОМБОРИ

Авторы изучали кальцитонино-подобную активность вытяжки плазмы, полученной методом Стутриджа и сотрудников, на основе действия, оказанного на уровень кальция в крови тест-крыс. Они установили, что плазма всех 18 исследовавшихся лиц обладала кальцитонино-подобным действием. Из этого результата авторы сделают вывод, что кальцитонин участвует в поддержании нормального обмена кальция и обмена веществ костей.

ЛИПОИДНЫЙ ОБМЕН ЛЕГКИХ И ЕГО ИЗМЕНЕНИЯ ПРИ ЭКСПЕРИМЕНТАЛЬНОЙ ЖИРОВОЙ ЭМБОЛИИ

Э. ВАНДОР и Е. АНДА

Липоидный обмен легких и его изменения при жировой эмболии были изучены на собаках. В вытекающей артериальной крови активность липопротеиновой липазы была более высокой, чем в втекающей венозной крови. После эмболизации артерио-венозная разность показала тенденцию к дальнейшему повышению. У животных контрольной группы уровень активности сывороточной липазы в течение экспериментального периода не показал изменения. Изменение после введения триолеина не было достоверным. В этом незначительном повышении активности легкие не могли играть роли.

Уровень свободных жирных кислот непрерывно повышался, как в контрольной, так и в подопытной группах. В контрольной группе последовательно появляющаяся, но статистически недостоверная артерио-венозная разность после введения триолеина исчезла.

После введения меченого J^{131} триолеина в радиоактивности липоидной фракции крови была выявлена артерио-венозная разность, что указывает на потребление триолеина легкими. Общий уровень жирных кислот был в группе эмболизированных животных достоверно выше, чем в контрольной группе. Ввиду того что такое сильное повышение не могло произойти за счет свободных жирных кислот и триолеина, то обще повышение уровня жирных кислот обуславливается прочими источниками липоидов.

Согласно результатам исследований, при нагрузке обмена жира легких наблюдаются специфические и неспецифические изменения. К первой категории относятся эндогенная активность липопротеиновой липазы и метаболизм свободных жирных кислот легких. К второй группе относится повышение общего уровня жирных кислот в плазме

ИЗМЕНЕНИЯ КОРРЕЛЯЦИИ ЩИТОВИДНАЯ ЖЕЛЕЗА-ЗОБНАЯ ЖЕЛЕЗА ПОД ВЛИЯНИЕМ ШИШКОВИДНОЙ ЖЕЛЕЗЫ. ПОГЛОЩЕНИЕ J^{131} ЗОБНОЙ И СЛЮННЫМИ ЖЕЛЕЗАМИ КРЫС

Д. ЧАБА, И. РЕТИ и Я. ФИШЕР

Авторы изучали действие удаления шишковидной и щитовидной желез и введения TSH, на поглощение радиоактивного йода зобной железой и подщелюстной железой и анализировали математическим методом корреляции, наблюдаемые между этими органами, используя в качестве индикатора J^{131}.

Они устанавливают, что зобная железа поглощает примерно в полтора раза больше йода, чем слюнная железа, и что это меняется в зависимости от вышеуказанных вмешательств. Удаление шишковидной железы повышает поглощение йода щитовидной железой, и оно, так же как и тиреоидектомия уменьшает поглощение йода зобной железой. Введение TSH не влияет на поглощение йода зобной железой, однако, после удаления эпифиза или щитовидной железы в значительной мере повышают его. Авторы устанавливают, что TSH является первичным, и шишковидная железа вторичным регулятором обмена йода щитовидной железы, а в случае зобной и слюнной желез наблюдается обратное положение. Обсуждаются предположительные механизмы этих эффектов, и они проводятся в связь с прочими корреляциями между бранхиогенными органами, а также взаимоотношениями между последними.

СТОЙКАЯ ГИПЕРТОНИЯ, ВЫЗВАННАЯ ЛИГИРОВАНИЕМ ПОЧЕЧНОЙ АРТЕРИИ У СОБАКИ

А. ФЕКЕТЕ

У собаки после лигирования левой почечной артерии при наличии невредимой правой почки наблюдалось развитие стойкой гипертонии, что привело к понижению функции почек и по истечении 420 дней к спонтанной гибели животных.

По истечении определенного времени удаление лигированной почки больше не приводит к нормализации гипертонии. Однако, если ишемизированная почка остается длительное время в организме, то время выживаемости животных сокращается.

Гипертония, возникающая у собаки после одностороннего лигирования почечной артерии при наличии невредимой другой почки, развивается по всей вероятности путем ренинового механизма. Гипертензивное действие ишемизированной почки распространяется также на несжатую, интактную почку, вызывая в последней некротизирующий артериолит. Вследствие прогрессирующей и необратимой дегенерации сосудов «интактная» почка поддерживает состояние хронической гипертонии, что может привести даже к изменению параметров почечной функции. Наблюдаемые на модели авторов изменения, нарушающие также функцию почек, можно ставить в аналогию с злокачественной гипертонией человека.

RUBEOSIS IRIDIS

Д. ИМРЕ

У 15 из 18 глаз с iris rubeosis минутный объем жидкости камеры был выраженно пониженным. Средняя величина F вместо нормальной величины в 2,3 мм³ в мин. была лишь 0,66 мм³ в мин. Следовательно при iris rubeosis нарушается секреция расничного тела и вследствие уменьшения тока жидкости камеры в последней повышается концентрация фактора васкуляризации. При закупорке сосудов глазного дна определение величины F имеет прогностическое значение.

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THIRD INTERNATIONAL MEETING OF THE INTERNATIONAL SOCIETY FOR NEUROCHEMISTRY

Budapest, Hungary, 5—9 July, 1971

The Meeting is open to members of the Society and all other persons interested in neurochemistry.
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3. Biochemistry of myelin.
4. Biochemistry of neuronal membranes.

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PRESS RELEASE

Congress on Congenital Malformations in Austria

Sponsored by the well-know "National Foundation — March of Dimes" of America the "Fourth International Conference on Congenital Malformations" will be held in the Hofburg Congress Centre, Vienna, Austria from 2 to 8 September 1973.

It is expected that this congres, which sofar has met with considederable interest throughout the world, will be attended by 1000 physicians, surgeons, geneticists and other specialists.

The previous conference was held in 1969 in the Hague, the Netherlands, and attracted 750 participants.

For all further information, please apply to the Congress secretariat, c/o Holland Organizing Centre, 16, Lange Voorhout, The Hague, the Netherlands.

The Hague. 8th July, 1970.

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HAEMODYNAMIC INVESTIGATIONS ON THE SKIN IN POLYCYTHAEMIA VERA

I. EFFECT OF EXPOSURE TO COLD AND OF VASODILATING STIMULI

By

Gy. NAGY, P. DARÓCZY and Cs. BALÁZS

FIRST DEPARTMENT OF MEDICINE AND DEPARTMENT OF DERMATOLOGY, UNIVERSITY MEDICAL SCHOOL, DEBRECEN

(Received January 19, 1969)

Haemodynamic changes involving the entire vascular system of the skin have been demonstrated in patients with polycythaemia vera, irrespective of the process being in a stage of exacerbation or remission.

In a previous paper we reported on capillary microscopic studies in polycythaemia vera [3] which showed definite capillary changes associated with the exacerbations of the disease; the changes were subsiding parallel with the spontaneous or therapeutic remissions. In view of the changes in the morphology of the peripheral vascular bed as well as in the haemodynamics it seemed of interest to examine the response to vaso-active stimuli in clinical cases of polycythaemia vera.

Material and methods

In these studies, 38 patients with polycythaemia vera (21 males, 17 females, of a mean age of 53.2 years, from 25 to 74) were involved. Haemodynamics of the skin were studied in 28 patients on one occasion, in 6 patients on two occasions, in 3 on three occasions and in one on four occasions. Our findings are thus based on the results of 53 tests, 38 of which were performed during exacerbation and 15 during remission due to successful treatment. RBC averaged 6.250,000, the haematocrit 56.5% during exacerbations and 4.530,000, and 41.8%, respectively, during remissions.

In accordance with the generally accepted criteria of polycythaemia vera [2, 5, 6] confirmed by our observations [11, 13], the diagnosis was based on the following evidence.

Polyglobulia: RBC over 5.500,000 per ml; haematocrit over 50 to 52%; haemoglobin over 110%.

Hyperplastic, hypercellular bone marrow with increased erythro-, myelo and thrombocytopoiesis (megakaryocytosis), and prevalence of erythropoiesis.

Exclusion of all possible sources (diseases, abnormal conditions) of secondary polyglobulism.

For comparative purposes, measurements were carried out in 60 subjects serving as controls (35 males and 25 females, with an average age of 39 and 44 years, respectively), with the diagnoses pyoderma in 27, trychophytia in 2, basal cell carcinoma in 6, herpes zoster in 1 and no evidence of disease in 24 subjects.

The patients as well as the controls were studied at 22 °C. After accommodation to this temperature, velocity of blood flow was examined on the nail folds of the right upper extremity. For exposure to cold the forearm and hand were immersed in water of 15 °C for five min. After the procedure, the time required by acrocyanosis to vanish altogether was registered. The nicotinic acid was administered by mouth and the time of appearance of flush as well as its duration were registered. The question how the capillaries of the skin were affected by the

hyperaemia thus produced was examined on the nail folds and in the middle third of the inner surface of the right forearm. When hyperaemia had vanished, NV ointment,* in the volume of a pea, was rubbed into the skin of the flexor surface of the forearm and the time of appearance and disappearance of the consecutive erythema were noted (application of the ointment was followed by the dilatation of the precapillary arterioles).

Results

According to our earlier findings, capillary flow rate is considerably reduced in the majority of subjects with polycythaemia vera, as compared to the controls [3]. Exposure to cold resulted in a further reduction of the flow.

Exposure to cold was followed by slight acrocyanosis vanishing slowly in polycythaemia vera, its time of disappearance being $55' \pm 3''$ in the subjects with this disease, as against $15'20'' \pm 12''$ in the controls.

In non polycythaemic subjects, ingestion of nicotinic acid was followed after a short interval averaging $43'' \pm 16''$ by slight erythema accompanied by a burning, itching sensation. In the subjects with polycythaemia vera this interval was longer, i. e. $4'29'' \pm 17''$.

The capillaries of the skin in polycythaemia vera showed no response whatsoever to nicotinic acid; dilation of the precapillary arterioles and the postcapillary venules as compared to their initial condition was of slighter extent than in the controls.

Hyperaemia of the skin in response to nicotinic acid has been found to disappear more rapidly in the controls than in the subjects with polycythaemia vera, the mean values being $13'17'' \pm 20''$ and $25'18'' \pm 23''$, respectively.

Application of NV to the skin of healthy subjects was followed after an average interval of $4'22'' \pm 19''$ by intensive cutaneous erythema with itching and burning, vanishing spontaneously in $1^h24' \pm 34'$. In the polycythaemic subjects the average time of appearance of the erythema was $12'20'' \pm 18$ and its average time of disappearance as long as $7^h19' \pm 32'$.

The abnormal responses were noted in the stage of exacerbation or remission of polycythaemia alike, in other words they failed to exhibit any appreciable relationship with the stage of the disease.

Table I sums up the results.

The difference between the mean vascular reaction times of the polycythaemic and the control subjects was in all cases within the significance limit of 1% ($p < 0.01$).

* Nonylic acid vanillylamide, 0.5% nicotinic acid beta-butoxyethylester, 2.5%.

Table I

Response of the vascular system of the skin to exposure to cold and to vasodilating stimuli, in patients with polycythaemia vera

Diagnosis	Number of patients	Number of tests	Time required for disappearance of cyanosis	Appearance of nicotinic acid erythema	Disappearance of nicotinic acid erythema	Appearance of N—V erythema	Disappearance of N—V erythema
Polycythaemia vera	38	53	15′ 20″ ± 12″	4′ 20″ ± 17″	25′ 18″ ± 23″	12′ 20″ ± 18″	7h 19′ ± 32′
Control	60	60	4′ 55″ ± 9″	43″ ± 16″	13′ 17″ ± 20″	4′ 22″ ± 19″	1h 24′ ± 34′

Discussion

Capillary microscopy lends itself to the study of the structural and functional properties of the precapillary arterioles, the capillaries and the postcapillary veins. Abnormalities revealed in this part of the vascular bed are characteristic of the entire vascular system of the body.

Polycythaemia vera is characterized by high erythrocyte, haematocrit and haemoglobin values as well as an increase in circulating blood volume and a high blood viscosity [1, 2, 16] which obviously modify the haemodynamic conditions. The increase in circulating blood volume and viscosity are alone sufficient to account for a reduction in flow rate. Long-standing polyglobulia is bound to result in circulatory failure and to lead in this manner to a further deterioration of the haemodynamic conditions [11]. All these changes, together with the impaired oxygenation of blood [14], if they persist for longer periods, will not fail to affect the vascular wall as confirmed by studies of the eye-grounds [12, 17].

In literature there is scanty evidence relating to the capillary microscopic features of polycythaemia vera.

Lavskij [9] demonstrated signs of a relative vascular proliferation and dilatation in the stage of exacerbation which were found to subside for the greatest part during remissions. Davis and Landau [4] found distinct capillary dilatation and an increase in the number of capillaries of the nail folds while Harders [7] could demonstrate similar abnormalities of the capillaries of the tongue in subjects suffering from polycythaemia.

The capillary microscopic pattern demonstrable during exacerbations and subject to partial regression during remissions consists in a distinct dilatation and characteristic proliferation of the capillaries and a reduction in the flow rate. Moreover, in the precapillaries the central cellular flow and the mar-

ginal plasma flow, clearly discernible under normal conditions, merge into each other in polycythaemia vera.

According to the present findings, the responses of the cutaneous vessels to local or systemic vasoactive stimuli are marked by an excessive sluggishness. This may account for some common signs of the disease, such as the acrocyanosis, the hypersensitivity to cold, and the itching [10, 11]. At the same time, these changes are indicative of the vascular system of the skin.

While the capillary microscopic abnormalities revealed by our earlier studies [3] were closely associated with the exacerbations of the process and were partially or totaly absent during remissions, the haemodynamic changes which thus seem to affect the entire vasculature of the skin are demonstrable during exacerbations and remissions alike.

For a closer study of the microcirculatory and thermoregulatory changes in polycythaemia vera, the use of sympathomimetic and parasympathomimetic drugs offers a promising avenue of approach. Studies along these lines are in progress and will form the subject of a later report.

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Dr. György Nagy
Dr. Pál Daróczy
Dr. Csaba Balázs

Orvostudományi Egyetem I. Belgyógyászati klinika, Debrecen, Hungary

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EXCITABILITY AND CONDUCTIVITY OF HUMAN PERIPHERAL NERVE IN HEXOBARBITAL SLEEP

By

F. MECHLER, F. KAJTOR and J. HIDASI

DEPARTMENT OF NEUROLOGY AND PSYCHIATRY, UNIVERSITY MEDICAL SCHOOL, DEBRECEN

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In 20 subjects with no evidence of neuromuscular abnormality, the excitability (stimulus threshold), conduction velocity and refractory period of the motor fibres of the ulnar nerve under the effect of hexobarbital-Na administered by the intravenous route have been studied. Stimulus threshold, distal latency period and conduction velocity have been found to vary with the concentration levels of hexobarbital. The results and the possible mechanisms accounting for the findings are discussed in the light of the literary data.

After the classical studies of HELMHOLTZ [1], HODES et al. [2] were the first to measure the velocity of motor conduction in man. Since then the method is widely used in electrophysiology as well as in clinical pathology.

The action of the hypnotics, in the first line of barbiturates, on the central nervous system has been widely explored. However, their effect on the human peripheral nerves has received little attention. In 1932 BISHOP [3] advanced the claim that anaesthetics produced nervous block by reducing neuronal excitability. HEINBECKER and BARTLEY [4] then showed that pentobarbital and ether produced an elevation in the stimulus threshold of the frog nerve while prolonging the refractory period and reducing the conduction velocity. LARRABEE et al. [5, 6] found that pentobarbital-Na was a ten times stronger depressor of synaptic transmisson than of the conductivity of the praeganglionic axon in cat. In contrast, ethanol proved to be a more potent depressant of axonal than of synaptic conductivity. LOW et al. [7] studied the effect of ethanol on human peripheral nerve and found that while motor conductivity was not significantly affected, there was a reduction in residual latency. According to GOODMAN and GILMAN [8], conduction velocity decreases in nerves exposed to the direct effect of anaesthetics. PIHKANEN et al. [9] found that under combined barbiturate + phenothiazine poisoning, particularly in hypo- or hyperthermia, conduction velocity in the ulnar nerve was significantly reduced as compared to the rate observed after recovery. As regards hexobarbital-Na, data on its action on human peripheral nerves are scanty and on its selective effects, non-existent. The present study has been concerned with the question how the substance affects the excitability and conduction velocity of the human peripheral nerve.

Material and method

The study involved 20 subjects (16 males, 4 females), ranging in age from 12 to 50 years (mean 25 years), free from any sign or symptom related to the limbs or from any evidence of neurologic abnormality. All but one had been abstaining from drugs for some time. The measurements were carried out on the ulnar nerve which was stimulated with bipolar surface electrodes 20 mm apart, fixed to the region of the elbow and the wrist flexibly but firmly so as to prevent displacement. Muscle action potentials were picked up by the aid of DISA-type bipolar plate electrodes placed 20 mm apart on the hypothenar musculature (m. flexor and abductor digiti minimi) and registered with a Galileo EMG apparatus, at 5 c/sec and 30 kc/sec filter position, 100 cm/sec (occasionally 200 cm/sec) beam velocity and 100 μV/mm amplification. The stimuli were delivered through a DISA Multistim stimulator, with 2 to 50 V, 2 cm/sec frequency, 0.7msec impulse width. Room temperature, 23 to 25 °C. First the weakest stimulus eliciting a response had been determined; subsequently, the intensity was gradually raised and the increase in response potentials was registered. Finally, supramaximal stimuli were applied. On repeated stimulations the gradual increments in stimulus intensity were invariably the same in the same person. In calculating the conduction velocity the difference in the latencies of supramaximal stimuli at the elbow and wrist was determined and the elbow—wrist distance was divided by the difference in stimulation latencies measured at these two points. Conduction velocity was expressed in terms of m/sec. Latency corresponds to the interval between the time of stimulation and the appearance of the initial point of the elicited muscle potential; it was expressed in msec. The latency figures shown represent the means of 5 to 10 estimations; their accuracy was in the order of ± 0.1 msec. Distal latency time means the latency at stimulation at the wrist (10). Control stimulations were performed in the alert resting state, then the stimulations were applied at 1 to 2 minute intervals during slow intravenous administration (25 mg/15 sec) of hexobarbital and at slightly longer intervals after the injection had been completed. Hexobarbital was administered until the phase of deep anaesthesia characterized by myosis and the absence of a reaction to painful stimuli. The figures shown represent the mean from estimations in 20 subjects.

Double-impulse stimulation of the motor fibres of the ulnar nerve for the measurement of the refractory period was performed in the alert state and during hexobarbital sleep in 3 cases.

Results

Mean conduction velocity in the alert state (control values) was unaffected during the first three minutes of the hexobarbital effect; subsequently it decreased until the end of the injection (4 to 9 minutes; $p < 0.01$, $s = 2.1$). This was followed by an increasing tendency, and for 20 to 25 minutes after completion of the injection the mean conduction velocity slightly exceeded that found in the alert state ($p < 0.01$, $s = 1.9$), to return subsequently to normal values (Fig. 1).

Hexobarbital was found to affect nerve excitability more conspicuously than its motor conduction velocity. The changes in the potential amplitudes under the effect of periliminal stimuli to the proximal and distal part of the ulnar nerve were analyzed separately. A significant reduction in comparison to the control values reflecting an elevation of the stimulus threshold under the effect of hexobarbital was found in both cases (Figs 2, 3). On plotting the mean values resulting from proximal stimulation it was found that excitability fell by approximately 50% during the first two minutes; this reduction persisted with slight oscillations until the end of the 4th or 5th minute after

completion of the injection; this was followed by a transitory increase in excitability to the control level for another 5 minutes; a subsequent reduction for the 22nd minute was then followed by a rapid tendency to normalization (Fig. 4). The pattern of response to distal stimulation was similar but the reduction in excitability was even more marked, culminating in the 4th minute after hexobarbital administration and showing a more protracted increase for the 12th minute than in the former case without, however, attaining the control values. As sleep tended to become more superficial,

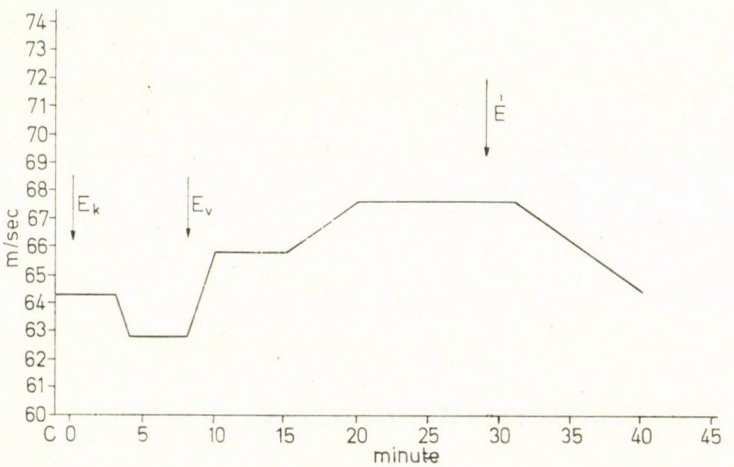

Fig. 1. Mean motor conduction velocity of ulnar nerve during hexobarbital sleep, in m/sec. C = alert control. E_k = start, E_v = completion of hexobarbital administration. É = first signs of awakening

excitability increased again and from the 27th minute onward, in the phase of recovery, excitability increased to a degree exceeding the control values (Fig. 5). Mean distal latency (Fig. 6) showed a slight reduction during hexobarbital administration (1 to 8 minutes) which yielded to a relative increase between the 2nd and 7th minute after injection, correlating on the whole with the reduction in distal excitability registered during this period. The subsequent phases, that of superficial sleep and recovery, was marked by a distinct reduction in distal latency ($p < 0.01$, $s = 0.2$) which on its part also correlated with the increased rate of nerve impulse propagation registered during this period (Fig. 1).

The behaviour of the absolute and relative refractory periods (ARP and RRP) at the peak of the hexobarbital effect, as compared with the alert state, was estimated in three subjects. A consistent increase (by 60%) was noted in the values of ARP as well as of RRP (by 50%) (Fig. 7).

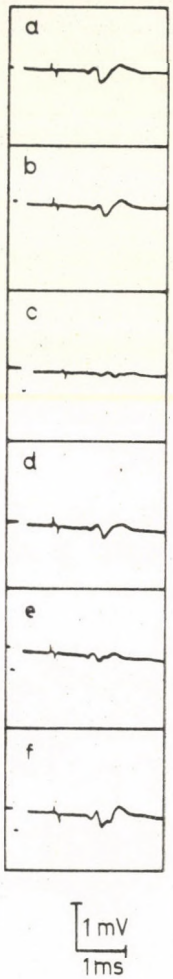

Fig. 2. Excitability of ulnar nerve in response to proximal stimulation with 10 V, 0.7 msec; 100 μV/mm, 100 cm/sec. *a)* alert control, *b)* 2nd minute during hexobarbital injection; *c)* 4th minute during hexobarbital injection; *d)* 6 minutes after completion of hexobarbital injection; *e)* 10 minutes after completion of hexobarbital injection; *f)* 25 minutes after completion of hexobarbital injection

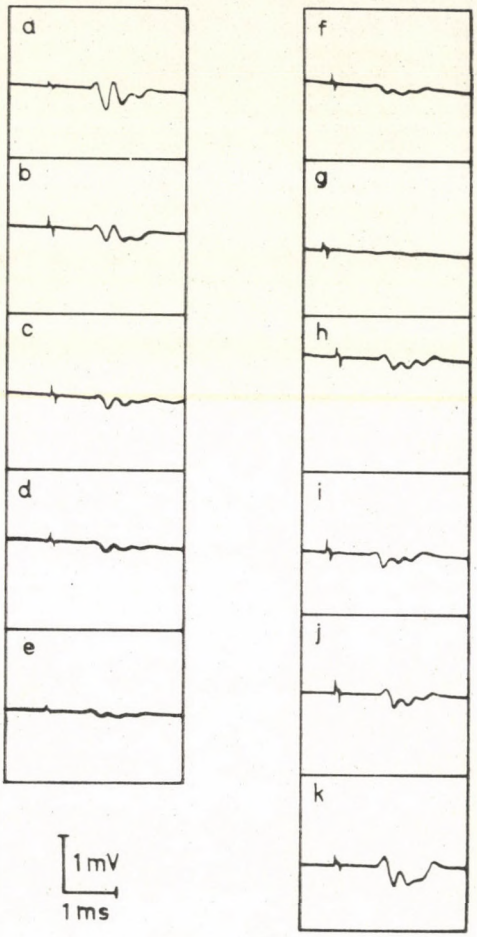

Fig. 3. Excitability of the ulnar nerve in response to distal stimulation with constant parameters (8 V). *a)* alert control, *b)* 2nd; *c)* 3rd; *d)* 4th; *e)* 6th; *f)* 8th minute during hexobarbital injection (completion of the injection); *g)* at 4th; *h)* 6th; *i)* 8th; *j)* 10th; *k)* 25th minute after completion of hexobarbital injection

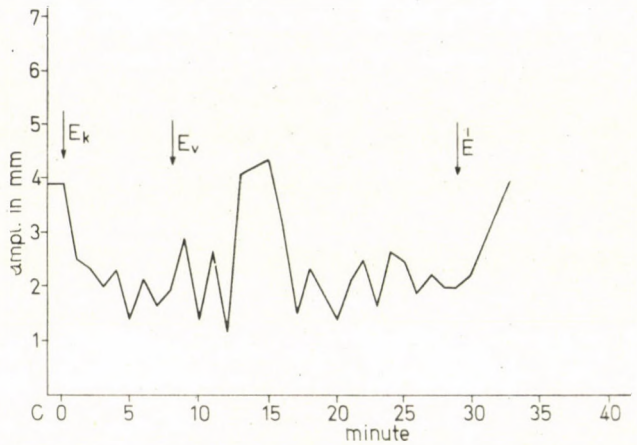

Fig. 4. Mean ulnar nerve excitability in response to proximal stimulation (elbow) under the effect of hexobarbital. The ordinate represents the action potentials amplitudes in mm, induced by periliminal stimuli with the same parameters. Other symbols as in Fig. 1

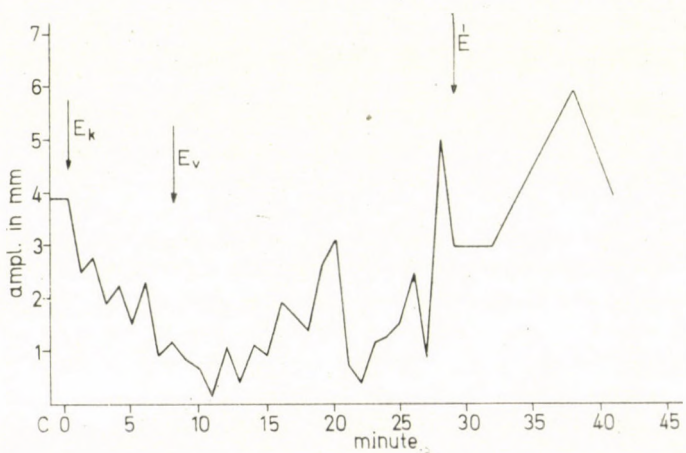

Fig. 5. Mean ulnar nerve excitability in response to distal stimulation (wrist) under the effect of hexobarbital. Symbols as in Fig. 4

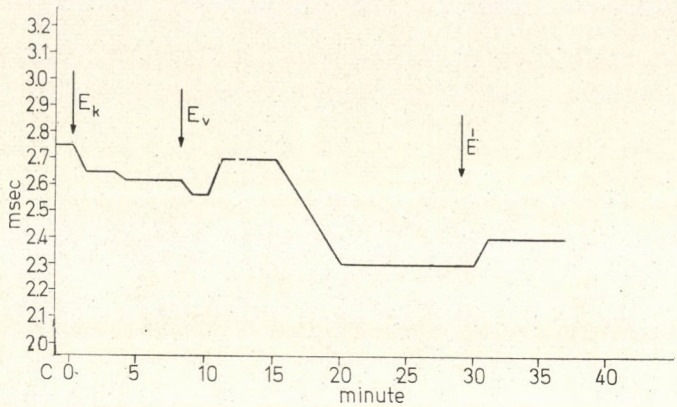

Fig. 6. Mean latency response to stimulation of the distal end of the ulnar nerve during hexobarbital sleep. The ordinate represents the distal latency periods in terms of msec. Other symbols as in Fig. 1

Case	ARPmsec		RRPmsec	
	Control	Hexobarbital	Control	Hexobarbital
D. S.	1.0	1.6	3.0	4.0
Sz. J.	0.8	1.6	3.0	5.0
D. J.	1.2	1.6	2.7	4.0
Mean:	1.0	1.6	2.9	4.3

Fig. 7. Refractory period of the motor fibres of the ulnar nerve (in msec.) under the effect of hexobarbital referred to the alert control state. ARP = absolute refractory period; RRP = relative refractory period

Discussion

The depressant action of barbiturates on synaptic transmission in the mono- and multisynaptic reflex arcs has been revealed by the studies of Brooks and Eccles [11] and of Wikler [12]. The blocking effect of massive doses of pentobarbital on peripheral nerve conduction was demonstrated by Toman [13]. Experimental evidence connecting this depressant effect with an increase in the electric resistance of the cell membrane (reduced permeability, increased stability) was produced by Eccles [14, 15].

The present results agree in certain respects with those obtained by hexobarbital in laboratory animals, major doses having been found to elevate the stimulus threshold; in other words, to reduce the excitability and to prolong the refractory period of the human ulnar nerve. Excitability of the nerve measured at its proximal and distal ends was consistent as regards the tendency of the changes, not their extent. The changes in question appeared in the

first minute, culminating 2 to 3 minutes after completion of the injection (0.6 to 1.0 g of hexobarbital). Response of the distal end (including the neuromuscular junction) was somewhat prompter and more intense than that of the proximal, mainly axonal, section, but the difference was slighter than that reported by LARRABEE et al. [5] on the grounds of pentobarbital studies on sympathetic ganglia of cats. This not only indicates that there is no complete analogy between the neuromuscular junction and the synapses of the sympathetic ganglion in respect of nerve impulse transmission, but also that the effects of pentobarbital and hexobarbital might also differ to some degree. We cannot, however, account for the rebound phenomenon, the relative reduction in the stimulus threshold at 5 to 12 minutes after completion of the injection, a reaction more transitory and more distinct at the proximal (axonal) portion of the nerve than at its distal end including the neuromuscular junction. This coincides with the phase when deep barbiturate sleep tends to become superficial, and presumably involves special changes in membrane permeability.

As regards the refractory periods, our results agree with the published evidence relating to the effects of pentobarbital in laboratory animals. The possible influence of temperature may be disregarded since in the present study the changes were registered in the same individual at identical room temperatures. The behaviour of the latency periods at the distal end of the nerve may be attributed particularly to the effect of hexobarbital on the neuromuscular junction. Distal latency comprises the following factors: 1. velocity of impulse passage along the distal part of the nerve trunk and the fine terminal nerve fibres; 2. velocity of neuromuscular transmission (synaptic delay); 3. velocity of passage of muscle action potential from the end plate to the point of recording. On closer analysis of the distal latency period under the effect of hexobarbital (Fig. 6) it was found that the maximum reduction in this time interval was associated with those concentrations of hexobarbital which are found in the phase of superficial sleep and recovery (mild effect). Parallel with the reduction in distal latency demonstrable in this phase a relative increase in the excitability of the distal portion has been noted (Fig. 5); a finding for which the increase in the rate of impulse transmission demonstrable in this phase fails to account in all respects (Fig. 1) unless we presume the existence of a factor acting on neuromuscular transmission. Thus, hexobarbital at certain concentrations slightly accelerates this transmission instead of slowing it. This is consistent with the findings of LOW et al. [7] that ethanol reduces the latency measured in the distal portion of the ulnar nerve; in other words, it facilitates neuromuscular transmission, an effect ascribed in the first place to the antiesterase activity of alcohol. It is difficult to find an adequate explanation for the action of this type exhibited by hexobarbital, unless the concentration at which the effect takes place cannot yet shift the membrane depolariza-

tion in the direction of stability. This might in fact account for the behaviour of conduction velocity during hexobarbital sleep (Fig. 1). As the concentration of hexobarbital increases, the conduction velocity of the most rapidly conducting motor fibres diminishes, and, after the administration of hexobarbital has been discontinued, during the phase of superficial sleep the values even exceed the control figures to which they return after complete recovery.

Although the fact that stimulation was done across the skin warrants caution in the interpretation of the figures, the results seemed to indicate that the different concentration levels of hexobarbital-Na involve different kinds of action on the membrane structure of the neurone (axon, neuromuscular junction).

Another possible interpretation of the barbiturate effects would be the activity on cellular metabolism. QUASTEL et al. [16, 17] found that barbiturates inhibit O_2 consumption by brain tissue in vitro. According to KETY [18] pentothal suppresses neuronal activity thus reducing the O_2 demand of the neurones during the inactive phase. LORENTE DE NÓ [19] questioned the exclusive role attributed to abnormal oxidation processes, an opinion shared by LARRABEE et al. [20]. The studies of DOTY and GERARD [21] also indicate that the impairment of nervous metabolism by anaesthetics need not affect O_2 consumption. Other authors emphasize the possible significance of neuroenzymatic processes. An involvement of the mitochondrial enzyme system has been suggested by BRINK et al. [22] as well as DAVIES and KREBS [23]. However, changes affecting the cell membrane as well as cell metabolism may well join in the mechanism responsible for the effects of hexobarbital on peripheral nerves.

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Dr. Ferenc MECHLER
Dr. Ferenc KAJTOR
Dr. József HIDASI

Orvostudományi Egyetem Ideg- és Elmeklinika, Debrecen, Hungary

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EFFECT OF SYNTHETIC ACTH ON ADRENOCORTICAL FUNCTION IN MAN AND IN THE RAT

By

F. A. László, K. Kovács, I. Faredin, L. Czakó,
F. Durszt, I. Szijj, I. Tóth, A. Biró and M. Julesz

FIRST DEPARTMENT OF MEDICINE, UNIVERSITY MEDICAL SCHOOL, SZEGED

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The biological activity of human ACTH synthetized by Bajusz et al. has been confirmed by the present study in rats as well as in human subjects, the hormone having displayed a significant stimulating activity on the adrenal cortex. No side-effects were observed after its administration, even to patients having proved hypersensitive to natural ACTH preparations.

After Smith in 1926 had shown that the adrenal cortex is under the control of a substance secreted by the pituitary gland [27], extensive research was directed at the purification of ACTH and at the identification of its structure. For human ACTH this was successfully achieved by Lee et al. [18] in 1961. The hormone consists of 39 amino acids, the amino acid sequence differing only slightly from those of porcine, bovine, or ovine ACTH which contain the same number of amino acids. Exploration of the structure of this hormone went hand in hand with attempts at its synthesis. After the production of various short-chain polypeptides, Bajusz et al. accomplished the synthesis of human ACTH in 1967 [1, 2]. The amino acid sequence of the synthetic hormone corresponds to that established by Lee et al. [18]. The pharmacological studies relating to the hormone were performed by Szporny et al. [29]. The present research team had been entrusted with the quantitative evaluation of its biological activity and with the study of its clinical effects. The results of these studies were published earlier in the form of brief preliminary notes [14, 15]. The present report gives a more detailed account of these studies.

Experimental part

I. Animal experiments

The biological activity of synthetic human ACTH was first studied in laboratory animals, using R Amsterdam albino rats of either sex and 170 to 200 g body weight; the diet was standard rat food.

In agreement with the results of the bioassays of Szporny et al. [30] each mg of the synthetic hormone was taken to correspond to 108 IU. The

Table I

Effect on endocrine organ weight of synthetic human

Group	Number of animals	Initial body weight	Final body weight	Adrenals
I Solvent	10	169±2.7*	175±1.8	19.3±1.7
II Synthetic ACTH	10	168±3.1	171±2.3	28.8±1.5
III Purified porcine ACTH	10	165±3.4	167±2.0	34.0±0.9
		I/II:		$p<0.001$
Probability		I/III:		$p<0.001$
		II/III:		$p<0.01$

Solvent: 0.4 ml s. c. subcutaneously T. I. D. for 4 days
ACTH: 0.4 ml (2.0 IU) T. I. D. for 4 days

* standard error of means

potency of the hormone was compared to that of adequately standardized, highly purified porcine ACTH (Cortrophine, Organon).

The following studies were carried out.

Weight of endocrine organs

ACTH was administered subcutaneously in doses of 2.0 IU T. I. D. at 8-hour intervals for four days; the control group was treated with physiological saline. Approximately 12 hours after the last injection, i. e. on the 5th day of treatment, the animals were killed by ether overdosage, the organs were carefully freed from accessory tissues, weighed without fixation, and, after formalin fixation, subjected to histological study.

Table I shows the results. It can be seen that administration of the synthetic hormone was followed by an enlargement of the adrenals parallel with a reduction in thymus and spleen weight. No increase in thyroid, ovarian or uterine weight was noted, indicating that the hormone had no thyrotrophic or gonadotrophic activity. After the administration of porcine ACTH, enlargement of the adrenals was more marked and the ovaries as well as the uterus were moderately enlarged.

The animals were found to tolerate the treatment without losing weight or exhibiting toxic reactions. Apart from a broadening of the adrenal cortex, histology revealed no significant change.

and of purified porcine ACTH

Thyroid	Ovary	Uterus	Thymus	Spleen	Kidney
		mg/100 g body weight			
9.2±0.6	26.8±1.0	213.9±16.3	124.7±5.7	147.3±3.2	753.4±21.5
11.5±0.6	28.7±1.4	195.5±13.5	93.7±8.3	130.2±3.1	707.5±19.3
11.7±0.7	33.1±1.6	241.4±14.2	114.2±6.3	148.9±2.9	767.8±16.0
$p < 0.02$ $p > 0.01$	$p > 0.05$	$p > 0.05$	$p < 0.01$	$p < 0.01$	$p > 0.05$
$p < 0.02$ $p > 0.01$	$p < 0.01$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
$p > 0.05$	$p > 0.05$	$p < 0.05$ $p > 0.02$	$p > 0.05$	$p < 0.001$	$p < 0.05$ $p > 0.02$

Sacrifice: on 5th day, about 12 hrs after the last injection, by ether overdosage
Weighing: without fixation.

Eosinophil and total leukocyte count

Rats were injected intraperitoneally with 0.5, 1.0 and 2.0 IU human ACTH. The dose of the natural hormone was 1.0 IU. The control group received the solvent. Eosinophil and total leukocyte counts were made from the blood of the tail vein before and 4 hrs after injection.

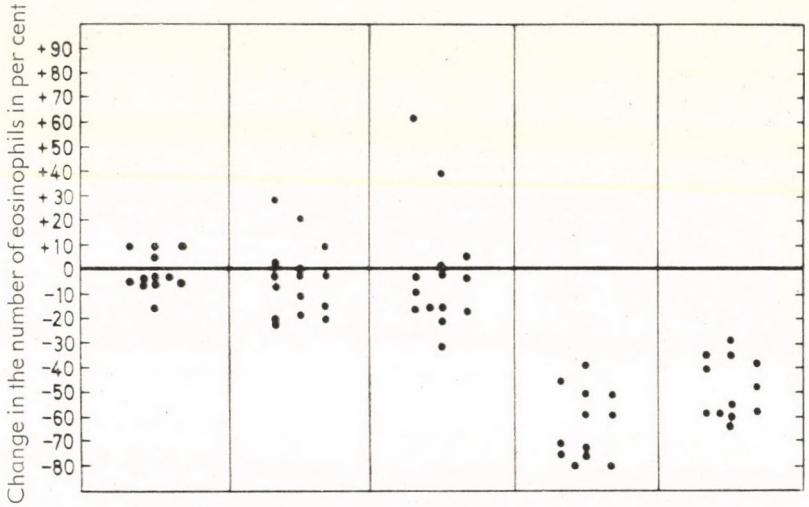
The results are presented in Fig. 1. It can be seen that neither the solvent nor 0.5 and 1.0 IU of the synthetic hormone produced any notable change in the parameters under study. On the other hand, injection of 2 IU resulted in a significant reduction of the eosinophil and leukocyte count, while 1.0 IU of porcine ACTH caused marked eosinophil depletion.

Plasma corticosterone level

The experiments were carried out in rats hypophysectomized from the parapharyngeal approach 2 to 3 hrs earlier and were studied by the method of LIPSCOMB and NELSON [20]. The ACTH preparations were administered in two different doses in a volume of 0.2 ml/100 g body weight; the controls were treated with physiological saline. Thirty minutes after the injection the animals were decapitated, and the plasma corticosterone level was estimated by the method of FRANKEL et al. [9] using an Opton Spectrofluorimeter. Complete removal of the pituitary gland was checked at necropsy.

Adrenal ascorbic acid content

Rats hypophysectomized 2 or 3 hrs earlier were killed, the adrenals were removed, carefully freed from accessory tissue, weighed and their ascorbic acid content was estimated according to ROE and KUETHER [25]. Ascorbic acid depletion induced by ACTH was expressed in per cent of the control value.



Treatment (intraperitoneal)	Solvent 0.2 ml/rat	Human ACTH 0.5 IU (0.2 ml/rat)	Human ACTH 1.0 IU (0.2 ml/rat)	Human ACTH 2.0 IU (0.2 ml/rat)	Porcine ACTH 1.0 IU (0.2 ml/rat)
Number of animals	12	15	14	12	12
Body weight, g	185±2.4*	197±4.4	180±1.4	180±1.6	195±4.9
Eosinophil count 0 hr	337	346	318	414	353
Eosinophil count, 4 hrs	330	327	302	158	181
Change in eosinophil count, %	−1.1	−5.9	−2.9	−62.7	−48.0
Leukocyte count, 0 hr	10 100	10 200	10 600	11 500	10 300
Leukocyte count, 4 hrs	10 300	10 500	10 800	7 800	10 900
Change in leukocyte count, %	+1.9	+2.9	+1.9	−32.2	+5.5

* Standard error of mean

Fig. 1. Leukocyte and eosinophil count in rats treated with synthetic human and purified porcine ACTH

Table II

Effect of synthetic human and of purified porcine ACTH on plasma corticosterone level and adrenal ascorbic acid content in hypophysectomized rats

Group	Treatment	Number of animals	Body weight g	Adrenal weight mg	Plasma corticosterone level μg/100 ml	Vitamin C content of adrenals	
						mg/100 g	change %
I. Control	0.9% NaCl	14	179±3.5*	26.1±0.6	4.9±0.6	530±19.7	—
II. ACTH -treated	0.8 mμ/100 g b.w. human ACTH	14	174±3.2	27.0±1.0	11.9±1.2	445±35.4	−15.9±3.1
III. ACTH -treated	3.2 mμ/100 g b.w. human ACTH	14	175±2.1	27.1±1.0	26.5±3.1	395±24.1	−25.5±4.3
IV. ACTH -treated	0.8 mμ/100 g b.w. porcine ACTH	10	176±3.8	26.7±0.8	15.9±1.7	493±31.2	− 6.9±2.4
V. ACTH -treated	3.2 mμ/100 g b.w. porcine ACTH	10	181±3.5	27.2±1.1	34.1±2.9	387±34.4	−27.0±5.1

* Standard error of mean

Probability

I/II:	$p<0.001$	$p<0.001$	—
I/III:	$p<0.001$	$p<0.001$	—
I/IV:	$p<0.001$	$p>0.05$	—
I/V:	$p<0.001$	$p<0.001$	—
II/IV:	$p>0.05$	$p>0.05$	$p<0.05$
III/V:	$p>0.05$	$p>0.05$	$p>0.05$

Table II shows the results. The plasma corticosterone level was significantly increased by synthetic, and still more so by natural ACTH. On the other hand, the ascorbic acid level was more significantly reduced by human ACTH at least by minor doses.

From Table III it emerges that despite minor differences, the two preparations are similar in their effects. Since the small number of animals the wide individual scatter did not allow calculations on a unit/mg basis, the standard units established by SZPORNY et al. [30] were used for human studies.

II. Studies in humans

The studies were performed in 20 subjects, 10 males and 10 females, of 29 years average age, with no evidence of endocrine or other clinical disease. ACTH was administered in intramuscular doses of 12.5 U T. I. D. at 8-hour intervals for 3 days. Ten subjects were given an additional dose of 6.25 U intravenously on another day. Intracutaneous sensitivity tests were performed in every case with 0.25 U hormone in 0.05 ml fluid and this had to prove negative before starting the treatment. Results were evaluated by Student's *t*-test.

Plasma hydrocortisone level

The plasma cortisol level was determined prior to and at 1, 2, 4 and 6 hrs after the intravenous injection (10 cases), by the method of MATTINGLY [21] as modified by SPENCER-PEET et al. [28], using an Opton-Spectrofluorimeter.

The results seen in Fig. 2 showed that the ACTH induced a significant increase in plasma hydrocortisone level, the initial values having doubled by the end of the 1st and 2nd hrs. The diagram also illustrates the time relations of this effect, showing clearly that by the end of the 4th hr the cortisol level had returned to the initial value and at 6 hr was still further diminished.

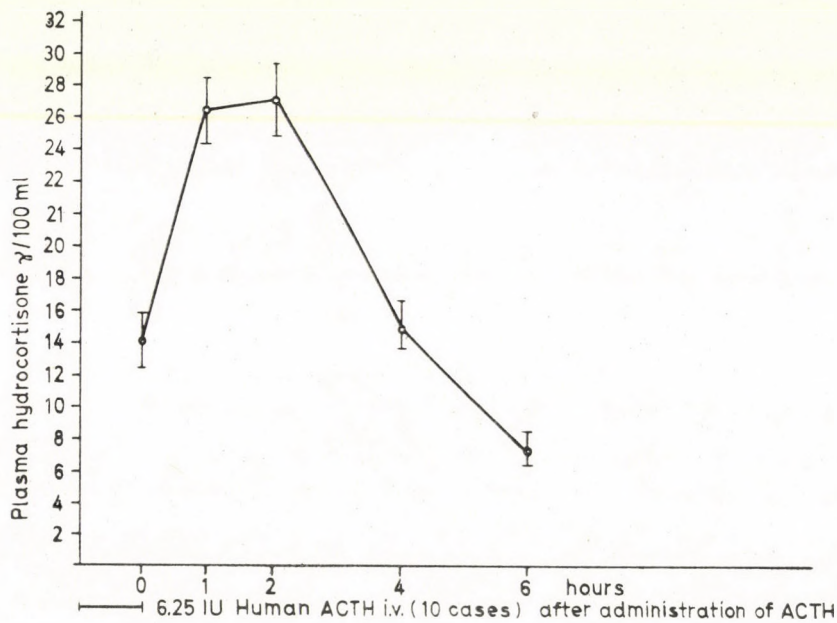


Fig. 2. Effect of synthetic human ACTH on plasma hydrocortisone level

Urinary output of neutral total-17-ketosteroid, total-17 α-hydroxycorticosteroid and pregnanetriol

Urinary total-17-ketosteroid was determined by the method of HOLTORFF and KOCH [12] as modified by FAREDIN et al. [6], total-17α-hydroxycorticosteroid (Porter—Silber-chromogens) and pregnanetriol (5 β-pregnane-3α, 17α, 20α-triol) by the method of FAREDIN and TÓTH [5, 7].

Steroid output was studied during two successive three-day periods, the first prior to the start of, and the second, during treatment. Results were expressed in mg/24 hrs. The reliability of urine collection was checked by 24-hour clearances.

Table III shows that the synthetic ACTH induced a significant increase in urinary total-17-ketosteroid and pregnanetriol excretion. The excretion of total-17α-hydrocorticosteroids displayed the most significant rise; their amounts on the second and third day of treatment corresponded to four to fivefold of the initial average values.

17-ketosteroid fractions

These studies were done in 3 female and 3 male patients. Prior to and during ACTH administration, the 17-ketosteroid fractions were separated by thin-layer chromatography [8]. Of the samples collected during the three-day period of treatment, the one revealing the maximum total ketosteroid excretion was subjected to separation. Table IV clearly shows a 50% elevation of the dehydroepiandrosterone as well as of the androsterone fraction. Etiocholanolone and 11-hydroxy-17-ketosteroid excretion was doubled under the effect of the hormone, especially in the female subjects.

Other studies

The circulating eosinophil count (20 cases) significantly fell after the injection of 12.5 U, from 172 ± 13.3 to 79 ± 7.6 at 4 hrs. Blood pressure, blood sugar, serum cholesterol, sodium, potassium and chloride levels, urinary sodium and creatinine excretion, endogenous creatinine clearance were practically unaffected. On the other hand, an increase in urinary potassium excretion was noted; this amounted prior to treatment (average of 3 days) to 38.6 ± 1.9 mEq/24 hrs, and after it (average of three days) to 48.2 ± 3.4 mEq/24 hrs $\left(P \begin{matrix} < 0.05 \\ > 0.02 \end{matrix} \right)$.

Tolerance to the hormone was satisfactory in all the 20 subjects. No secondary effects, signs of toxicity or hypersensitivity reactions were noted.

Intracutaneous sensitivity reaction after various ACTH preparations

Intracutaneous tests were carried out in 7 subjects who had proved hypersensitive to other ACTH preparations. The test, details of which have been given earlier, was evaluated as follows: 0 = no reaction; + = erythema measuring 10 mm in diameter at the site of injection at 24hrs (early reactions were absent); ++ = urticarious nodule or papulous infiltration (larger than 5 mm), with a hyperaemic area (the latter disappearing within 48 hrs) at the site of injection; +++ = oedematous, bullous reaction, larger than 5 mm ("—" the test has not been performed). Systemic reactions (fever, generalized urticaria, etc.) were absent even in the positive cases. In addition to synthetic human ACTH, the following preparations were used: Exacthin (Richter),

Table III

Effect of synthetic human ACTH on urinary total 17-ketosteroid, total 17-hydroxycorticosteroid and pregnanetriol excretion, mg/24 hrs

Steroid	Number of cases	Before ACTH treatment (day)			During ACTH-treatment (day)		
		3	2	1	I.	II.	III.
Total 17-ketosteroid	20	12.1 ± 0.22*	11.6 ± 0.25	12.0 ± 0.22	16.1 ± 1.26	19.0 ± 0.31	18.5 ± 0.25
Total 17-hydroxy-corticosteroid	20	3.9 ± 0.38	4.0 ± 0.30	3.7 ± 0.10	12.3 ± 1.16	23.1 ± 3.43	19.0 ± 2.66
Pregnanetriol	20	0.9 ± 0.06	0.9 ± 0.08	0.8 ± 0.09	1.2 ± 0.09	2.4 ± 0.09	2.3 ± 0.08

Steroid	Number of cases	Increase, in response to treatment			Probability		
		I−1	II−1	III−1	I/1	II/1	III/1
Total 17-ketosteroid	20	4.1 ± 1.13	7.0 ± 1.08	6.5 ± 1.76	$p<0.01$	$p<0.001$	$p<0.001$
Total 17-hydroxy-corticosteroid	20	8.6 ± 1.27	19.4 ± 3.51	15.3 ± 2.56	$p<0.001$	$p\ll0.001$	$p\ll0.001$
Pregnanetriol	20	0.4 ± 0.08	1.6 ± 0.07	1.5 ± 0.29	$p<0.01$	$p<0.001$	$p<0.001$

* = standard error of mean

Table IV

Effect of synthetic human ACTH on urinary

No of case	Sex	Age (year)	Total 17-ketosteroid, mg/24 hrs			Dehydroepiandrosterone mg/24 hrs		
			before treatment	during ACTH treatment	increase	before treatment	during ACTH treatment	increase
1	female	26	16.0	26.0	10.0	4.6	5.6	1.0
2	female	36	11.7	17.7	6.0	3.3	4.7	1.4
3	female	46	10.2	32.8	22.6	2.9	6.5	3.6
4	male	16	16.1	22.6	6.5	3.5	5.4	1.9
5	male	27	12.5	23.9	11.4	4.7	6.0	1.3
6	male	32	14.1	23.2	9.1	4.2	6.4	2.2
		Average	13.3	24.4	10.9	3.8	5.7	1.9
			±0.97*	±2.02	±2.48	±0.29	±0.27	±0.38

* standard error of mean

retard (Z), purified and highly purified porcine ACTH (all three: Cortrophine, Organon).

Table V shows the results. Negative reactions to the native preparations were confined to one case in seven. In contrast, the reactions to synthetic human ACTH were negative in all subjects but one. The reaction in this single case was reproducible and consisted of a slight erythema 3 to 5 mm in diameter (graded ±).

Discussion

Recent literature contains reports by various research teams [10, 11, 13, 19] on the synthesis of polypeptides being able to stimulate the adrenal cortex. On the evidence of these studies even different fragments of ACTH have a biological activity of this kind. Fragments consisting of not more than 10 amino acids exhibit a stimulating effect of some degree on the adrenal cortex [23] and those made up of 23 or 24 amino acids display a significant biological activity [3, 4, 17, 22, 24, 26, 31]. On the grounds of the present study, synthetic human ACTH consisting of a sequence of 39 amino acids may also be regarded as highly active. The preparation lends itself to diagnostic purposes in endocrinology as well as to clinical use. It may be employed, in addition, as a standard in radio immuno assays for the determination of plasma ACTH [16]. On the evidence of the present observations, synthetic

17-ketosteroid fractions

Androsterone mg/24 hrs			Etiocholanolone mg/24 hrs			11-hydroxy-17-ketosteroid, mg/24 hrs		
before treatment	during ACTH treatment	increase	before treatment	during ACTH treatment	increase	before treatment	during ACTH treatment	increase
4.3	6.2	1.9	4.3	9.7	5.4	2.5	3.7	1.2
4.1	6.1	2.0	2.3	3.0	0.7	1.6	2.4	0.8
2.6	5.4	2.8	3.2	12.0	8.8	0.8	6.5	5.7
4.5	5.8	1.3	6.4	9.8	3.4	1.7	1.6	−0.1
2.3	5.7	3.4	3.6	6.4	2.8	1.4	3.6	2.2
5.0	7.1	2.1	3.7	8.0	4.3	1.2	1.4	0.2
3.8	6.0	2.2	3.9	8.1	4.2	1.5	3.2	1.7
±0.44	±0.24	±0.38	±0.56	±1.28	±1.12	±0.23	±0.77	±0.86

Table V

Intracutaneous hypersensitivity of various ACTH preparations

No.	Name	Age, sex	Diagnosis	Exacthin (Richter)	Cortrophine (Organon)			Synt. human
					retard.	purified	highly purified	
1.	H.S.	43 ♀	Melanosis	+	+	+	+	⌀
2.	B.M.	39 ♀	Hypo-adrenocorticism treated hyperthyroidism	+	+	+	—	⌀
3.	S.S.	41 ♀	Addison's disease, treated hyperthyroidism	++	—	+	+	⌀
4.	Sz.I.	29 ♀	Diffuse scleroderma	+++	++	++	++	⌀
5.	Cs.Z.	32 ♀	Cushing's disease, previous, left subtotal, right total, adrenalectomy	++	+	—	+	±
6.	T.M.	29 ♀	Hypo-adrenocorticism	++	++	—	⌀	⌀
7.	J.B.	40 ♀	Hypo-adrenocorticism	++	—	+	+	⌀

human ACTH may be used in patients having developed hypersensitivity to natural ACTH; its detailed comparison to other ACTH preparations will need further studies.

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Dr. Ferenc A. László
Dr. Kálmán Kovács
Dr. Imre Faredin
Dr. László Czakó
Dr. Ferenc Durszt
Dr. Ilona Szijj
Dr. István Tóth
Dr. András Biró
Dr. Miklós Julesz

Orvostudományi Egyetem I. Belklinika,
Szeged, Hungary

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INTERRELATIONS OF THYROID AND MESENCEPHALIC ACTIVITY

By

L. Szántó, B. Mess and A. Reviczky

NATIONAL INSTITUTE OF RHEUMATOLOGY AND PHYSICOTHERAPY, BUDAPEST, AND INSTITUTE OF ANATOMY, UNIVERSITY MEDICAL SCHOOL, PÉCS

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The clinical and experimental aspects of the interactions of the diencephalon and the thyroid are analyzed. The observation that the substitution therapy of congenital myxoedema requires excessive doses of thyroid hormone without involving the risk of thyroid hyperactivity is suggestive of developmental abnormalities affecting the corresponding regions of the diencephalic nuclei. In the rat, karyometric variations in the regions of the diencephalic, ventromedial and habenular nuclei, always associated with methylthiouracil-induced myxoedema, were fully normalized on adequate substitution therapy, in opposition to similar diencephalic lesions accompanying congenitally induced myxoedema in laboratory animals where thyroid substitution therapy was no longer capable of restoring conditions to normal.

Two children with congenital myxoedema, unresponsive to substitution therapy, have been observed. Thyroid hormone administered far in excess of adult requirements failed to ensure clinical improvement. To account for the failure, the eventual role of extrathyroidal factors was studied. The possibility of developmental defects affecting the mesencephalic nuclear regions where the vegetative centres are represented, was considered in view of the evidence concerning their close involvement in thyroid-cerebral feedback. To prove the validity of this claim, it had to be shown in animal experiments that the cerebral lesions associated with acquired myxoedema, however extensive, were temporary, i. e. reversible on thyroid substitution therapy whereas those accompanying congenital myxoedema were unresponsive to such treatment. More precisely, the diencephalic changes due to acquired myxoedema had to be restored to normal by thyroid administration, whereas those accompanying congenital myxoedema in a generation brought up on antithyroid drugs had to prove unresponsive to prolonged administration of major thyroid doses. In brief, the question was, whether or not, the diencephalic lesions due to myxoedema are reversible.

Material and methods

A group of Wistar rats aged 3 to 4 months were treated with massive doses (50 mg daily) of methylthiouracil and subsequently with massive doses of thyroid hormone 100 μg thyroxine or 1 μg triiodothyronine, daily, for two months. This group served as control to the group of congenital hypothyroidism, which was induced as follows. Female Wistar rats of 200 g body weight were started on 50 mg of methylthiouracil daily three months prior to mating, and administration of these doses was continued through pregnancy and lactation.

The actual test animals, the young rats, were started on methylthiouracil at birth and maintained on it until the end of the second month. Half of these animals with congenital hypothyroidism induced in the above manner were subjected to thyroid hormone treatment with the same doses of thyroxine or triiodothyronine as in the animals of the first group with induced adult myxoedema. The other half served as controls. The "adult" series was made up of two groups of 30 animals each and the series of congenital myxoedema also comprised 60 animals.

Serum protein-bound iodine, chromatographic pattern of thyroid hormones and the histologic appearance of the thyroid were studied in all the animals, and were found invariably in harmony with the prevailing state.

The diencephalic nuclear regions were submitted to karyometric study (for technical details see [19]). Material for the karyometric study of the ventromedial nucleus was invariably derived from its basal (ventrolateral) part corresponding to the frontal plane of the arcuate nucleus. For karyometry of the medial habenular nucleus the cellular nuclei of its macrocellular ventral and lateral areas at the mid-level of the habenular nucleus in the anteroposterior direction were studied. The microcellular dorso-medial part was not included in the present study, though on the evidence of earlier investigations [20] the microcellular area also exhibits shrinking of the nuclei in response to thyroidectomy or to thiouracil treatment.

Results

Figs. 1 and 2 present the karyometric variations in the ventromedial and habenular nuclei in the group with myxoedema induced by chemical thyroidectomy and with induced congenital myxoedema, respectively.

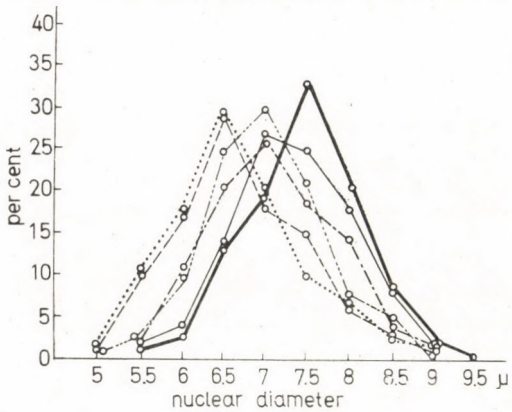

Fig. 1. Abnormal shift in the karyometric variations (nuclear shrinking) in the ventromedial nucleus in rats with congenital hypothyroidism. Symbols as in Fig. 2

It can be seen that, while in the thyroxin-treated group of thiouracil-induced adult myxoedema (continuous thin line) the karyometric abnormalities demonstrable in the ventromedial nucleus were insignificant as compared to the normal controls (continuous heavy line), the curve obtained in the group of induced congenital hypothyroidism after thyroxin substitution therapy showed a marked shift to the left, i. e. in the direction of nuclear shrinking. As to the habenular nucleus, here thiouracil induced swelling of the cell nuclei, which in the group of congenital myxoedema were no longer responsive to subsequent thyroxine or triiodothyronine administration.

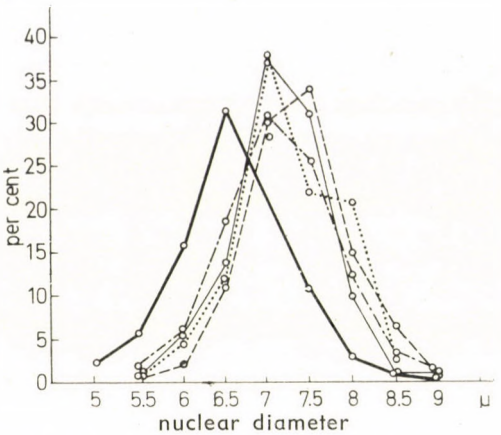

Fig. 2. Abnormal shift in the karyometric variations (nuclear swelling) in the habenular nucleus in rats with congenital hypothyroidism. ▬▬▬ = normal control, ... = rats with congenital myxoedema, - - - - - - - = rats with congenital myxoedema; thyroxine treatment, ·-·-·-·-·-·- = rats with congenital myxoedema; methylthiouracil + thyroxine treatment, ——————— = adult rats: pretreatment with thiouracil, subsequent treatment with thyroxine

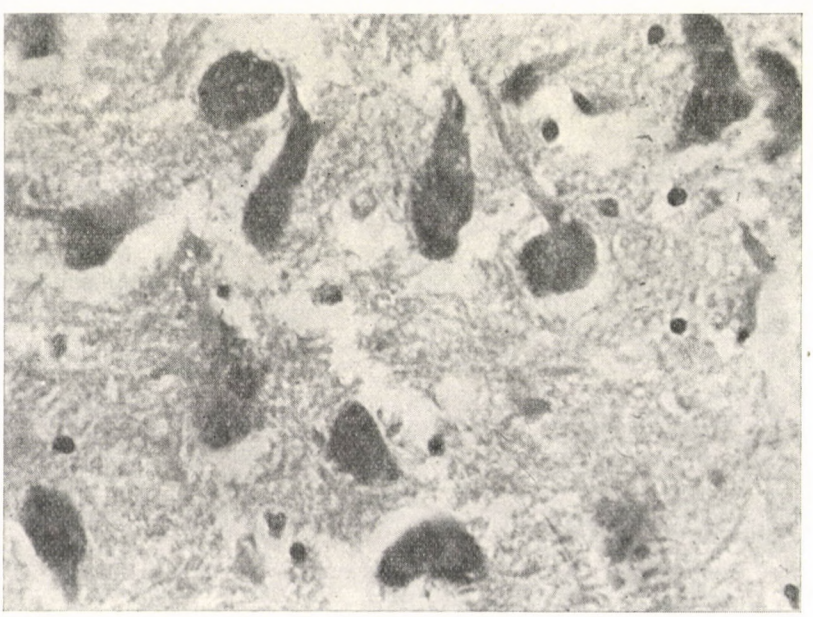

Fig. 3. Histologic appearance of mesencephalon. Wear-and-tear pigments as a sign of degeneration

Qualitative histologic study revealed in the groups with hypothyroidism minor chromatolysis, largely confined to the ventromedial nucleus. No accumulation of wear-and-tear pigments was demonstrable.

HALÁSZ et al. [16] and MESS and KUTAS [20] have shown that the karyometric abnormalities in the dorso- and ventromedial and the habenular nuclei do not affect uniformly all cells of the central nervous system in an unselective manner. Certain hypothalamic nuclei, e. g. the arcuate nucleus, remain intact while others, e. g. the paraventricular nucleus, exhibit an inverse response. The consistent karyometric variations demonstrable in the present experiments have clearly proved the presence of consistent structural, and commensurate functional, changes in the corresponding nuclear regions. From the curves and the consistent response to every influence involving the thyroid it clearly follows that the lesions affecting the diencephalic nuclei of animals with congenital hypothyroidism are of permanent character and show very good responsiveness to changes in the blood thyroid hormone level.

Discussion

In recent years some insight has been gained into the relationship between the thyroid and the central nervous system, in particular the diencephalon. TAUROG et al. [29] and COURRIER et al. [7] have shown that 4 hours after administration of ^{131}I-thyroxine and ^{131}I-triiodothyronine the thyroid hormone concentration in the hypothalamus exceeds the plasma level 2 to 4 times. FORD and GROSS [13], on administering ^{131}I-triiodothyronine to rabbits, found the highest hormone concentrations in the region of the paraventricular nucleus.

The thyroid gland and the diencephalon are connected by a two-way mechanism accounting for the feedback regulation of thyroid function. It is generally agreed that increased thyroxine levels are influencing thyroid function by acting directly on the hypophysis, and reduced levels, by an action on the hypothalamus [24].

Stereotactic studies were undertaken with the aim of establishing which of the hypothalamic nuclei were involved in the production of TSH by the hypophysis. Lesion to the area enclosed by the optic chiasm, the paraventricular nucleus and the medial eminence was followed by a definite reduction in TSH secretion and, consequently, in the secretory activity of the thyroid. BOGDANOVE [2], and BOGDANOVE and HALMI [3] found morphologic and functional changes characteristic of thyroid hypofunction after lesion to the ventral area of the medial eminence. D'ANGELO and TRAUM [8] noted a reduction in TSH secretion after lesions of similar localization. MESS [19] and KOVÁCS,

LISSÁK and ENDRŐCZI [18] found a reduced pituitary TSH content together with histologic signs of thyroid hypofunction after destruction of the area between the optic chiasm and the medial eminence. HARRIS [17], CAMPBELL et al. [6], and SHIZUME [26] showed that electric stimuli applied to the ventral part of the hypothalamus increased the release of ^{131}I, while after a similar intervention D'ANGELO and SNYDER [9] demonstrated reduced hypophyseal concentrations of TSH, parallel with a rise in its blood level.

There are data in favour of the neurohormonal nature of the influence of the diencephalon on TSH secretion. The existence of a thyrotropin-releasing-factor (TRF) of hypothalamic origin was proved by SHIBUSAWA [25], while AVERILL et al. [1] were able to produce an elevation in the serum TSH level by direct intrahypophyseal infusion of crude hypothalamic extract.

The habenular nuclei are also involved in the neural feedback mechanism but their role is limited to a sensitizing influence either co-ordinated to neural feedback or controlling it [21].

In agreement with data in the literature, our earlier observations in a fatal case of hypothyroid coma [27, 28] were also suggestive of an involvement of the diencephalic regions. The cause of the coma was an acute cerebral oedema induced by a slight injury in a patient with myxoedema. The acute thyroid hormone deficiency elicited central nervous signs and these were soon followed by coma. All the preterminal signs such as loss of consciousness, bradycardia, bradypnoea, hypotension, hypothermia must have been connected with a functional disturbance of the corresponding diencephalic areas. The wear-and-tear pigments demonstrated in the diencephalon had originated from the macrocellular area of the anterior pituitary; they were regarded as a sign of degeneration.

Production and course of the thyrotoxic crisis represents a counterpart to hypothyroid coma. Here too the condition of utmost severity is not limited to a peripheral organ but involves the central nervous system, producing a disastrous breakdown of its functions [28]. The excessive central-nervous involvement in grave thyroid disease does not mean that in its common, i. e. peripheral, type the central nervous system should be considered a negligible factor. In fact, the results of various studies definitely suggest the contrary. FÖLDI et al. [14] have shown in crossed circulation dogs that early thyroxine tachycardia cannot be elicited without central nervous mediation. POLICZER [23] emphasized the existence of close relationships between increased thyroid function and the central nervous system. The same applies to certain types of myxoedema, as pointed out by BREWER [5]. The correlation between thyroid function and the central nervous system has been discussed by us in 1962 in the context of the clinical observation of two cases of congenital cretinism. The condition was due to an enzyme defect inhibiting the organic binding of iodine. Data of these patients are presented in Table I.

Table I

Clinical and laboratory data of the two patients with congenital myxoedema

S. J., female: aged 14 at the time of observation	O. K., female; aged 16 at the time of observation
History: Retarded development; mental deficiency. Thyroid disease had not been diagnosed.	History: asphyxia at birth. Mental deficiency. Thyroid disease had been diagnosed at age seven. Sporadic thyroid substitution therapy only a year before admission.
State: Diffuse, apple-sized goitre. Typical myxoedema.	State: Fist-sized diffuse goitre. Typical myxoedema.
Serum protein-bound iodine 1 μg per 100 ml.	Serum protein-bound iodine 1.7 μg per 100 ml.
Basal metabolic rate $-10\ \%$.	Basal metabolic rate -28%.
Chromatogram: triiodothyronine not demonstrable; thyroxine 0.26%; mono- and diiodotyrosine: increased.	Chromatogram: not performed.
^{131}I-retention curve	
per cent	per cent
1 h: 20	1 h: 28
2 h: 39	2 h: 40
6 h: 68	3 h: 68
24 h: 89	4 h: 20
48 h: 80	6 h: 10
	24 h: 13
	48 h: 11

^{131}I-retention test repeated 3 weeks later in combination with potassium perchlorate-loading, 2.0 g being administered by mouth at 3hrs after ingestion of the isotope. This resulted in the following retention curves:

^{131}I-retention curve	
per cent	per cent
1 h: 16	1 h: 22
2 h: 48	2 h: 35
6 h: 80	3 h: 57
24 h: 90	4 h: 30
48 h: 94	6 h: 8
	24 h: 12
	48 h: 18

As to the doses for substitution therapy, the patients were started on 40 μg triiodothyronine daily, with daily increments of 20 μg; it was striking to find that the daily dose had to be raised to 600 μg. Dry thyroid extract in doses of 100 mg daily successively increased to 800 mg daily likewise failed to produce any toxic effect, in contrast to the general experience in non-congenital myxoedema where we cannot be careful enough with the substitution doses, since despite utmost caution and minimum daily increments, toxic effects due to thyroid hyperactivity may appear before any notable benefit has been achieved as emphasized also by ZONDEK et al. [30, 31].

The special response to thyroid hormone found in sporadic cretinism—unlike that seen in non-congenital myxoedema—raised the possibility that the metabolic disturbance associated with congenital myxoedema causes an irreversible damage to the central nervous system by depriving it from the thyroid hormones necessary for normal development. The unresponsiveness of our patients to thyroid doses far in excess of those normally used for substitution would suggest that the classical syndrome of tachycardia, tremor and sweating results from an indirect mechanism involving the agency of the central nervous system, rather than from a direct metabolic response of the target organ, the thyroid. The symptoms in question may thus be connected with the nuclear regions of the diencephalon (vegetative centres) which react readily to thyroid hormone. In the case of failure or inadequacy of central regulation, even massive doses of exogenous thyroid hormone are inefficient.

Congenital myxoedema is associated in our view with developmental defects of the central nervous system, as confirmed by numerous experimental and clinical observations. Multiple lesions affecting the structural development of the central nervous system have been demonstrated in rats with congenital myxoedema induced by goitrogens or by radio-iodine [15]. The pathologic significance of lesions reproduced with the aid of close localization techniques has also been examined. These studies have shown that in patients with congenital hypothyroidism, the brain is reduced in weight and has a characteristic shape, its breadth being out of proportion with its length. The histology of the cerebral cortex has also provided valuable information. The main abnormalities thus demonstrated consist in narrowing of the neuronal perikaryon [10], delayed axonal myelinization [4], reduced density of the axonal network [11]. The rate of growth of dendrites is also slower than in the healthy progeny. The cerebral vessels also reveal abnormalities, i. e. increased capillary permeability [22] and considerable loss of capillary surface which is closely involved in metabolism [12].

To account for the failure of sporadic cretins to develop symptoms of thyroid overdosage, it is suggested that, during the process of development, the brain (diencephalon) lacks the thyroid hormones essential to its normal development. This results in defects of certain diencephalic nuclear structures. In acquired hypothyroidism, sensitivity of the diencephalic centres to hormonal influences is preserved and an excess of thyroid hormone will elicit their response.

The present studies seem to have proved that the effect of substitution therapy in human myxoedema (acquired or congenital) depends on the reversibility or irreversibility of the damage to the diencephalic structures.

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Dr. László SZÁNTÓ
Dr. Alice REVICZKY } Budapest, II. Frankel Leó-u. 17—19 Orfi

Dr. Béla MESS Pécs, Dischka Győző-u 5. Anatómiai Intézet

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LYMPHADENOPATHY RESULTING FROM LONG-TERM USE OF HYDANTOIN

By

GY. SZEGEDI, G. KRASZNAI, P. SORSZEGI and G. SZABÓ

FIRST DEPARTMENT OF MEDICINE, INSTITUTE OF PATHOLOGY AND DEPARTMENT OF NEUROLOGY AND PSYCHIATRY, UNIVERSITY MEDICAL SCHOOL, DEBRECEN

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In 170 epileptic subjects the incidence and histomorphologic characters of lymphadenopathies associated with the long-term use of hydantoin derivatives has been studied. A particular type of lymph node enlargement persisting for years named chronic hydantoin lymphadenopathy (CHL), was demonstrable in 15%. Lymph node biopsy performed in 10 cases revealed atrophy of the lymphoreticular tissue, angiofibrous tissue proliferation and, in a few cases, nodular transformation of the lymph node (nodular fibrosis). No sign of malignant transformation was demonstrable in any of the cases. The possible relationships between the lymph node changes and the autoimmunogenic properties attributed to hydantoin derivatives are discussed.

The pathomechanism of autoimmune diseases closely involves, in addition to an endogenous predisposition, various exogenous factors, very often drugs, which may in fact provoke the process as it has been shown by clinical observations and experimental evidence relating to the autoimmunizing influence of the antihypertensive drug hydrazinophthalazine [1, 4, 6, 22, 23, 24, 28, 29]. Later, procainamide [14, 19, 36], isoniazid [20, 36] and various antiepileptic drugs [2, 12, 20, 21, 25, 30, 32, 34, 41] had to be added to the list of drugs carrying similar hazards.

On the evidence of clinical observations, hydantoin derivatives, in addition to their auto-immunizing effects, possess blastomogenic properties [11, 13] affecting the lymphoreticular system, in particular the lymph nodes; HYMAN and SOMMERS [11] reported on cases of lymphogranulomatosis or malignant lymphoma.

These observations prompted prospective and retrospective studies on a large scale directed at the detection of lymphadenopathies, serologic abnormalities or autoimmune processes which might be connected with long-term antiepileptic therapy.

Material and method

Three hundred patients subjected to long-term antiepileptic therapy were seen the last five years. Of these 170 could be continuously followed up owing to the co-operation of the Rehabilitation Department for Epileptics, Hencida, and the Institute for Defective Children, Debrecen, and were regularly examined by us every six months. Lymph node enlargement, mostly of symmetrical cervical or axillary localisation, occasionally of generalized character, was noted in 15% of the 300 cases, particularly where treatment had been of longer duration and where hydantoin had been used. Lymph-node biopsy was carried out in 10 from among the 170 patients who were kept under continuous observation and whose serologic abnormalities indicative of autoimmune disease were of relative severity.

Results

Table I shows the main data relating to these ten patients.

Pathohistologic study. The lymphoglandular structure is generally discernible. The capsule is uniformly thickened and consists of firm, collagenous fibrous tissue. The cortical area is atrophied, follicles are sparse, of reduced cell population, poorly outlined, made up of small lymphocytes or lymphoblasts (Fig. 1). Germinative centres constituted by reticular cells or lymphoblasts, are practically absent. The moderately broadened medullary bundles consist of mature lymphocytes. The intermediary and medullary sinus system shows dilatation,

Table I

No.	Name	Sex	Age	Drug	Duration of treatment, years	Lymph node	Serologic abnormalities
1.	B.M.	♂	17	primidone trimethadione phenacemide	3	Cervical	LE-cells: negative ANR: positive Rose-test: 1 : 32
2.	B.I.	♂	19	mephenytoin trimethadione	10	Cervical Axillary	LE-cells: negative ANR: positive Rose-test: 1 : 8
3.	B.I.	♂	29	mephenytoin primidone	10	Cervical Axillary	LE-cells: negative ANR: negative Rose-test: positive
4.	B.Á.	♂	32	mephenytoin primidone	15	Cervical Axillary	LE-cells: positive ANR: positive Rose-test: positive
5.	K.E.	♂	26	mephenytoin phenytoin	10	Cervical	LE-cells: negative ANR: negative Rose-test: positive
6.	O.L.	♂	36	mephenytoin primidone	15	Cervical Axillary	LE-cells: rosette formation ANR: negative Rose-test: positive
7.	T.J.	♂	37	mephenytoin primidone	16	Cervical	LE-cells: negative ANR: negative Rose-test: positive
8.	V.R.	♂	22	mephenytoin phenacemide	3	Cervical	LE-cells: negative ANR: negative Rose-test: negative
9.	K.B.	♂	28	mephenytoin phenacemide	4	Cervical	LE-cells: rosette formation ANR: positive Rose-test: positive
10.	B.L.	♂	24	phenytoin	3	Generalized	LE-cells: negative ANR: positive Rose-test: positive

Rose-test: positive $\geq$ 1 : 64. ANR = Antinuclear reaction

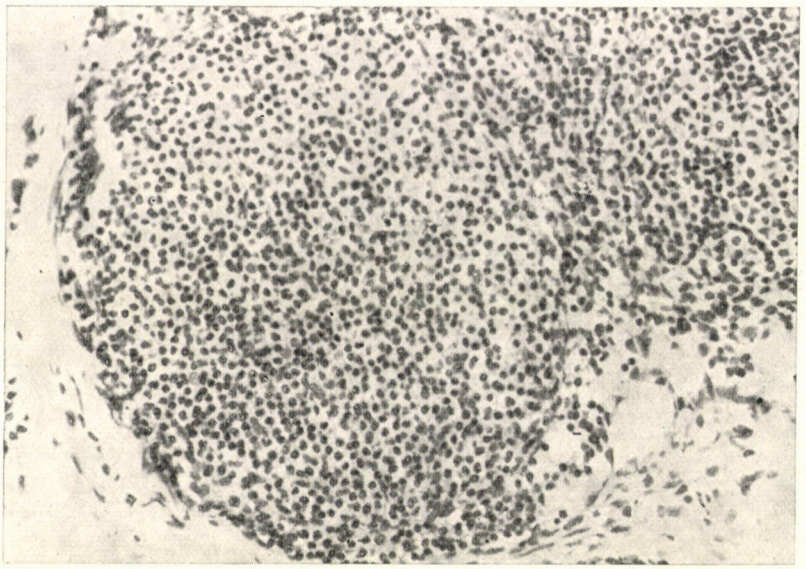

Fig. 1. Detail of atrophied cortex with poorly outlined follicle made up of small lymphocytes. H.E. ×120

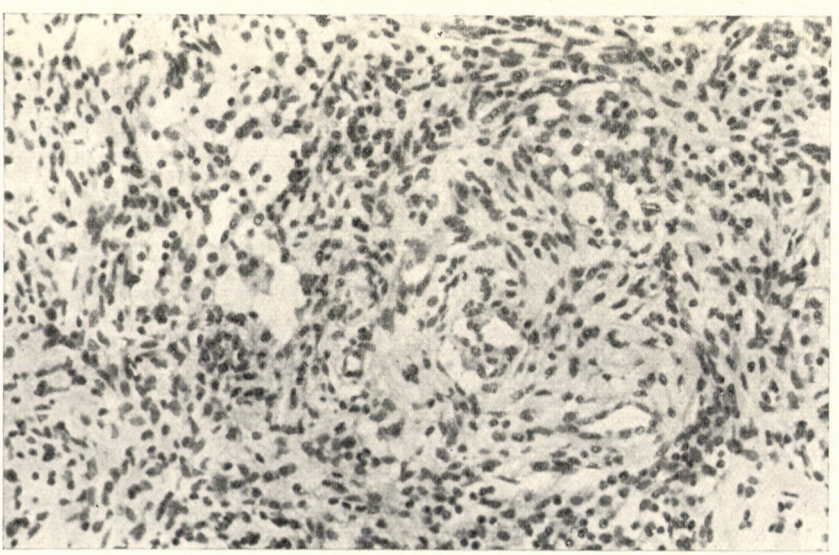

Fig. 2. Angiofibrous proliferation involving the medullary substrate of lymph node. H.E. ×160

thickening of the walls, and contains histiocytes with large, loose as well as with narrow, dark eosinophilic cytoplasm, furthermore lymphocytes and eosinophils in the lumen (sinus histiocytosis).

Gross angiofibrous proliferation with abundant interstitial fibres and blood vessels extends from the hilar region to the medulla and also involves the cortical area. It occupies almost entirely the hilus and replaces the lymphoreticular tissue of the medullary substance

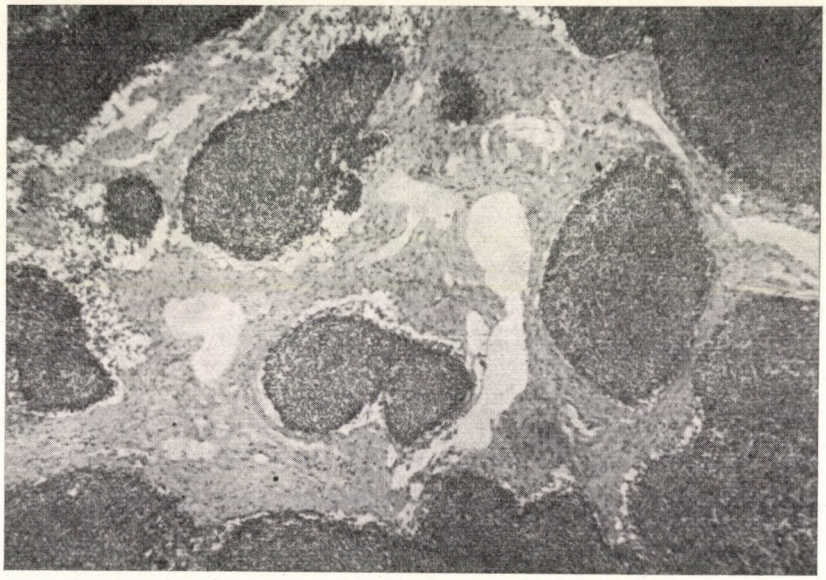

Fig. 3. Lymph node divided into focal areas by broad bundles of collagenous fibre rich connective tissue. H.E. ×80

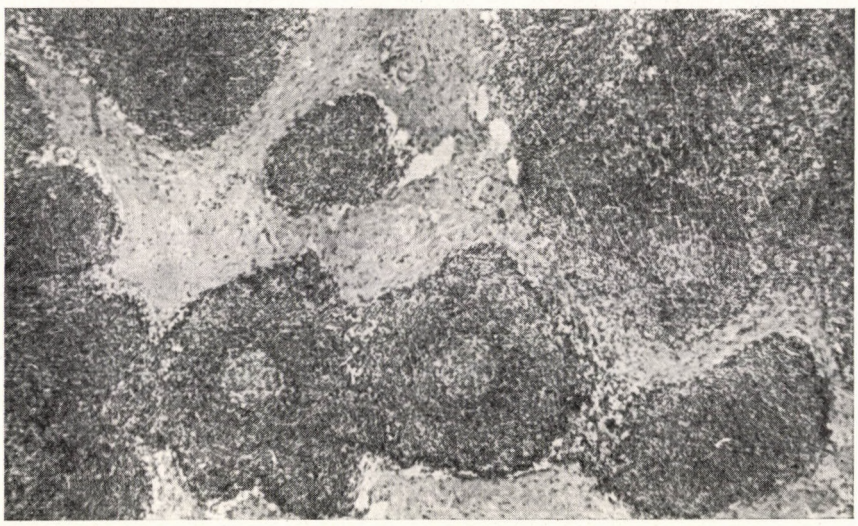

Fig. 4. Nodular fibrosis

to a variable degree. It may even divide up the entire lymphoglandular substrate and separate the medullary bundles into focal areas which are surrounded by broad zones of firm collagenous connective tissue, i.e. nodular fibrosis (Figs 2, 3, 4).

Small foci of hyalinization occur principally in the medullary bundles, partly also in the cortical area. Sinus histiocytosis is absent from the marginal sinus system. The subcapsular sinus system is collapsed, practically cell-free. The residual lymphoreticular tissue is made up of small lymphocytes and lymphoblasts exhibiting no evidence of transformation into immunocytes. Sporadic plasma cells are seen among the lymphocytes (Fig. 5).

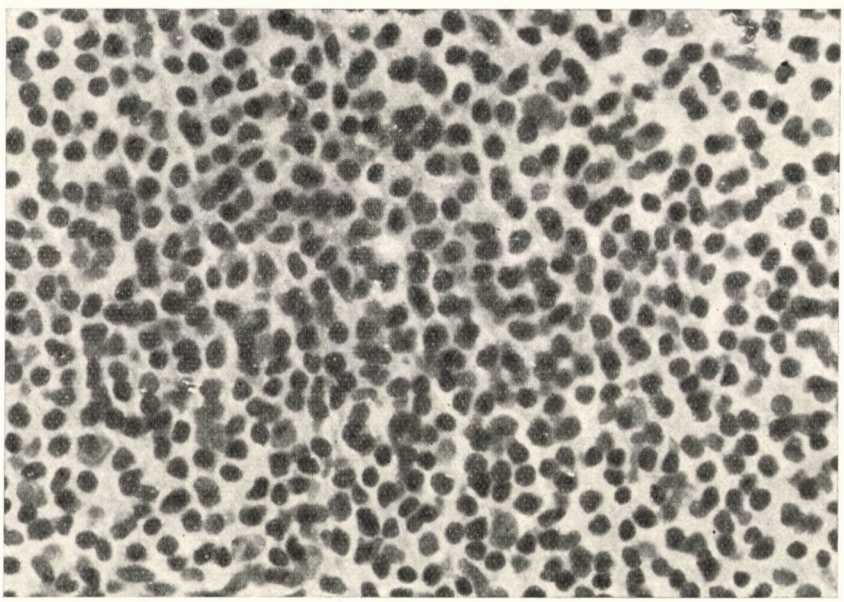

Fig. 5. Residual lymphoreticular tissue, consisting of small lymphocytes and sporadic plasma cells. H. E. ×380

The histomorphologic changes in the lymph nodes are thus characterized by follicular atrophy, sinus histiocytosis affecting the intermediary sinus system, distinct angiofibrous proliferation, nodular fibrosis and focal hyalinization. No reticular cell proliferation or transformation of immunocompetent cells into immunocytes were demonstrable. Diagnosis: Chronic hydantoin lymphadenopathy (CHP).

Discussion

Lymph node enlargement induced by antiepileptic drugs has been described as a sign of drug-induced systemic reaction of allergic nature [3, 5, 17, 18, 35, 37]. Lymphadopathy of this origin is regarded as lymphoreticular tissue proliferation of blastomatous character accompanied by vascular reactions indicative of an allergic state. All allergic manifestations including an adenopathy subside spontaneously after elimination of the responsible

allergen, i. e. the suspension of the drug treatment. The lymphadenopathy accompanying the hypersensitivity response is termed hydantoin-lymphadenopathy (HP) [7, 16]. HYMAN and SOMMERS [11] have observed six cases of true lymphoma during antiepileptic treatment and in contradistinction to these, refer to the lymph node enlargement of allergic nature as pseudolymphoma. In their estimate true lymphoma and pseudolymphoma show much the same incidence during antiepileptic therapy. In the three cases reported by GAMS et al. [7] the lymphadenopathy of allergic appearance successively assumed the features of true lymphoma. These authors suggested the term pseudo-pseudolymphoma for this particular group. These three cases, together with those of HYMAN and SOMMERS point to the possible blastomogenous properties of hydantoin derivatives. Experimental evidence in support of this claim has been furnished by JUHÁSZ [13]; in his experiments, 10 out of 50 mice subjected to long-term diphenylhydantoin treatment were found to develop lymphoreticulo-sarcoma or leukaemia.

In the last 5 years we have noted hypersensitivity reactions associated with the long-term use of hydantoin preparations in 17 cases. Lymph node biopsy has been performed in 12 cases. The histological features of lymphadenopathy were characteristic of HP, or according to the terminology of HYMAN and SOMMERS, of pseudolymphoma. None of the patients have thus far shown any sign of malignant lymphoreticular proliferation. As a matter of course, the drug responsible for the reaction has been substituted by some other compound.

Lymph node enlargement in the absence of any sign of allergic hypersensitivity has been found in 15% of our patients on chronic antiepileptic treatment. Palpability of the enlarged lymph nodes was checked on repeated occasions. The average duration of antiepileptic therapy was 9 years.

Instead of the expected lymphoreticular tissue proliferation the lymph nodes of the patients subjected to long-term hydantoin therapy revealed atrophy of lymphoreticular tissue, lymphoid cell depletion, angiofibrous proliferation and fibrosis. Fibrosis and marked vascular proliferation were found to go hand in hand and to result occasionally in a nodular transformation of the lymphoglandular structure, a process we have been referring to as nodular fibrosis. None of the blastomatous features of HP, i. e. extensive foci of reticular cell proliferation, plasmocytic and eosinophil infiltration, allergic necrotizing vasculitis, haemorrhagic necrosis and thrombotic microangiopathy [16, 17, 18], were demonstrable. As far as we were able to ascertain, the present observation of nodular fibrosis seems to be the first of its kind reported in the literature.

The stimulating effect of hydantoin preparations (phenytoin, mephenytoin) on the production of collagen fibres noted earlier in the fibrous tissue of the gum [40, 42] was confirmed by experimental evidence [15]. MOORE

[27] observed pulmonary fibrosis, HOUCK at al [10] hyperplasia of the collagenous fibres of the skin during hydantoin therapy. Certain authors connect the process with an increased fibroblast activity [38], some others regard it as the end stage of a local hypersensitivity reaction, marked by permanent hyperplasia of the collagenous fibre rich connective tissue [40].

It is a point of interest that the antihypertensive agent hydrazinophthalazine, well known for inducing systemic lupus erythematosus, has a stimulating effect on the production of collagenous fibres [26]. According to the interpretation offered by MEILMAN et al. [26] hydralazine combines with the specific receptors of the collagen molecule thus modifying its antigenic character and initiating the autoimmune process. Others regard the liberation of histamine and histamine-like products as the primary factor [7]. Hydrazinophthalazine and mephenytoin were found by us to cause histamine and serotonin depletion [39]. Methysergide, likewise an antiseroton in compound, has been found to produce fibrosis, particularly in the retroperitoneal tissue, in a number of cases.

Hyperplasia of collagenous fibres associated with the use of hydantoin preparations is of generalized character: fibrosis and increased production of collagenous fibres may involve the gum, lungs and, on the evidence of our observations, the lymph nodes. We attribute direct collagen-forming properties to hydantoin and ascribe the production of fibrosis to an effect of this kind rather than to a local hypersensitivity reaction of the tissues. No manifestations of hypersensitivity — fever, rash, arthralgia, haematological abnormalities — have been observed in association with CHP. The incidence of CHP in the subjects under study was far higher than that of HP accompanied by systemic reactions of allergic nature. CHP was noted in 15% of the cases of epilepsy as against 17 cases of HP. Only 2 out of the 17 patients belonged to the continuously controlled series of 170 cases which signifies that the incidence of pseudolymphoma during long-term hydantoin therapy is far below that of CHP.

Malignant lymphoreticular proliferation has thus far been observed in none of the patients treated with antiepileptics neither did the 17 subjects with pseudolymphoma reveal signs of malignant transformation. These observations are by no means incompatible with a possible blastomogenous effect of the antiepileptic drugs; they do, however, suggest that, in contrast with the view of HYMAN and SOMMERS, association of antiepileptic therapy with malignant lymphoma is much rarer than with allergic HP.

Serologic abnormalities, i. e. LE. cell phenomenon, antinuclear factor, rheumatoid factor, modifications of immunoglobulin levels, were demonstrable in a greater number of patients with CHP than in those having been on other kinds of long-term antiepileptic therapy. (These observations will be reported elsewhere). In the 10 patients under study the following abnormalities were

found: LE. in one, antinuclear factor in five, rheumatoid factor with abnormal titre revealed by the Rose test, in six instances. A joint study of the morphologic changes induced in the lymphoreticular system and the consecutive functional changes of the immune system seems to be the most promising angle of approach to clarify the mechanism of the autoimmunogenic effects of hydantoin preparations. This is in agreement with the view [31, 33] attributing the effect of the said drugs in eliciting autoimmune diseases to a direct influence on the immune organs rather than to peripheral binding and consecutive antibody-response. The changes thus produced may be imperceptible or merely of functional character, but in the case of long-term use and a suitable individual constellation they may manifest themselves morphologically and even assume malignant features.

Hydantoin derivatives may also act as allergens and as such form antibodies which, however, are unsuitable for the transfer of autoimmune reactions. We must therefore attribute a more significant role to their direct influence on the immune system in the production of drug (hydantoin)-induced syndromes.

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Dr. Gyula SZEGEDI
Dr. Gábor SZABÓ
I. Belklinika, Debrecen, Hungary
Dr. Géza KRASZNAI, Kórbonctani Intézet, Debrecen, Hungary
Dr. Pál SORSZEGI; Ideg és Elmegyógyászati Klinika, Debrecen Hungary

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IDIOPATHIC DIABETES INSIPIDUS: AUTOIMMUNE PROCESS IN PATHOGENESIS; CORTICOID TREATMENT IN EARLY PHASE

PRELIMINARY COMMUNICATION

By

V. HOLEČEK, J. CHARVÁT and Z. JEŽKOVÁ

THIRD DEPARTMENT OF MEDICINE, LABORATORY FOR ENDOCRINOLOGY AND METABOLISM, FACULTY OF MEDICINE, CHARLES UNIVERSITY, PRAGUE, AND INSTITUTE OF HAEMATOLOGY AND BLOOD TRANSFUSION, PRAGUE

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1. In the pathogenesis of some cases of idiopathic diabetes insipidus, an autoimmune process may play a role. The process is probably preceded by some other (e. g. inflammatory) damage to the supraoptic and paraventricular nuclei; yet, it may be responsible for the clinical manifestation.

2. In persons in whom the inflammatory and/or autoimmune process has a role in the development of diabetes insipidus, corticoid treatment in the early phase of the disease offers a hope for permanent recovery.

3. Serum antibodies against gray or white brain matter were absent in 27 patients in whom diabetes insipidus lasted more than one year. In 9 of these patients, prednisone did not influence the severity of the diabetes insipidus.

The hypothalamo-hypophyseal system may be suppressed by primary and/or metastatic tumours, inflammatory diseases, thesaurismosis, granulomatosis, traumatic injuries and vascular lesions. The data on the incidence of each of these conditions differ considerably with regard to sampling of the patients and the interpretations of clinical data.

This paper deals with the investigation of 86 patients suffering from diabetes insipidus, recorded since 1954. In 73% of these patients, diabetes insipidus was classified as idiopathic; in 16 patients of this group febrile diseases preceding the appearance of polyuria by 2—6 weeks were mentioned in the history.

Since 1962, the blood of 30 consecutive patients with diabetes insipidus was examined for the presence of gray and white brain matter antibodies. The results of these investigations were negative in 27 patients in whom clinical symptoms of diabetes insipidus had appeared more than one year before the examination, and were positive in 3 patients, in whom clinical symptoms of diabetes insipidus had been observed in the year preceding the examination. In these 3 patients administration of prednisone was ineffective, but in one patient the vasopressin dose necessary for substitution decreased after discontinuing prednisone treatment. In 9 patients, in whom the diabetes insipidus had lasted more than one year, corticoid therapy impaired polyuria and polydipsia. The temporary improvement following the discontinuation of the corticoids could not be confirmed to the extent reported by RUZIČ and BULIC [1].

This paper will report on the course of diabetes insipidus and the results observed in 3 patients.

Case 1. O. P., male, 40, had fallen ill with fever and signs of an inflammation of the upper respiratory tract. Three days later he had become unusually thirsty and had to drink up to 8 l of water daily. Another three days later fluid consumption temporarily decreased to 5—6 l, but increased again after few days. Three weeks after the onset of the disease the daily consumption had amounted to 10 l; the thirst had not ceased during nights.

The patient was admitted on November 27th, 1962. Physical examination and basic laboratory data were within normal limits. The diagnosis of diabetes insipidus was confirmed by the dehydration test (max. specific weight of urine,

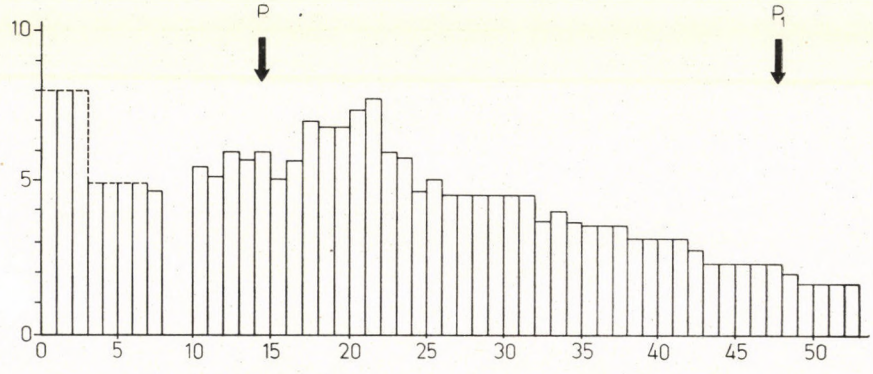

Fig. 1. P — Prednisone started, P_1 — Prednisone stopped

1004; loss of body weight, 3.5 kg in 12 hours). The biological method [2] routinely used in our laboratory for the determination of antidiuretic hormone failed to demonstrate ADH activity in dehydration or after an intravenous injection of saline solution. Neurological examination revealed residues after parainfectious encephalitis. The complement fixation test [3] showed the presence of antibodies against gray brain matter in the blood, whereas white brain matter antibodies were not found. On the basis of the history and our findings, an autoaggressive process was assumed as pathogenetic factor and it was attempted to block it by 30 mg of prednisone daily. Polyuria and polydipsia improved and three weeks later diuresis became normal without any substitution of ADH. Prednisone was administered for 34 consecutive days. During this time, the patient's ability to react to dehydration by renal concetration was restored. No relapse of polyuria and polydipsia occurred. The data are shown in Fig. 1.

Case 2. M. V., female, 30, had had measles and parotitis in childhood and then repeated episodes of tonsillitis. The present disease had begun in November, 1966, with painful retrobulbar pressure, vertigo and subfebrility followed by intense headache localized in the occipital and parietal regions.

Polyuria and polydipsia had suddenly developed on December 20th, 1966 and fluid intake had increased to 10—14 l daily. The patient had been admitted to a district hospital with the diagnosis of diabetes insipidus. As ADH substitution did not stop the polyuria and polydipsia, she was admitted to our Department.

Physical findings and basic laboratory data were normal. The dehydration test, the hypertonic solution test and attempts at the stimulation of vasopressin secretion by amylnitrite inhalation [4] confirmed the diagnosis of diabetes insipidus. Even following these stimuli, neither antidiuretic activity in the blood nor gray or white brain matter antibodies in serum were demonstrated. Nonetheless, in view of the history and the short duration of the diabetes 30 mg of prednisone daily was prescribed. After a temporary impairment of polyuria, fluid intake dropped to normal in the 6th week of prednisone therapy. After its discontinuation the patient had no symptoms of diabetes insipidus and has been without vasopressin substitution in the last 4 years. In November, 1967, however, 8 microunits of antidiuretic activity per ml of serum were found after amylnitrite stimulation.

Case 3. V. S., male, 29. Since August, 1967, he had suffered 3 times from tonsillitis, although before he had not had this disease. Then polyuria and polydipsia had developed within 5 days, fluid intake amounted to 10 l, later 14 l, daily. Physical findings were normal. In December, 1967, the dehydration and Hickea—Hare tests confirmed the diagnosis of diabetes insipidus. Antibodies against gray and white brain matter were not found. In February, 1968, however, high amounts of gray brain matter antibody were demonstrated in the blood. Following this observation, the patient was treated with 30 mg of daily prednisone for 6 weeks. Polyuria rose to 16 l per day then decreased and at present, 26 months after prednisone treatment had stopped, the diuresis amounts to 3—4 l daily without vasopressin substitution.

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MuDr V. Holeček, CSc, Doc.
MuDr J. Charvát, DSc, Professor of Medicine,
Member of Czechoslovak Academy of Sciences,
PhDr Z. Ježková, CSc

Praha 2,
U nemocnice 1,
III. Int. Klinika,
ČSSR

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EFFECT ON ASCITES OF THORACIC DUCT FISTULA AND OF CERVICAL LYMPHATIC-VENOUS SHUNT

By

GY. SZABÓ, ZS. MAGYAR and P. SERÉNYI

NATIONAL INSTITUTE OF TRAUMATOLOGY, BUDAPEST

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In the normal dog as also in animals with constrictive pericarditis there is no constant pressure gradient between the thoracic duct and the cervical veins. In ascites induced by cava inferior constriction, pressure in the thoracic duct exceeds that of the jugular vein by 6.9 ± 0.7 mm Hg. This is consistent with a functional narrowing at the junction of the thoracic duct with the venous system. A thoracic duct fistula leads to a reduction of the ascites and a fall in the portal (cava inferior) pressure in these animals. The effect is transitory and suspension of free lymphatic drainage is followed by the reappearance of ascites and congestion. The cervical lymphatic-venous shunt reduced experimental ascites due to cava inferior constriction in 50% of animals. However, ascites and venous congestion may spontaneously subside in a number of cases. Despite this fact, the shunt seems to have been of definite benefit in a number of animals. In a patient with liver cirrhosis with ascites resistant to all therapeutic measures, the creation of a cervical lymphatic-venous shunt brought the situation under permanent control. Cervical lymphatic-venous anastomosis is recommended in properly selected cases of liver cirrhosis associated with extensive ascites.

Postsinusoidal venous stasis in systemic congestion or in cirrhosis of the liver results in an increased intrahepatic production of extracellular fluid and of lymph [18, 21, 35, 37]. The former factor leads to a further impairment of blood flow in the organ enclosed by a firm envelope, and the latter to fluid accumulation in the abdominal cavity in consequence of the dynamic failure of lymph flow. This implicates that any intervention which improves lymphatic drainage must result in a reduction of portal pressure and, consequently, of ascites. This has been confirmed by the results of thoracic duct drainage in experimental ascites and, to a certain degree, also in ascites accompanying cirrhosis of the liver [2, 7, 10, 23, 26]. The drawbacks of the procedure are, however, obvious [14, 39, 40]. A fistula of the thoracic duct cannot be maintained for unlimited periods, and as soon as it is closed, portal pressure rises and the ascites reappears. Moreover, ascites is associated with a substantial increase in lymph flow which involves a loss of lymphocytes, protein, fluids and electrolytes through the open thoracic duct [2, 9, 20, 23, 26, 39]. Attempts have therefore been made to return the lymph leaving the thoracic duct into the venous system by creating a lymphatic-venous anastomosis [4, 40, 42]. Interventions of this kind are, however, not rational, unless the lymph flow inside the thoracic duct, particularly if increased, meets with a considerable resistance at its entry to the venous system [7, 13, 26]. The resistance

may be of mechanical or organic nature and the obstacle to the lymph flow into the venous system may be constituted by an organic stricture, possible by a ring of little compliance. In this case, pressure in the lymphatic trunk is increased in contrast to that in the cervical veins and this will result in a significant lymphatic-venous pressure gradient.

An obstacle to lymph flow may furthermore be constituted by an increased central, i. e. cervical, venous pressure [15, 16, 32, 38] (mechanic obstacle to lymph flow) in which case central venous as well as lymphatic pressure is high, and there is no significant pressure gradient between them.

A narrowing at the orifice of the thoracic duct can be eliminated by transecting the thoracic duct and constructing a new orifice. An anastomosis of this kind may be created between the cervical section of the duct and some suitable cervical vein. On the other hand, if the obstacle to the lymphatic flow is a high pressure prevailing in the cervical veins, the anastomosis should be formed intrathoracally, as suggested by COLE et al. [4], between the thoracic duct and an intrathoracic vein, pressure in these being lower than in those of the neck. If, however, central venous pressure is increased, as in congestive heart failure, the only suitable shunt is one between the thoracic duct and the pulmonary veins [5].

Some of these problems have been given close attention, but evidence is on the whole conflicting. The present study had the aim to examine the subject under its various aspects.

Material and methods

Mongrel dogs of either sex were used. The interventions were performed under intravenous chloralose anaesthesia (0.1 g/kg).

For the elevation of systemic venous pressure, experimental constrictive pericarditis was induced by our method described earlier [32, 34]. The chest was entered through the 2nd costal interspace, the pericardium was incised and acid-free asbest wool was placed into the pericardial sac. The pericardium was sutured and the chest was closed. The investigations were performed 8 to 14 days later when the signs of systemic venous congestion (high systemic venous pressure, oedema, ascites) had become manifest.

In the other group of animals ascites was produced by chronic supradiaphragmal constriction of the cava inferior vein. Of the interventions used for this purpose that described by ORLOFF et al. [25] has been widely adopted; we found it, however, most unsatisfactory, having no control over the degree of narrowing thus produced. While excessive constriction proved mostly fatal, no ascites at all was produced if the ring was too loose. This difficulty was overcome by constricting the vein to a lesser degree over a longer segment, i.e. by 40 to 50% instead of 60 to 70%, with the aid of a Teflon tube corresponding to 2/3 of the v. cava inferior in diameter. Of the tube 2 to 3 cm long piece was slit open in its entire length, placed around the supradiaphragmal portion of the vena cava inferior and tightened as desired by stitches passed through its cut edges. Ascites was induced by this method in 50 animals; the method proved successful in 90% of the cases, ascites having developed by the end of the 4th to 10th (average, 6th) postoperative day.

Pressure was measured in the thoracic duct and in the innominate vein at the orifice of the thoracic duct. A special, fine-bore needle (No. 30) was used for this purpose with its tip against the direction of blood flow; this technique was adopted because an obstruction of lymph flow by the needle would produce continuously rising pressure figures and consequently

be easily detected. In earlier studies [32] we introduced a cannula through a cervical side branch into the thoracic duct, as also through the jugular vein into the angulus venosus and compared the figures with those obtained by the needle technique. We found the results congruent. Pressure in the v. cava inferior was measured by the aid of a cannula inserted through the saphenous vein. The pressures were measured with a Statham strain gauge connected to an Elema electromanometer.

The animals were weighed and their abdominal circumference was measured daily so as to follow the production of ascites. The volume of circulating plasma (Evans blue dilution and the extracellular fluid compartment (NaSCN-space) were checked at regular intervals.

In a group of 9 dogs, 4 to 6 days after constriction of the cava inferior, the cervical section of the thoracic duct was exposed, divided and a polythene cannula was inserted into its lumen. When an adequate amount of lymph had drained off, the thoracic duct was connected by the aid of a teflon tube with the external jugular vein which had been likewise divided beforehand. On the following day the communication was closed and lymph was allowed to drain freely. The procedure was repeated one to four times.

A further group of 16 dogs was subjected to the following procedure. As soon as ascites had become manifest thus reflecting a significantly increased pressure prevailing in the caval system, an end-to-end anastomosis was formed between the thoracic duct and the external jugular vein by invaginating the distal stump of the cervical section of the thoracic duct into the proximal stump of the vein and securing the connection with 2 to 3 stitches of 5/0 atraumatic sutures. The procedure proved adeaquate; leakage of lymph, if occurring at all, was slight and transitory. In view of the enhanced lymph flow, clotting was infrequent. Patency of the shunts was checked by repeated lymphographies and at necropsy.

A further group of animals served as controls. Here congestion and ascites were induced by constriction of the v. cava inferior, but no anastomoses were performed. The animals were kept under observation, and intracaval pressure and accumulation of abdominal fluid were checked at regular intervals.

Results

I. Pressure-gradient at the orifice of the thoracic duct

Pressure measurements in the terminal section of the thoracic duct and in the cervical vein were done in 13 dogs with constriction of the cava inferior and 10 with induced constrictive pericarditis. The control values (23 normal dogs) have been derived from an earlier study [33]. In a group of animals the thoracic duct was opened after completion of the measurements, cannulated, and the volume of lymph escaping from the fistula under atmospheric pressure was determined. This was 0.97 (S. D. 0.47) ml/min in 10 normal animals (body weight between 11 and 26 kg, average 17.4 kg), while 4.0 (S. D. 2.04) ml/min in 10 animals with constrictive pericarditis (body weight between 11 and 27 kg, average 15.1 kg), and 5.9 (S. D. 3.59) ml/min in 9 animals with cava inferior constriction (body weight between 8 and 18 kg, average 15.1 kg).

Pressure in the cervical veins of 23 normal dogs was 5.85 ± 0.40 mm Hg and in the thoracic duct 5.15 ± 0.46 mm Hg, the difference was not significant statistically. In other words, no pressure gradient of constant nature has been found between the thoracic duct and the cervical vein. Excessive central venous pressure is likewise associated with the absence of a pressure gradient. In the 10 animals with pericarditis the pressure in the innominate vein was

15.8±1.2 mm Hg and in the thoracic duct, 15.7±1.2 mm Hg. In contrast, a high pressure gradient was found at the orifice of the thoracic duct in the animals with ascites induced by constriction of the vena cava inferior. In these cases, probably as a result of the relative hypovolaemia, central venous pressure was considerably reduced, and pressure in the cervical veins was as low as 1.0±0.35 mm Hg. Pressure in the thoracic duct was higher, or at least not lower, than in the normal animals, i.e. 7.9±0.8 mm Hg (Table I).

Table I

Thoracic duct — jugular vein pressure-gradients (mm Hg)

	Control			V. cava constr.			Pericarditis		
	V. jug.	D. thor.	Diff.	V. jug.	D. thor.	Diff.	V. jug.	D. thor.	Diff.
1.	6.5	5.5	—1.0	1.5	5.0	3.5	13.0	17.5	4.5
2.	5.0	6.0	1.0	1.5	9.0	7.5	20.0	16.0	—4.0
3.	7.5	6.5	—1.0	0.5	7.0	6.5	13.0	14.0	1.0
4.	8.5	9.0	0.5	0.5	6.0	5.5	12.0	11.5	—0.5
5.	7.5	7.0	—0.5	0.0	10.0	10.0	12.0	10.0	—2.0
6.	4.5	3.0	—1.5	1.0	15.0	14.0	11.5	11.0	—0.5
7.	7.0	5.0	—2.0	1.0	9.0	8.0	20.5	17.5	—3.0
8.	10.0	10.0	0.0	2.0	9.5	7.5	20.0	21.0	1.0
9.	8.0	6.5	—1.5	0.0	8.0	8.0	17.0	17.5	0.5
10.	4.0	3.5	—0.5	1.0	7.0	6.0	18.5	20.5	2.0
11.	8.0	5.0	—3.0	4.5	8.0	3.5			
12.	9.0	8.0	—1.0	—0.5	3.0	3.5			
13.	5.0	3.0	—2.0	0.0	6.0	6.0			
14.	2.0	2.0	0.0						
15.	3.0	10.0	7.0						
16.	4.0	2.0	—2.0						
17.	7.5	1.0	—6.5						
18.	6.0	4.0	—2.0						
19.	1.0	2.0	1.0						
20.	6.5	4.0	—2.5						
21.	7.0	5.0	—2.0						
22.	3.5	7.0	3.5						
23.	3.5	3.5	0.0						
M.	5.8	5.1	0.7	1.0	7.9	6.9	15.8	15.7	0.1
S.	0.4	0.5	0.5	0.3	0.8	0.8	1.2	1.2	

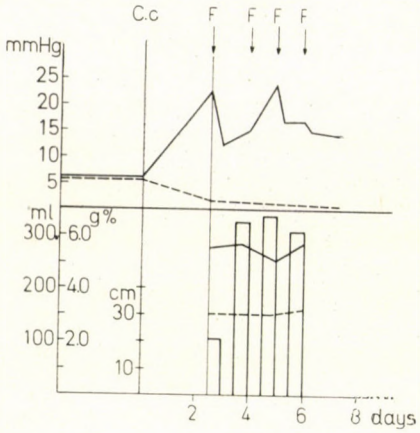


Fig. 1. Effect of thoracic duct fistula on congestion and ascites induced by cava inferior constriction in dogs. Upper part: continuous line = pressure in cava inferior; interrupted line = pressure in jugular vein; C.c. = time of caval constriction; F = time of lymph drainage. Lower part: continuous line = serum protein concentration (g per 100 ml); interrupted line = abdominal circumference (cm); columns = volume of lymph withdrawn

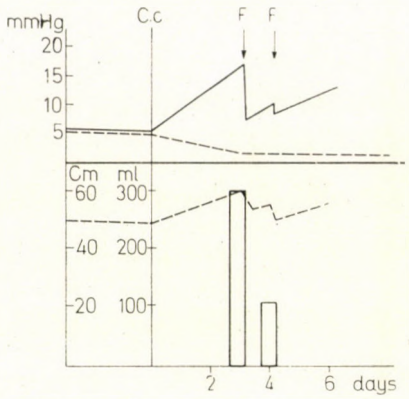


Fig. 2. Effect of thoracic duct fistula on ascites and venous pressure. Symbols as in Fig. 1

II. Effect of thoracic duct drainage on experimental ascites

The effect of thoracic duct drainage on venous congestion involving the cava inferior system was examined in 9 animals. Lymph was withdrawn between the 3rd and 7th day after the intervention 1 to 4 times, 100 to 400 ml on each occasion. Withdrawal of lymph was followed by a reduction of the ascites and of caval pressure in nearly all of the cases (Table II and Figs 1, 2). The effect was, however, transitory. Although the thoracic duct was not closed after completion of lymph withdrawal but united with the external jugular vein so as to provide for drainage, on the evidence of body weight and abdominal circumference, ascites had usually increased by the next day, and this was also true for caval pressure.

III. Effect of lymphatic-venous shunt

A technically adequate lymphatic-venous shunt was produced in 16 animals. The intervention was performed 4 to 15 days (average 6 days) after cava inferior constriction with the exception of two animals where the interval between caval constriction and anastomosing was 31 and 110 days, respec-

Table II

Effect of thoracic duct fistula in dogs

No.	Day	Δ Weight kg.	L. ml.	Venous pressure mmHg		Abdominal circumference	
				before	after	before	after
60	4	1.0	330	28	13		
	6	1.6	350	24	17		
	7	2.3	300	17	16		
120	5	2.5	430	15	13		
118	5	1.0	200	15	12	17	6
	6	0.7	230	12	10	9	6
218	3	3.0	100	90	6	9	8
	4	3.9	—	17		16	
	5	4.0	—	23		18	
242	3	0.5	150	18	14	4	2
	4	0.8	120	15	14	8	6
265	3	0.7	300	17	8	10	5
	4	0.8	100	9	8	6	2
	5	0.8	—	13		7	
277	4	2.5	100	12	7	10	8
	5	2.4	—	11		10	
279	3	1.5	120	16	13	4	2
	4	1.0	120	16	13	3	0
	5	0.4	150	14	8	5	2
325	5	1.0	250	16	7	6	2

Day: from the day of caval constriction
L. ml: volume of lymph drained off
Δ weight: postoperative increase in weight
Venous pressure: in cava inferior, before and after drainage
Δ abdominal circumference: difference referred to values prior to caval constriction

Table III

Effect of thoracic duct–jugular vein shunt on ascites produced by cava inferior constriction in dogs

No.	Weight kg	Abdominal circumference cm	Preoperative changes				Changes within the first post-op. week			Maximum changes			Time of observation
			Day of operation	Weight kg	Abdominal circumference cm	Systemic ven. pressure mmHg	Weight kg	Abdominal circumference cm	Systemic venous pressure mmHg	Weight kg	Abdominal circumference cm	Systemic pressure mmHg	
371	13.80	41	7	+2.30	+16.5	+10	−2.6	−11.5	−10	+3.60	15.5	−10	66
355	14.00	55	6	+3.30	+ 6.5	+11	−2.7	− 4.0	−11	−3.0	− 4.5	−10	14
406	10.00	41	7	+0.25	+ 8.5	+ 9	−0.6	− 7.0	− 7	−1.3	−10	− 8	52
405	9.00	39	7	+2.10	+11.0	+10	+0.6	− 0.5	− 1	−3.3	− 8.5	−10	32
192	17.50	54	11	+3.00	+13.0	+14	−3.0	−10.0	− 2	−3.3	−10	− 7	58
348	17.75	56	14	−1.30	− 3.0	+10	+1.0	+ 5.0	+ 2	+4.2	+ 5.5	+ 8	43
349	14.80	51	15	−1.00	+ 2.0	+10	−1.0	+ 2.5	− 1	−1.0	+ 2.5	− 1	22
386	10.80	43	6	+1.70	+12.0	+16	+1.0	+ 9.0	− 1	+2.3	+10.0	− 1	22
387	19.83	53	5	+2.00	+ 9.0	+ 7	−0.5	− 2.0	0	+3.3	+18.0	—	21
414	12.10	42	4	+1.70	+10.0	+ 6	−0.2	+ 1.0	+ 3	+1.8	+ 9.0	+ 4	33
366	15.50	53	5	+0.70	+ 0.5	+10	−1.7	− 0.5	+ 1	−1.7	− 0.5	− 3	20
365	10.50	39	4	+1.10	+ 8.0	+11	+3.4	+13.0	+ 1	+3.4	+13.0	+ 1	22
380	10.90	42	5	+2.60	+11.5	+ 6	+0	+ 0	+ 4	+1.4	+ 0.5	+ 4	13
1	25.00	60	110	+6.00	+20.0	+16	−1.8	− 4.0	− 1	+0.6	+ 4.0	− 1	146
2	26.80	61	5	+2.90	+12.5	+10	+2.3	+ 9.0	+ 0	+1.7	+10.0	+ 0	66
43	32.2	65	31	+2.20	+60	+ 7							31
M				1.85	9.0	10.2	−0.2	− 0.3	− 2.2	−0.05	− 0.2	− 2.7	
S				0.43	1.4	1.5	0.6	2.4	1.5	0.86	3.1	1.7	

Preoperative changes referred to the condition prior to caval constriction.
Changes within the first postoperative week and maximal changes = referred to the condition prior to lymphaticovenous anastomosis.
The values enclosed by the heavy line have been disregarded in calculating the average figures (the animals having had no ascites prior to operation).

tively. At the time of the shunt operation, ascites with elevation of venous pressure in the caudal part of the body had been present in all but three animals (body weight, abdominal circumference and venous pressure having risen by 1.85 ± 0.43 kg, 9.0 ± 1.4 cm and 10.2 ± 1.5 mm Hg, respectively).

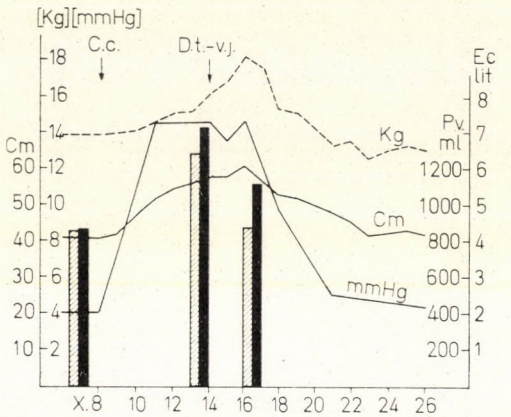


Fig. 3. Effect of cervical lymphatic-venous shunt in dogs with cava inferior constriction. C.c. = time of caval constriction; Dt.-vj = time of anastomosis; interrupted line = body weight (kg); heavy line = abdominal circumference (cm); thin line = pressure in cava inferior (mm Hg); shaded columns = circulating plasma volume; filled columns = extracellular space (kg)

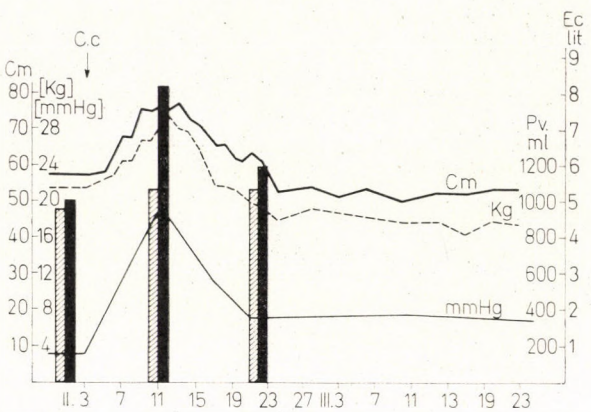
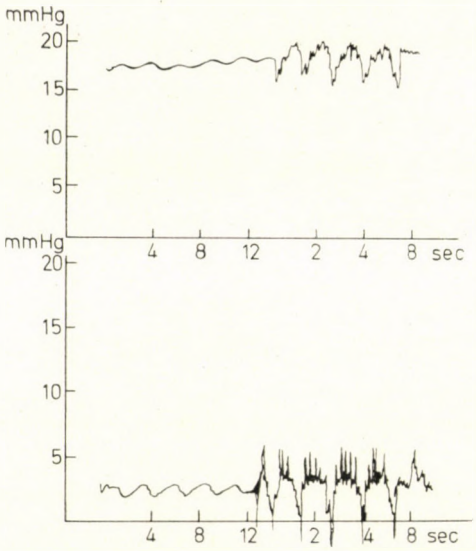


Fig. 4. Control study. Changes consequent upon cava inferior constric tion. Symbols as in Fig. 3

Evaluation will be confined to those 11 experiments where the interval between caval constriction and anastomosis was less than 15 days and where significant ascites had been present prior to the second intervention. (The reason why those two animals which despite considerable ascites had the second intervention at a far later date were excluded from the evaluation was that they had been in a terminal state at the time of the second intervention

and actually died soon afterwards). Since the condition of animals with ascites induced by caval constriction is liable to considerable spontaneous variations, the second intervention was not regarded as successful, in other words, improvement was not ascribed to the lymphatic-venous shunt unless the changes ensued within the first week after the anastomosis.



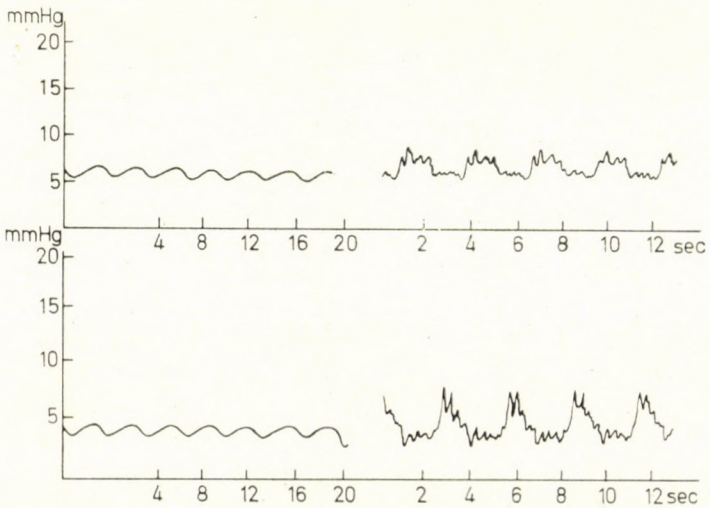


Fig. 5. Pressure in jugular vein and thoracic duct. *a)* in an animal with ascites due to cava inferior constriction; *b)* in the same animal after thoracic duct-jugular vein anastomosis; Upper part: pressure in thoracic duct; Lower part: pressure in jugular vein; left: damped pressure-curves; right: undamped pressure curves

Table IV

Changes in the examined

No.	Weight cm	Abdominal circumference cm	Changes in 7 days reffered to control values				Changes in 15 days		
			Venous pressure mm Hg	Weight kg	Abdominal circumference cm	Venous pressure mm Hg	Weight kg	Abdominal circumference cm	Venous pressure mm Hg
1.	25.0	60.5	5	−0.3	2	6	1.9	3	1
23.	32.2	65.0	4	2.2	6	7	1.2	8	2
41.	17.0	49.0	5	−0.3	8	6	1.3	3	0
143.	18.6	52.5	5	1.4	6	10	4.5	13	1
146	12.5	43.5	5	4.0	14	6	−3.0	−1	0
42.	21.4	57.0	5	5.3	18	5	−5.0	−9.5	0
54.	15.0	53.5	4	−0.5	4	13	1.0	5	0
8.	19.0	51.5	4	2.1	8	15	2.0	11.5	−7
M.				1.74	8.1	8.5	0.45	4.1	−0.4
S.				0.66	1.9	1.3	1.12	2.5	

Changes in 7 days = referred to values prior to caval contriction.
Changes in 15 days and maximum changes = referred to the 7th day after caval constriction.

Improvement ensued within the first week after the anastomosis in 5 out of 11 animals. Accordingly, the changes in body weight, abdominal circumference and caval pressure, referred to the entire group, were not significant (0.20 ± 0.6 kg; -0.3 ± 2.4 cm; -2.2 ± 1.5 mm Hg, respectively). This was valid for the entire period of observation (13 to 66 days) as well as for the average maximum variations (body weight, -0.05 ± 0.86 kg; abdominal circumference, -0.2 ± 3.1 cm; venous pressure, -2.7 ± 1.7 mm Hg).

Lymphography revealed obstruction of the shunt in one out of six unsuccessful interventions. In this animal a new end-to-end anastomosis was created between the thoracic section of the thoracic duct and a side branch of the left pulmonary vein. On the evidence of lymphography performed on the 5th postoperative day, this anastomosis too had become obstructed. As ascertained by repeated lymphographies and at necropsy, the shunts functioned adequately in all the other cases; there was intensive flow through the orifice as well as through the neighbouring collaterals. No obstruction or clot in the lymphatic duct was demonstrable.

Though the operation proved technically faultless, it failed to reduce the ascites and even to prevent its further increase, in 50% of the cases. Therefore, a control series was set up in which the v. cava inferior was constricted but subsequently no shunt was formed. In 6 out of 8 animals of this

parameters in control studies

Maximum changes			Maximum increase			Maximum reduction			Time of observation (days)
Weight kg	Abdominal circumference cm	Venous pressure mm Hg	Weight kg	Abdominal circumference cm	Venous pressure mm Hg	Weight kg	Abdominal circumference cm	Venous pressure mm Hg	
6.3	17.5	11	6.3	17.5	11	5.0	2.0	0	146
−2.2	8	2	1.2	8	2	−2.4	0	0	31
−1.4	6.5	3	1.4	6.5	3	0	−3	0	152
4.5	13	1	4.5	13	1	−1.1	5	−1	60
2.3	10	6	2.3	10	6	1.5	4	0	58
−8.5	−20	−3	1.5	1	0	−8.5	−20	−3	82
−3.2	−10	−12				−3.2	−10	−12	125
4.0	23	0	4.0	23	0				51
0.55	5.4	1.0	3.00	11.3	3.3	−2.67	−5.10	−2.3	
1.62	3.0	5.6	0.54	7.6	2.2	1.59	14.3	2.8	

Maximum increase and Maximum reduction = highest and lowest values noted beyond 7th day, as referred to 7-day-values.

group a significant increase in venous pressure and ascites occurred in 7 days and body weight, abdominal circumference and caval pressure having increased by 1.74 ± 0.66 kg, 8.1 ± 1.9 cm and 8.5 ± 1.3 mm Hg, respectively.

The figures quoted above were regarded as the preoperative basic values. Referred to these, significant reduction in ascites at the end of the first week was confined to a sole animal of the entire group and a significant reduction in the venous pressure was demonstrable in another animal.

The entire group revealed a significant further increase in abdominal circumference amounting to 4.1 ± 2.5 cm, while body weight and venous pressure were not significantly affected. Similar values were obtained on referring the maximum changes noted over the entire period to the "preoperative" (7 day) values: body weight, $+0.55 \pm 1.62$ kg; abdominal circumference, $+5.4 \pm 1.7$ cm; venous pressure, $+1.0 \pm 2.4$ mm Hg.

Even as compared to suitable controls, the rise of the thoracic duct—venous shunt is difficult to assess. The main obstacle lies in the failure of caval constriction to induce a steady state. In the control group, too, the ascites, and to a lesser degree the venous pressure, while steadily increasing in some animals, tended to diminish after an initial rise in certain others, and again in others the entire condition showed spontaneous variations, i.e. deterioration of the congestive signs after an initial amelioration. The figures in Table IV shown under the headings "maximum increase" and "maximum

Table V

Laboratory data of patient with liver cirrhosis (operation November 27, 1968)

	1968			1969		
	October 13	November 17	December 6	March 14	April 8	July 15
Body weight (kg)	86	77	72.5	72.5	72.5	71.5
Serum total protein (g per 100 ml)	6.6	7.4	7.1	6.5	5.6	8.0
Erythrocytes (million/cu. mm)	4.0	4.2	3.8	4.5		4.4
Serum bilirubin (mg per 100 ml)	3.8	1.4	0.9			1.6
Thymol test	3 U	2 U	2 U			1 U
Gold sol test	3+	∅	∅			∅
Se—Na (mEq/l)	129	135	133	135	139	137
Se—K (mEq/l)	2.9	4.3	4.2	3.2	3.7	4.6

reduction" are illustrative of these relationships by representing the maximum changes in both positive and negative directions, with reference to the basic values obtained on the 7th day after caval constriction.

A further factor making proper evaluation difficult was the adverse effect of caval constriction on the general condition of the animals. Parallel with the increase of ascites, a number of animals were losing weight and displayed an increased susceptibility to infections, particularly of enteral ones. The change in body weight was, therefore, no reliable indicator of the ascites. In the assessment of its amount we had to rely on the abdominal circumference.

IV. Lymphatic-venous shunt in a patient with liver cirrhosis

Although the value of the thoracic duct—jugular vein anastomosis could not be consistently proved in experimental ascites, we have none the less attempted this type of operation in a patient suffering from liver cirrhosis with ascites.

The patient, a 45 year old male had been disabled by liver cirrhosis with excessive ascites for the last five years requiring repeated hospitalization and oral diuretics. The latest admission had been made necessary by a further increase in ascites despite high doses of hydrochlorothiazide. On admission the abdomen was enlarged, tense, with engorgement of the collateral veins. There was moderate oedema of the lower limbs. After removal of the ascites the liver was palpable four fingerbreadths below the costal margin. X-rays revealed oesophageal varices.

A salt-free diet and diuretic therapy with mercurophylline and spironolactone caused a substantial reduction but no full control of ascites, complete disappearance of oedema and improvement of the general condition. In four weeks the patient lost 9 kg in weight, but the condition remained stationary and became unresponsive to further therapy. In the absence of any further improvement in another three weeks, surgery was indicated.

The operation was performed on November 27, 1968. From an approach between the two heads of the sternocleidomastoid, after transection of the muscles the external jugular vein was prepared free and retracted medially. The thoracic duct was identified by separating it from the loose connective tissue. The 7 mm thick lymphatic trunk was transected between two ligatures. Now the jugular vein was isolated in its entire length, slipped to the thoracic duct below the belly of the lateral scalenus muscle and joined with it end-to-end by invagination. Lymph immediately rushed through the vein at a high speed. A drain was left in place and the wound was closed.

All therapy including the salt-free diet was discontinued after operation. There was none the less a reduction in body weight and abdominal girth. Despite the patient's good appetite which was in accordance with the improvement in his general condition, he lost 4.5 kg and all signs of the ascites gradually disappeared.

Lymphography was performed 6 weeks after the operation. The rate of lymph flow was high, the contrast medium attained the plane of anastomosis in a few minutes and, despite its large amount (30 ml of Lipiodol UF) failed to give continuous filling.

Subsequently, the condition seemed to be stable and the patient was discharged without any dietary restriction or prescription of drugs. Seen 3.5 months later, he was symptom-free and had no evidence of ascites. Then he contracted influenza and his physician prescribed furosamide twice weekly. One month later the patient had slight hypopotassaemia without ascites or oedema. Despite his good appetite and over-indulgence in eating he had not increased in weight. Diuretic treatment was discontinued. One year after the operation he was symptomfree without requiring drugs or dietary restriction. Summing up, in this patient the ascites has thus been brought under permanent control by the creation of a lymphatic-venous shunt.

Discussion

The experience with thoracic duct cannulation has shown that the disorders in the production and transport of lymph play a decisive part in the development of the hepatic cirrhosis syndrome [1, 20, 20, 31]. It is assumed that lymphatic-venous drainage is inhibited at the cervical section of the thoracic duct, at its entry to the venous system. The obstacle may be con-

stituted by the increased venous pressure itself, or by a limited compliance of the wall or of the orifice of the lymphatic trunk, or by some morphological anomaly of these structures [16, 32, 41, 12].

Cinelymphangiographic studies [3, 19, 22] have shown that, if lymph production is not increased, there is no continuous flow in the thoracic duct, the contrast medium being forwarded dropwise, without any regularity or rhythm. Although on the ground of morphological studies, the thoracic duct does not seem to possess sphincter-like structures, its terminal portion often divides into two or three branches tapering off in the end. Their oblique course across the wall of the subclavian vein may be adverse to their evacuation [3]. It may be further assumed that parallel with the distension of the thoracic duct resulting from an excessive production of lymph, its orifice is drawn apart, becomes deformed, slit-like instead of its originally round or oval shape (NAITOVE cit. [12]). The absence of a continuous flow in the case of normal lymph production may also be due to the lack of a constant pressure gradient between the thoracic duct and the cervical veins which might maintain the lymph flow [32, 33]. There is, however, evidence to the contrary too, i.e. suggestive of the presence of a positive gradient [17, 27]. The discrepancy in the results is due to the fact that the quoted authors had left the integrated mean venous pressure out of account and assumed that as soon as the venous pressure rises above the thoracic duct pressure, the orifice closes. Therefore they considered only those phases of the venous pressure curve in which venous pressure was lower than the lymphatic pressure; in other words, it was only the lowest values which they accepted as venous pressure.

Lymph produced in normal amounts presumably does not fill entirely the lymphatics and part of the lymphatic bed is totally or partially collapsed. Under these conditions lymph transport in the peripheral pathways as also in the thoracic duct is maintained partly by extraneous factors of temporary character such as muscle contraction, intestinal peristalsis, respiratory movements, etc., partly by autonomous contractions of the lymph vessels. The flow of lymph, if its production remains within normal limits, is therefore little affected by a relative narrowing of the orifice of the thoracic duct. If, however, lymph production is excessive, resistance at the orifice greatly affects the dynamics of flow in the engorged vascular bed. An inverse (exponential) correlation has been in fact demonstrated between resistance and lymph flow velocity [32] and there are observations of excessively high thoracic duct pressure in liver cirrhosis [6, 13]. Other authors, however, failed to demonstrate an increased resistance at the lymphatic-venous junction in the laboratory animal in which excessive lymph flow had been induced by caval constriction or by forcing fluid in massive amounts through the thoracic duct. An elevation of the pressure gradient to 4.5 cm H_2O was attained only if the rate of flow was as high as 25 ml/min [29, 36]. The pres-

ent findings are at variance with these results. In caval constricted dogs with average rate of lymph flow of 5.9 ± 3.6 ml/min, we found a pressure gradient of 6.9 ± 0.8 mm Hg as against -0.7 ± 0.5 in normal animals. The difference in comparison to normal animals was probably due to a reduction in central venous pressure. Production of such a gradient would have been none the less impossible had lymph flow been unhindered. We therefore assume that with an enhanced lymph production there is a relative narrowing of the orifice which interferes with flow.

In the animals with induced pericarditis on the other hand, it is the high venous pressure which poses a mechanical obstacle to the evacuation of the thoracic duct. Correspondingly, thoracic duct and jugular mean pressure are nearly the same; in other words, there is no significant gradient between the two systems. Finally, both with caval constriction and with an increased central venous pressure, transection of the cervical portion of the thoracic duct eliminates the resistance interfering with lymph flow and thus promotes evacuation of the thoracic duct.

In accordance with data in the literature [2, 7, 10, 13, 20] we have been able to reduce both the ascites and portal (v. cava inferior) pressure by the cannulation of the thoracic duct. Some other observations were less consistent, and especially if the fluid and electrolyte losses have been corrected no effect on portal pressure and ascites accumulation was observed [14, 23, 26]. The effect of abdominal puncture or of diuretic therapy might be regarded in fact as basically similar to that of thoracic duct drainage. However, their effect is transitory and, as confirmed by the present study and earlier data [6, 8, 14], closure of the thoracic duct fistula is followed by a rapid accumulation of ascites and a rise in portal pressure.

In view of the obvious shortcomings of thoracic duct fistula, various procedures for the surgical construction of a new connection between the lymphatic and venous systems have been devised. The purpose of these interventions is not only to remove the obstacle to lymph flow as does a thoracic duct fistula but also to connect the lymphatic trunk with the venous system at a level where pressure is lower than in the cervical veins so as to avoid the obstacle to lymph flow represented by the high venous pressure. This latter consideration has led to attempts to return the lymph by the oesophagus [11, 24], a technique which has proved impracticable due to the formation of clots rapidly obliterating the lymphatic duct. The fact that pressure in the intrathoracic veins is lower than in those of the neck has resulted in techniques for the union of the intrathoracic section of the thoracic duct with the azygos and hemiazygos veins [40, 42]. If systemic venous pressure is elevated (congestive cardiac failure), the lymphatic-venous shunt must be formed at the level of the pulmonary veins [5, 40].

The construction of an intrathoracic lymphatic-venous anastomosis

represents a major surgical intervention. On the other hand, at that stage of liver cirrhosis where the necessity for a lymphatic-venous shunt may arise, the patient's condition is usually too poor for a grave surgical stress. Thus, the shunt must consist of a cervical anastomosis which involves a smaller stress and, theoretically, should be no less efficient. On the evidence of our experimental results, in experimental liver cirrhosis the central, i.e. cervical, venous pressure is low. Although in clinical cases of liver cirrhosis, central venous pressure is not constantly reduced, usually there is a significant gradient between the thoracic duct and the cervical veins.

The surgical technique described in the foregoing has proved successful. According to lymphangiographic and necropsy findings, cervical lymphatic-venous anastomosis by invagination remained patent in 15 out of 16 dogs. The effect of the shunt on the ascites and the portal hypertension was, however, less consistent. Disappearance of the ascites in the first week and a parallel reduction in the venous pressure were confined to 50% of the animals. Evaluation, however, is difficult in view of some factors inherent in the model employed in our experiments.

As it has been demonstrated in the control animals, ascites induced by constriction of the cava inferior vein is far from remaining stable. In the long run, there are spontaneous variations in portal pressure and in the amount of abdominal fluid accumulation. The causes of these variations are partly nonspecific such as the nutritional state, intercurrent infections, etc., and partly specific, inherent in the model. In the course of time, persistent post-sinusoidal congestion will not fail to affect the circulation and the structure of the liver. Such animals display gross hepatic changes. On the other hand, a collateral network develops or grows more extensive between the cava inferior and superior systems. This has been confirmed by our observation that in the animals with long-standing experimental narrowing of the vena cava inferior, constriction of the vena cava superior was immediately followed by a significant elevation of the pressure in the vena cava inferior. All these factors greatly affect the results of the lymphatic-venous shunt in experimental ascites.

The present model failed to prove conclusively the value of a lymphatic-venous shunt and all it permitted to predict with due caution was the probability that the intervention would be of benefit in some cases of ascites due to postsinusoidal congestion, we none the less decided for it in a patient with liver cirrhosis, having come to realize that to establish the value of this theoretically well-founded intervention, experimental models are not informative. Besides, the creation of a cervical shunt is a minor operation with practically no hazard.

The construction of a lymphatic-venous anastomosis in liver cirrhosis has had numerous advocates but it was not until the present studies were in

progress that actual observations on human patients were published [28]. The report in question concerns three patients. Two of them died soon after the intervention, but they had been poor risk cases. In the third patient, the sole survivor, the operation resulted in a complete disappearance of ascites. In conformity with this observation, in the present case too, ascites which had been unmanageable for years ceased soon after the operation without reappearing during the observation period of one year. Two successful cases are obviously inconclusive but certainly indicate that the cervical shunt may have a beneficial effect in liver cirrhosis associated with ascites. Correct indications and a proper selection of the cases are naturally basic requirements.

Enhancement of lymph drainage in liver cirrhosis was originally advocated for the reduction of portal hypertension and prevention of haemorrhage from oesophageal varices [2, 10, 12]. Our observations failed to contribute direct evidence to this controversial problem, it was, however, established that a lymphatic-venous shunt reduces congestion due to cava inferior constriction in a number of cases. Since, however, ascites induced in animals or accompanying liver cirrhosis responded favourably to the shunt, we must regard the presence of ascites as a basic indication.

As regards contraindications, the presence of hepatocellular failure in liver cirrhosis makes the intervention unwarranted. The observation that in the two animals with long-standing ascites the operation was soon followed by death illustrates the necessity for a critical attitude in the selection of cases. In view of the entry of massive amounts of fluid into the blood stream in consequence of the intervention [28], it should be avoided in diseases of the circulatory organs until conclusive observations have been collected. It must be noted that in the presence of systemic venous congestion a cervical shunt is useless.

Thus, the candidate for a lymphatic-venous anastomosis is a patient not too advanced in years (preferably under 60), with liver cirrhosis accompanied by ascites, no evidence of impaired liver function, a normal central venous pressure, and in fair general condition with no sign of malnutrition.

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Dr. György SZABÓ
Dr. Zsuzsa MAGYAR
Dr. Pál SERÉNYI

Budapest VIII,
Mező Imre út 17,
Hungary

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STEROIDS IN HUMAN SKIN AND HAIRS

V. QUANTITY OF FREE CHOLESTEROL AND CHOLESTERYL ESTERS IN THE HUMAN SKIN

By

I. TÓTH, I. FAREDIN and M. JULESZ

FIRST DEPARTMENT OF MEDICINE (DIRECTOR: PROF. M. JULESZ), UNIVERSITY MEDICAL SCHOOL, SZEGED

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Free cholesterol and cholesteryl ester contents of human skin has been studied in females with no endocrine abnormality, females with hirsutism and males with acne. The whole skin specimens, excised during surgery, were obtained from various regions of the human body. A portion of 80 to 100 mg of the whole skin was freezed with carbon dioxide ice on a freezing microtome and cut into 20 to 25 μ thick slices. The skin slices were extracted by soaking in ether–ethanol mixture (1 : 1). The extracted free and esterified cholesterols were separated on silica-gel-G plates by double thin-layer chromatography and they were estimated by the ZLATKIS, ZAK and BOYLE colour reaction. Total cholesterol of whole female abdominal skin (hairless) was found to consist approximately to two thirds of free cholesterol and nearly one third of esterified cholesterol. Pubic skin (hairy) contained more free as well as esterified cholesterol than did abdominal skin. The abdominal skin (hairy) of hirsute females with adrenocortical origin did not differ from the controls in respect of free or esterified cholesterol contents. The back-skin of male patients with acne contained significantly more free and esterified cholesterol than the skin of control males of similar age.

It has been shown that human skin synthetized cholesterol from acetate (BLOCH and RITTENBERG 1945, ANKER 1948, SRERE et al. 1950). Moreover, it was found that it is one of the most active cholesterol-synthetizing organs of the body besides the liver and the adrenal glands. Consequently, the steroids of the human skin have been intensively studied. Interest in this field has received new impulse by the discovery that the skin contains all the enzyme-systems necessary to catalyze the metabolism of steroids (OERTEL and TREIBER 1969) including the androgenically active steroids (FAREDIN et al. 1966 a, 1967 a, b, 1968, 1969, GALLEGOS and BERLINER 1967, BERLINER et al. 1968, GOMEZ and HSIA 1968, WILSON and WALKER 1969, MAUVAIS-JARVIS et al. 1969). According to these investigations androgenically active steroids can also be formed in the skin. Therefore the study of all sterane compounds which may be regarded as possible precursors of biologically active steroids (JUNGMANN 1968) acquired particular interest (OERTEL et al. 1968).

In an earlier study we showed that human skin contained cholesterol in considerable amounts (TÓTH et al. 1966, FAREDIN et al. 1966 b). The present study has been concerned with the quantitative considerations of free and esterified cholesterol contained in human skin.

Material and methods

Reagents and materials

Reagents and materials used as well as the method of thin-layer chromatography on silica-gel-G (Kieselgel-G nach Stahl, Merck, Darmstadt) were previously described (JULESZ et al. 1970).

Sterols for the study, i.e. cholesterol (cholest-5-ene-3β-ol), cholestanol (5α-cholestane-3β-ol), cholesteryl stearate (cholest-5-ene-3β-ol-stearate) and cholesteryl oleate (cholest-5-ene-3β-ol-oleate) were purified by thin-layer chromatography on silica-gel-G plates (Table I).

Destruction of the skin tissue and extraction of cholesterol

The surface of the whole skin specimen, excised during surgery, was wiped off with a cotton-swab wetted with physiological saline to remove any disinfectant. The skin slices were then freed of the subcutaneous fatty tissues. A portion of 80 to 100 mg of the whole skin was freezed with carbon dioxide ice on a freezing microtome and cut into 20 to 25 μ thick slices and weighed. The skin slices were repeatedly frozen with 1 ml of distilled water and stirred vigorously with a glass-rod.

The skin suspension thus obtained was shaken vigorously with 20 ml of ether—ethanol mixture (1 : 1) and left to stand for two hours. The extracting solvent was subsequently decanted from the skin residue, and after addition of further 20 ml of fresh solvent was allowed to stand for 22 hours with occasional shaking. Then the skin residue was soaked for another 5 days in 20 ml portions of ether–ethanol (1 : 1) changing the extracting solvent every day.

The ether was removed from the collected ether-ethanol extracts (140 ml) by distillation and the remaining extract was evaporated to dryness in vacuo.

The entire cholesterol content of the skin was extractable with this method. Namely, we subjected the remaining skin residue to alkaline destruction in a few instances, however, yielded neither free nor esterified cholesterol (TÓTH et al. 1966).

Purification of the cholesterol extract

The dry residue of the cholesterol extract obtained from the skin suspensions contains a considerable amount of impurities, therefore it had to be purified before separation of free and esterified cholesterol. The dry residue was dissolved in ether and was washed successively with 2 N NaOH solution and distilled water. The purified ether phase was dehydrated with anhydrous Na_2SO_4, filtered and evaporated to dryness.

Separation of free and esterified cholesterols by thin-layer chromatography

The dry residue of the purified cholesterol extract was dissolved in 10 ml of benzene, divided into two equal parts (extracts No. I and No. II) and evaporated again to dryness. The free and esterified cholesterols were separated on a 10×20 cm silica-gel-G plate (thickness: 0.25 mm). The thin-layer was divided on the glass plate into four strips measuring 4×2 cm (strips No. 1, 2, 3, 4), the starting point was formed 20 mm from the lower end of the plate. Extracts No. I and No. II were applied in 3×0.2 ml of benzene to strips No. 1 and No. 2 and dried in a stream of hot air (40 to 50 °C). Authentic cholesterol (40 μg) and authentic cholesteryl stearate (40 μg) were dropped onto strip No. 3, while strip No. 4 provided the blank. This was followed by the first run in 110 ml of a chloroform—carbon tetrachloride mixture (12 : 88, v/v) up to a line drawn 5 mm from the upper end of the glass plate.

The cholesteryl ester spots separated in this manner were located by the aid of an analytical quartz lamp (Desaga UV 131 000) and a separating line was drawn 5—10 mm below the lower edge of the spots.

Then the run was repeated in 110 ml of an ethanol–benzene mixture (6 : 94, v/v) up to the separating line (second run), thus separating the free cholesterol from fats and contaminants (Table I).

Table I

R_f values of free and esterified cholesterols on a silica-gel-G thin-layer

Cholesterols	Chloroform-carbon tetrachloride (12 : 88, v/v)		Ethanol-benzene (6 : 94, v/v)	
	Run, cm	R_f	Run, cm	R_f
Cholesteryl stearate	10.6	0.54	17.0	0.94
Cholesteryl oleate	9.6	0.49	17.0	0.94
Cholesterol	0.6	0.03	7.3	0.42
7-dehydrocholesterol	0.6	0.03	7.2	0.42
Cholestenol	0.4	0.02	7.0	0.40
20α-hydroxycholesterol	—	—	5.7	0.32
20α, 22-dihydroxycholesterol	—	—	4.0	0.23

Detection and elution of cholesterols

Strips No. 1 and No. 4 were covered with glass plates while spraying strips No. 2 and No. 3 with sulphuric acid—ferric chloride reagent. The bluish violet spots of extract No. II on strip No. 2 and those of the authentic cholesterol and the cholesteryl stearate on strip No. 3 appeared within a short time. 7-dehydrocholesterol, 7-ketocholesterol, 20α-hydroxycholesterol, 20α, 22-dihydroxycholesterol, cholest-7-ene-3β-ol, 5α-cholestane-3β-ol failed to give the said colour reaction.

The areas corresponding to the spots detected of the free and esterified cholesterols were marked on the strip No. 1 and No. 4, the adsorbents were scraped into centrifuge tubes and the cholesterols were eluted with 4×2 ml of an ethanol—ethyl acetate mixture (1 : 1). The collected eluates were evaporated to dryness in vacuo at 45 °C.

According to our present investigations 86—89% of cholesterol, 91—97% of cholesteryl stearate and 87—95% of cholesteryl oleate were recovered from the silica-gel-G layer.

Quantitative measurement of free and esterified cholesterols

The dry residue of the free and esterified cholesterols was submitted to the colour reaction described by ZLATKIS et al. (1953). The bluish violet colour was estimated photometrically against the corresponding blanks obtained by the dry residue of the extract eluted from the corresponding area of strip No. 4. The values obtained for the free and esterified cholesterol fractions were read from calibration curves, which were prepared by authentic cholesterol (20, 40, 80 and 120 μg) and authentic cholesteryl stearate (20, 40, 80 and 120 μg). The values thus obtained were referred to g wet skin-tissue and the final results were reported in terms of mg/g. The amounts for cholesteryl esters were given as cholesteryl stearate and free cholesterol.

Results

The point which seemed of primary interest was the amount of free and esterified cholesterol contained in whole abdominal skin. This was studied on surgical specimens of abdominal skin of non-hirsute females, who were operated on for various reasons, and did not have any endocrine disorders. The skin surface had been cleansed owing to the rutine preoperative procedures and the subcutaneous fat from the skin specimens was removed prior to processing.

Table II

Free cholesterol and cholesteryl ester contents of the abdominal skin in non-hirsute women

No.	Age (years)	Free cholesterol mg/g	Cholesteryl ester		Total cholesterol mg/g	Cholesteryl ester per cent
			in stearate mg/g	in cholesterol mg/g		
1	22	0.70	0.29	0.17	0.87	19.7
2	29	0.70	0.32	0.19	0.89	21.3
3	33	0.67	0.53	0.31	0.98	31.9
4	34	0.68	0.48	0.28	0.96	29.4
5	39	0.61	0.36	0.21	0.82	25.9
6	42	0.73	0.60	0.36	1.09	32.7
7	43	0.87	0.55	0.33	1.20	27.2
8	45	0.67	0.61	0.36	1.03	35.0
9	46	0.69	0.64	0.38	1.07	35.4
10	55	0.67	0.36	0.21	0.88	24.1
11	65	0.50	0.29	0.17	0.67	25.6
12	75	0.69	0.47	0.28	0.97	28.7
Limits		0.50—0.87	0.29—0.64	0.17—0.38	0.67—1.20	19.7—35.4
Average		0.68	0.46	0.27	0.95	28.1
Standard error		±0.025	±0.037	±0.022	±0.04	

Table III

Free cholesterol and cholesteryl ester contents in skin of females without and with hirsutism

No.	Age (years)	Source of skin	Free cholesterol mg/g	Cholesteryl ester		Total cholesterol mg/g	Cholesteryl ester per cent
				in stearate mg/g	in cholesterol mg/g		
			Non-hirsute women				
1	29	abdominal skin (hairless)	0.70	0.32	0.19	0.89	21.3
		pubic skin (hairy)	0.88	0.58	0.34	1.22	27.9
2	43	abdominal skin (hairless)	0.87	0.55	0.33	1.20	27.5
		pubic skin (hairy)	0.97	0.67	0.40	1.37	29.2
3	46	abdominal skin (hairless)	0.69	0.64	0.38	1.07	35.5
		pubic skin (hairy)	1.10	0.95	0.56	1.66	33.7
			Women with hirsutism				
1	30	hairy skin from thoracolumbal incision	0.76	0.64	0.38	1.14	33.3
2	33	hairy skin from thoracolumbal incision	0.94	0.65	0.38	1.32	28.8

Table IV

Free cholesterol and cholesteryl ester contents in back skin of males without and with acne

No.	Age (years)	Free cholesterol mg/g	Cholesteryl ester		Total cholesterol mg/g	Cholesteryl ester per cent
			in stearate mg/g	in cholesterol mg/g		
			Normal males			
1	22	1.03	0.25	0.15	1.18	12.71
2	24	1.01	0.41	0.24	1.25	19.20
3	19	0.55	0.43	0.25	0.80	31.25
4	20	0.50	0.31	0.18	0.68	26.47
Average		0.80		0.20		
Standard error		±0.143		±0.024		
			Males with acne			
1	19	1.59	0.45	0.27	1.86	14.52
2	17	1.65	1.02	0.60	2.25	26.66
3	18	1.79	0.90	0.53	2.32	22.84
4	18	1.60	0.93	0.55	2.15	25.58
Average:		1.66		0.49		
Standard error		±0.045		±0.075		
Statistical analysis:		free cholesterol		$t_{(6)} = 5.736$, $p<0.01$		
		esterified cholesterol		$t_{(6)} = 3.713$, $p<0.01$		

Table II shows the results obtained from abdominal skin of twelve non-hirsute females (between 22 and 75 years of age). The free cholesterol content of the whole (hairless) abdominal skin ranged between 0.50 and 0.87 mg/g limits with an average of 0.68 mg/g (S.E. = ±0.025). The cholesteryl ester content as expressed in free cholesterol is much less in these 12 abdominal skins. The two limits were 0.17 and 0.38 mg/g, the average was 0.27 mg/g (S.E. = ±0.022). Cholesteryl ester accounted for 19.7 to 35.4% of the total cholesterol, average: 28.1%.

In three females (aged 29, 43 and 46 years, respectively) free and esterified cholesterols were determined both in abdominal and in pubic skin. The pubic hairs having been removed preoperatively, the skin specimens were hairless. On the evidence of these investigations (Table III) pubic skin was containing more free and esterified cholesterol then the abdominal skin. The cholesteryl ester accounted for 27.9 to 33.7% of the total cholesterol of the pubic skin and for 21.3 to 35.5% of that of the abdominal skin.

Specimens of hairy abdominal skin obtained at surgery from two hirsute female patients (aged 30 and 33 years respectively) of adrenocortical origin revealed no significant difference from the hairless abdominal skin of non-hirsute females in respect of free and esterified cholesterol contents (Table III).

The skin excised from the back of four male patients with acne (between 17 and 19 years of age) was investigated for free and esterified cholesterol. The results are shown in Table IV. Back-skin specimens of four males, approximately of the same age, excised from the same regions served as controls. Comparison of the results revealed a significant increase both in free and in esterified cholesterols in the subjects with acne (free cholesterol: $t_{(6)} = 5.736$, $p < 0.01$; esterified cholesterol: $t_{(6)} = 3.713$, $p < 0.01$).

Discussion

Cholesterol content of the human skin has been studied by various methods (BÜRGER and SCHLOMKA 1928, MEYER 1931, KOOYMAN 1932). The procedures based on the Liebermann—Burchard colour reaction revealed that the skin contains free cholesterol, cholestanol and in addition to fast acting sterols, 7-dehydrocholesterol (cholesta-5,7-diene-3β-ol) (MILLER and BAUMANN 1954, REINERTSON and WHEATLEY 1959) and lathosterol (cholest-7-ene-3β-ol) (IDLER and BAUMANN 1952). 7-dehydrocholesterol has been found to be the precursor of vitamin B_3 formed in the skin (WINDAUS and BOCK 1937, WHEATLEY and REINERTSON 1958) and to be present, together with lathosterol intermediates of cholesterol. Cholesterols detected in the skin are found either free or in form of their esters with various fatty acids (stearic acid, palmitic acid, oleic acid etc.).

Free cholesterol and cholesteryl esters can be completely separated with our thin-layer chromatographic method. The sulphuric acid—ferric chloride colour reaction introduced by ZLATKIS et al. (1953) is specific for cholesterol and its esters. The interfering action of fast acting sterols and of cholesterol-metabolites (cholestanol, 7-ketocholesterol, 7-hydroxycholesterol) can be eliminated and information of the true cholesterol content of the whole human skin may be gained with this method.

According to our findings, in agreement wirh earlier results, the human skin is very rich in cholesterols. Free cholesterol accounts for nearly two thirds and esterified cholesterols for nearly one third of total cholesterol demonstrable in the whole abdominal skin of females (Table II). The average of total cholesterol resulting from the two values was in agreement with our earlier findings (FAREDIN et al. 1966 b). In pubic skin the cholesteryl esters also make up approximately one third of the total cholesterol content (Table III), thus the excess of total cholesterol in pubic skin as against abdominal skin is due to the higher values for both free and esterified cholesterols.

Considering that there is no noticeable difference between free and esterified cholesterol contents of skins of hirsute and non-hirsute females, we may presume that hirsutism does not involve disturbances in cholesterol metabolism of the skin (Table III). The situation is different in male patients with acne. The free and esterified cholesterol contents in back skins of male patients with acne were significantly greater than in back skins of control males of similar age (Table IV).

It is documented by numerous literature data that the epidermis and the dermis differ widely in lipid and sterol concentrations (LŐRINCZ and STOUGHTON 1958, MONTAGNA and LOBITZ, Jr. 1964). On the grounds of the present results, even the various regions of the human body present wide variations in respect of free and esterified cholesterol contents. In the case of abdominal versus pubic skin, it is probably the different number and function of hair follicles, sebaceous and sweat glands which account for the divergence.

*

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István TÓTH
Dr. Imre FAREDIN
Dr. Miklós JULESZ

First Department of Medicine,
University Medical School,
Szeged, Korányi rakpart 12, Hungary

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DIE PHYSIKALISCHEN GRUNDLAGEN DES ELEKTROENZEPHALOGRAMMS

DAS POTENTIAL DER DOPPELSCHICHT AN DER OBERFLÄCHE EINES GESCHLOSSENEN SPHÄRISCHEN RAUMS

Von

T. GERÉB

LANDESHEILANSTALT FÜR NERVEN- UND GEISTESKRANKHEITEN
(DIREKTOR: DR. B. MÁRIA), BUDAPEST

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Um die Analyse der Potentialschwankungen im EEG, die den epileptischen Anfall begleiten, zu ermöglichen, wurde das Potential der im Innern eines sphärischen Raums gelegenen Doppelschicht an dessen Oberfläche errechnet. Durch die Anwendung der erhaltenen Formeln werden einige sonst schwer deutbare Besonderheiten der Krampfpotentiale erklärt.

Trotz der klinischen Bedeutung der Elektroenzephalographie wurden ihre physikalischen Grundlagen bisher wenig erforscht. Der erste Schritt in dieser Richtung sollte die Bestimmung jener Beziehung sein, in der die von der Kopfoberfläche abgeleiteten Potentiale zu den Quellen der im Innern des Kopfes fließenden Ströme stehen. Man nimmt mit Recht an, daß diese Ströme aus den Nervenzellen der Hirnrinde auf einer mit dem gut bekannten Aktionspotential analogen Weise entspringen, der wesentlich langsamere zeitliche Verlauf dieser Potentialschwankungen weist aber darauf hin, daß der nähere Ort der Stromquellen in den auf die Hirnoberfläche senkrecht orientierten Dendriten von niederer Reizbarkeit zu suchen ist. Aus dem Gesichtspunkte der Physik suchen wir also das Potential einer mit der Hirnoberfläche gleichlaufenden Doppelschicht. Im unbegrenzten Medium wird das Potential der Doppelschicht durch eine einfache Formel ausgedrückt, in einem geschlossenen Raum aber wird das Kraftfeld wegen der Grenzflächen stark deformiert, besonders dann, wenn — wie im Falle des EEG — die Stromquellen in der Nähe der Grenzfläche — hier der Kopfoberfläche — liegen. Das Potential des Dipols an der Oberfläche eines geschlossenen sphärischen Raums wurde schon bei den Forschungen über das EKG bestimmt, das Potential der Doppelschicht am selben Orte ist aber bisher nicht angegeben worden und GEISLER und GERSTEIN, die Autoren, die sich mit dieser Frage zuletzt beschäftigten, ersetzten die zweidimensionale Doppelschicht mit einem hypothetischen »äquivalenten Dipol-Modell«, ohne eine Methode zur Bestimmung seiner Größe und Lage anzugeben.

Wir wollen vorläufig nur den einfachsten Fall betrachten, wo die paroxysmale Tätigkeit sich über Teile oder über die Totalität der Hirnrinde gleichzeitig verbreitet, also den Fall der lokalen oder generalisierten Epilepsie. Da

in diesem Falle im Innern der Hirnfurchen zwei in entgegengesetzter Richtung gerichtete Doppelschichten in gleicher Phase einander gegenüberstehen, deren Potential auf der Oberfläche des Kopfes in erster Näherung vernachlässigt werden kann, vereinfacht sich unsere Aufgabe auf die Ermittlung des Potentials der mit der Kopfoberfläche parallelen Doppelschicht.

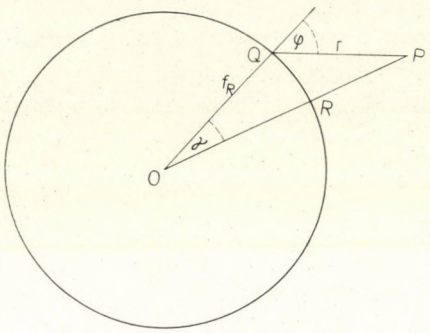

Abb. 1. Erklärung im Text

Der Ausgangspunkt unserer Untersuchung ist das Potential des im Innern eines geschlossenen sphärischen Raums gelegenen, auf die Oberfläche senkrechten Dipols an der Oberfläche dieses Raums:

$$V_D = \frac{M}{fR^2}\left(\frac{1-f^2}{(1+f^2-2f\cdot\cos\gamma)^{3/2}} - 1\right)$$

wo M das Dipolmoment, R den Radius der den Kopf vertretenden Kugel, fR den Abstand des Dipols vom Mittelpunkt der Kugel, also f eine Verhältniszahl kleiner als 1 (die am Kopf meistens zwischen 0,8 und 0,95 liegt), γ den Winkel zwischen dem zum Aufpunkt gezogenen Radiusvektor und der Verlängerung der Dipolachse (fR) bedeuten. Das wollen wir nun mit dem Flächenelement multiplizieren und an der Oberfläche der Kugel vom Halbmesser fR integrieren. Dann bedeutet M das Dipolmoment je Flächeneinheit. Mit Rücksicht darauf, daß

$$\frac{1-f^2}{f(1+f^2-2f\cdot\cos\gamma)^{3/2}} = \frac{2(\cos\gamma - f)}{(1+f^2-2f\cdot\cos\gamma)^{3/2}} + \frac{1}{f(1+f^2-2f\cdot\cos\gamma)^{1/2}}$$

ist und aus dem Dreieck OPQ (Abb. 1) wir erhalten

$$r = R\sqrt{1+f^2-2f\cdot\cos\gamma}$$

und

$$\cos\varphi = \frac{\cos\gamma - f}{\sqrt{1+f^2-2f\cdot\cos\gamma}}$$

und der räumliche Winkel eines Flächenelements dA

$$d\Omega = \frac{dA \cdot \cos\varphi}{r^2}$$

ist, kann das gesuchte Integral auf die folgende einfache Formel gebracht werden:

$$V_{DD} = M\left(2\int_{\Omega} d\Omega + \frac{1}{fR}\int_{A}\frac{dA}{r} - \frac{1}{fR^2}\cdot\int_{A} dA\right)$$

Das erste und das dritte Integral können wir gleich aufschreiben: Ω und A. Um das zweite Integral zu erhalten, schreiben wir in räumlichen Polarkoordinaten, r und $dA = (fR)^2 \sin\vartheta\, d\vartheta\, d(\varphi - \varphi_1)$ substituierend

$$\frac{1}{fR}\int_{A}\frac{dA}{r} = \int_{\varphi-\varphi'=0}^{2\pi}\int_{\vartheta=0}^{\Theta}\frac{f\sin\vartheta\, d\vartheta\, d(\varphi-\varphi_1)}{\sqrt{1+f^2-2f\cdot\cos\gamma}}$$

wo

$$\cos\gamma = \cos\vartheta\cos\vartheta_1 + \sin\vartheta\sin\vartheta_1\cos(\varphi-\varphi_1)$$

ist, γ den Winkel zwischen dem Radiusvektor zum Aufpunkt P und dem Vektor des Dipols, ϑ_1 und φ_1 die räumlichen Polarkoordinaten des Aufpunktes, ϑ und φ die des Vektors des Dipols bedeuten.

Bei achsensymmetrischer Lage des Aufpunktes, also wenn $\cos\gamma = \cos\vartheta$ bzw. $-\cos\vartheta$ ist, erhalten wir das Integral in geschlossener Form:

$$\frac{1}{fR}\int\frac{dA}{r} \quad \text{im Aufpunkt } P_0 = 2\pi\left[\sqrt{1+f^2-2f\cdot\cos\Theta} - (1-f)\right]$$
$$\text{im Aufpunkt } P_\pi = 2\pi\left[(1+f) - \sqrt{1+f^2+2f\cdot\cos\Theta}\right]$$

Wir wollen uns nun die Mühe des Rechnens durch die folgende graphische Methode möglicherweise ersparen. Man zeichnet um denselben Mittelpunkt zwei Kreise von den Halbmessern f und 1 und bildet an ihnen den Aufpunkt und im verkleinerten Maßstabe die Doppelschicht an (Abb. 2). Dann ist die Fläche der Kugelkalotte von der Höhe h im Verhältnis von $1 : R^2$ kleiner als die Fläche der Doppelschicht A auf der Oberfläche der Kugel vom Halbmesser fR. Der Kreis vom Radius f schneidet die Symmetrieachse im Punkte A. Man nimmt den Abstand von den Aufpunkten P_0 bzw. P_π bis die Berandung der Kugelkalotte in Zirkelweite und erhielt dadurch auf der Symmetrieachse die Punkte B bzw. C. Wie man in Abb. 2 sieht

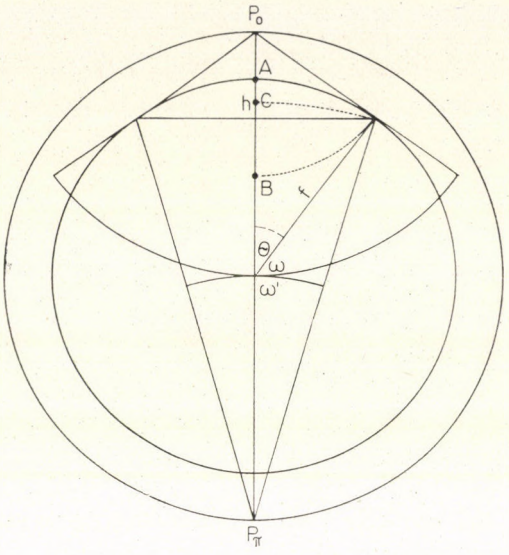

Abb. 2. Erklärung im Text

$$AB = \sqrt{1 + f^2 - 2f \cdot \cos\Theta} - (1 - f)$$

$$AC = (1 + f) - \sqrt{1 + f^2 + 2f \cdot \cos\Theta}$$

Die Fläche der Kugelkalotte von der Höhe h ist $2\pi fh$, die der Doppelschicht also $2\pi fR^2h$, so

$$\frac{A}{fR^2} = 2\pi h$$

Da der ebene Winkel des vollen Kreises 2π und der räumliche Winkel der ganzen Kugel 4π ist, um 2Ω zu erhalten, multipliziert man den im Längenmaß ausgedrückten Wert des am Papier gemessenen Winkels ω bzw. ω' mit 4. Der Arzt kann also das Potential elementar erhalten
oberhalb der Doppelschicht (in relativen Werten)

$$V_{DD}(P_0) = -[4\omega + 2\pi(AB - h)]$$

und auf der Gegenseite

$$V_{DD}(P_\pi) = 4\omega' + 2\pi(h - AC)$$

wo wir zugleich berücksichtigt haben, daß unsere Doppelschicht im Gehirn immer die negative Seite zur Oberfläche des Kopfes wendet.

Bei nur rotationssymmetrischer Lage des Aufpunktes können wir das zweite Integral noch in Kugelfunktionen ausdrücken

$$\frac{1}{fR}\int\frac{dA}{r} = 2\pi\left\{(1-x)f + \sum_{u=1}^{\infty}\frac{f^{n+1}}{2n+1}\,P_n(x_1)\,(P_{n-1}(x) - (P_{n+1}(x)\right\}$$

wo x und x_1 die Kosinusen von Θ und ϑ_1, P_n die entsprechenden, aus Tabellen entnehmbaren Kugelfunktionen bedeuten.

Da aber $(1-x)f = h$ ist, um das Potential einer auf die Oberfläche negativen Doppelschicht bei beliebiger Lage des Aufpunktes zu erhalten, addiert man zu 2Ω

$$2\pi\sum_{u=1}^{\infty}\frac{f^{n+1}}{2n+1}\,P_n(x_1)\left(P_{n+1}(x) - P_{n-1}(x)\right)$$

Wenn wir das Potential des auf die Oberfläche senkrechten Dipols in Kugelfunktionen ausgedrückt hätten,

$$V_D = \frac{1}{fR^2}\sum_{1}^{\infty}(2n+1)\cdot f^n\cdot P_n(x)$$

würden wir das Potential der Doppelschicht ebenfalls in Kugelfunktionen erhalten haben

$$V_{DD} = 2\pi\sum_{n=1}^{\infty} f^{n+1}\,P_n(x_1)\left(P_{n-1}(x) - P_{n+1}(x)\right)$$

dann aber müßte man die mühsamere numerische Errechnung und den Verzicht auf die Anschaulichkeit mit in Kauf nehmen.

Diese Formeln zeigen nun, wie die Potentialdifferenz je zweier beliebigen Punkte der Kopfoberfläche von der Lage und Verbreitung einer erregten Gehirnfläche abhängt. Man sieht, daß parallel mit der Verbreitung der Erregung über die Oberfläche des Gehirns das Potential im absoluten Wert zuerst wächst, dann aber abnimmt. Man versteht, warum zwischen zwei Elektroden auch dann eine Potentialdifferenz bestehen kann, wenn unter ihnen eine gleichstarke Erregung vorliegt. Wenn sich die Erregung über der unteren Oberfläche des Gehirns verbreitet, wird man aus den Elektroden am Scheitel — wenn überhaupt — eine positive Potentialschwankung registrieren. Diese Möglichkeit wurde bisher wegen des großen Elektrodenabstands kaum in Erwägung gezogen und ist bei der Annahme eines einfachen Dipols tatsächlich schwer vorstellbar. Unsere Formeln zeigen aber, daß, wenn sich die Erregung über eine größere Gehirnfläche verbreitet, die Höhe des Potentials der Höhe des von der Nähe eines Elektroden stammenden Potentials gleichkommen kann. Die allerdings sehr seltenen positiven Krampfentladungen wird man also mit Recht auf eine sich über der unteren Gehirnoberfläche verbreitenden paroxysmalen Tätigkeit zurückführen. Wenn sich eine gleichstarke Erregung — eine Doppelschicht vom konstanten Dipolmoment — über die ganze

Gehirnoberfläche ausbreitet, da dann $2\Omega = 0$ wird, ist das Potential an jedem Punkte der Kopfoberfläche

$$V_{DD} = 0 + 4\pi f - 4\pi f = 0$$

In ziemlich seltenen Fällen sieht man ja, daß der epileptische Anfall im EEG von einer allgemeinen »Desynchronisation«, also vom Verschwinden praktisch aller Potentiale, begleitet wird. Ist aber, wie meistens, die sich über der ganzen Hirnrinde verbreitete Erregung keine gleichmäßige, kann man von den beiden Seiten der Doppelschicht gleiche Mengen elektrischer Ladungen, da sie zum äußeren Potential nichts beitragen, im Gedanken so lange abnehmen, bis nur lokale Doppelschichten zurückbleiben, die die registrierte Potentialschwankung allein verursachen. Da die Erregbarkeit der Hirnrinde auf der motorischen Zone und über gewissen Teilen der temporalen Rinde am höchsten ist, ist es natürlich, daß bei einer generalisierten Krampftätigkeit irreführende »lokale«, besonders temporale und präzentrale Krampfpotentiale im EEG einen lokalen Herd vortäuschen können.

Es wird gehofft, daß ein besseres Verständnis der physikalischen Grundlagen des EEG, wie es schon aus diesen kleinen Winken ersichtlich ist, dem Elektroenzephalographer auch bei der Lösung diagnostischer Fragen Hilfe leisten wird.

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Dr. Tibor GERÉB; Budapest XI., Szakasits Árpád u. 10, Ungarn

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DETERMINATION OF PREGNANEDIOL IN URINE BY THIN-LAYER CHROMATOGRAPHY

By

T. FEHÉR, K. G. FEHÉR and L. BODROGI

FIRST DEPARTMENT OF MEDICINE AND SECOND DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY, SEMMELWEIS UNIVERSITY MEDICAL SCHOOL, BUDAPEST, HUNGARY

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A simple specific method is described for the determination of pregnanediol in urine by thin-layer chromatography. The method is suitable for estimating the steroid level in urine of non-pregnant and pregnant women as also of men and children. The technique enables a simultaneous determination of pregnanediol and pregnanetriol in the same specimen. Results of control experiments, normal values and estimates in typical endocrinopathies are summarized.

Pregnanediol (5β-pregnan-3α,20α-diol) has first been determined in urine by VENNING [1]. An improved method combining pregnanediol acetylation and column chromatography was worked out by KLOPPER et al. [2]. Recent control experiments have shown [3—5] that the purification step applied in these and some other classical methods resulted in a great loss of pregnanediol, further, the steroid fraction, despite circumstantial purifications, still contained considerable amounts of aspecific chromogens and other steroid contaminants. Thin-layer [6—10] or gas-liquid chromatography [11—18] allow an accurate and specific separation and purification of pregnanediol from urinary extracts [3—5].

A simple routine procedure for the determination of pregnanediol has been employed for years in the steroid laboratory of our Departments. The method appeared to be suitable for measuring the excretion rate of steroid in the urine of non-pregnant and pregnant women and in that of men and children. The technique has been controlled in several respects, and the normal values have been determined in a number of cases.

In this paper the method and its results will be reported in detail.

Method

To a 20 ml specimen of 24 hr urine, 0.25 ml of 10% formalin solution and 3 ml of concentrated HCl were added and the mixture was boiled for 20 min. After hydrolysis, the cooled material was extracted with 20 + 10 ml ether—ethyl acetate—ethanol (20 : 20 : 10). The pooled extract was washed with 5 ml 1 *N* NaOH and twice with 5 ml water, dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The dry residue was dissolved in chloroform. For non-pregnant women, and men or children, 0.5 ml, for pregnant women 1.0 ml of the solvent was used.

In first cases, 0.25 ml, in the second, depending on the time of gestation, 0.05 or 0.1 ml extract was applied to 1.5 cm strips of silica gel G (Merck AG) layer. The size of the plate was 200 × 200 × 0.2 mm. Ten, 20 and 30 μg authentic pregnanediol were also applied for quantitation. Every third strip contained 3 to 5 μg cortisone-bis-dinitrophenylhydrazone as marker. Ascending chromatography without equilibration was employed. The steroids were

chromatographed in the solvent system chloroform-acetone (90 : 10). To increase the resolving power, the chromatoplate was dried at room temperature after the first development and repeatedly chromatographed in the same direction and solvent system.

After development, the zones corresponding to the steroid dye were marked. An incidental shift of mobility on some strips was further controlled in UV-light using a violet filter (λ_{max} 400 nm) by comparing the mobility of fluorogens routinely observed in urinary extracts even without the use of any reagent. The zones were scraped into centrifuge tubes and suspended in exactly 4.0 ml of concentrated H_2SO_4. The tubes were incubated at 100 °C for 4 min, cooled and centrifuged at 5000 r.p.m/min.

Pregnanediol was estimated in the clear supernatant by a Spectromom-360 (MOM, Budapest) spectrophotometer at 390, 425 and 460 nm in 1 cm cuvettes. The pregnanediol standards served for calibration, and ALLEN's equation [19] was used for correction of values. The blank value obtained for a zone not containing steroid was consequently below E = 0.005.

For the simultaneous determination of pregnanetriol from the same urine sample, enzymatic hydrolysis was performed, 500 U/ml of β-glucuronidase (Reanal, Budapest), 500 U/ml of penicillin and 2.5 ml of 1 *M* acetate buffer (pH 4.8) were added to the urine, and it was incubated at 37 °C for 16 to 24 hrs. For the separation of pregnanediol and pregnanetriol, the solvent system chloroform-acetone (80 : 20) was used.

Results

Control experiments

Reliability of the method was controlled by recovery experiments. In 45 estimates, 10 to 250 μg/10 ml authentic pregnanediol was added to urine of non-pregnant and pregnant women and to that of men. As it can be seen in Table I, mean recovery was above 80% and the standard error (S.E.) below

Table I

Control of methodology

Recovery experiments			
Steroid added μg/10 ml urine	N	$\bar{x} \pm$ SE, %	
10	3	89.0±9.5	□, I
20	10	81.1±4.6	□, I
30	4	94.3±2.7	□, I
25—250	28	89.8±2.8	§, II
estimations in duplicate			
	N	$\bar{x} \pm$ SD, mg	
	21	2.4±0.4	□, I
	17	2.7±0.3	□, II
	12	34.8±3.8	§, II

N number of estimations
$\bar{x}$ mean
SD standard deviation
□ estimations from urine of men and non-pregnant women
§ estimations from urine of pregnant women
I material of First Department of Medicine
II material of Second Department of Obstetrics.

10%. Parallel pregnanediol determinations in the urine of 21 men and 17 non-pregnant women yielded a mean between 2 and 3 mg with a standard deviation (S.D.) of 0.4 and 0.3 mg, respectively.

Sensitivity of the method was determined by the lowest quantitative limit of sulphuric acid reaction performed in the presence of adsorbent. It was found between 1 and 2 μg of pregnanediol. Further control experiments showed Lambert—Beer's law to be valid in a range of 2 to 200 μg of the steroid. Based on these findings, the excretion rate of pregnanediol is expressed with a precision of 0.1 mg/24hr.

Specificity of the technique was controlled as follows. The resolution of 8 epimeric pregnanediols and most of the naturally occurring androgen, oestrogen and corticosteroid metabolites was studied by the thin-layer chromatographic method employed for the separation of 5β-pregnan-3α,20α-diol. As seen, of these steroids, a negligible amount of 5β-pregnan-3α,20β-diol may contaminate the 5β-pregnan-3α,20α-diol zone ($R_{f\text{-}diff} = 0.08$). Consequently, due to its occasional occurrence in human urine, the values presented below may include some of the 20β-epimer. According to our earlier experiments [20], the most faultless marking of the mobility of steroids on chromatoplates is done by steroid-dyes having similar chemical-chromatographic properties as the free steroids to be determined. By this method of marking, and by the UV-detection of the position of urinary fluorogens, the most frequent failure of thin-layer chromatography could be eliminated. Analysis of the UV absorption spectra of the pregnanediol zones obtained revealed a sharp maximum at 430 nm. No shift in the adsorption maximum of pregnanediol (pregnanetriol) was indicated by the sulphuric acid reaction performed in the presence of adsorbent. On the other hand, the chromatographic property of 5β-pregnan-3α,20α-diol-diacetate in the solvent system n-hexane-ethyl acetate (80 : 20) and that of 5β-pregnan-3,20-dione in benzene-ethyl acetate (85 : 15), studied after the acetylation or CrO_3-oxidation of the isolated steroid was found similar to that of authentic 5β-pregnan-3α,20α-diol derivatives. The detection of these chromatoplates by vanillin-perchloric acid [21] or anisaldehyde-H_2SO_4 [22] showed only a single steroid to be present.

Normal excretion

Values for the physiological excretion of pregnanediol as determined in the two laboratories are compiled in Table II. In the urine of children aged 4 to 10 years, little pregnanediol was found, and there was no correlation between steroid excretion and chronological age. Excretion of pregnanediol by non-pregnant women was studied in four periods of the cycle at days 4—8, 11—15, 19—23 and 26—30. In these cases the whole cycle could not be followed, the time of urine collection was related to the day of menstruation.

Table II

Normal urinary pregnanediol values

Subjects, age	N	Mean	Range
		mg/24 hr	
Children, 4—10 years	10	0.2	0.1— 0.5
Women			
days 4—8 of cycle	12	0.6	0.3— 1.0
days 11—15 of cycle	12	1.1	0.4— 2.1
days 19—23 of cycle	15	3.3	1.5— 5.6
days 26—30 of cycle	21	1.0	0.2— 2.2
Women, 46—60 years post-climacteric	6	0.6	0.3— 1.0
Men, 18—45 years	11	0.8	0.4— 1.4
Pregnant Women			
I—II month of gestation	5	10.1	6.1— 16.0
III—IV month of gestation	10	13.6	7.3— 22.7
V—VI month of gestation	9	22.7	16.6— 47.0
VII—VIII month of gestation	6	35.2	21.2— 51.0
IX—X month of gestation	18	47.7	26.0—110.0

N number of cases

Variation of pregnanediol values during the cycle showed a physiological ovarian function. Excretion of pregnanediol by healthy women in the post-climacteric stage decreased significantly, while in men it was similar to the values for the 4th to 8th days of the cycle of normal women.

Table II shows pregnanediol values in the urine of normal pregnant women in 5 periods of gestation. Such a limited number of cases did not allow a more detailed grouping of physiological pregnancy.

Pregnanediol was determined in the urine of 5 normal women on 4 occasions, on the 7th, 14th, 21th and 26th day of the cycle. Results are shown in Table III, which includes in addition values for some typical endocrinopathies. These estimations served as an additional control of the method. As it may be seen in Table III, pregnanediol excretion decreased in patients with disturbances in the cycle without androgen overproduction, and increased in those with persisting corpus luteum. An elevation of pregnanediol excretion in Cushing's syndrome and the adrenogenital syndrome, and a normal level or a decrease in subjects with simple obesity were observed. Corticotrophin, metyrapone and human chorion gonadotrophin induced an elevation of the pregnanediol values under physiological conditions.

Table III

Pregnanediol values in urine of healthy women and of patients with endocrinopathies

Subjects Disease Age, years Sex	Day of cycle			
	7	14	21	26
Normal				
18 F	0.5	0.6	1.3	0.2
21 F	0.7	0.7	3.4	1.1
22 F	0.3	0.7	2.7	0.7
26 F	1.0	1.8	2.2	0.8
38 F	0.3	1.2	1.8	1.0
Disturbances in cycle				
20 F	0.3	0.4	0.7	
23 F	0.1	2.1	1.0	0.1
25 F	1.0	1.9	1.1	
26 F		1.1	0.5	
32 F		1.1	0.7	0.7
Persisting corpus luteum				
22 F		1.8	3.4	2.3
26 F		1.7	1.2	4.6
28 F		1.7	0.5	1.6
Cushing's syndrome				
26 F	4.5			
30 F	3.5			
36 M			2.8	
45 F			0.8	
Obesity				
23 F	0.4			
24 F	0.3			
26 F	0.6			
28 F			1.0	
ACTH			2.1	
Adrenogenital syndrome				
4 F			1.3	
6 M			1.6	
22 F			4.0	
Normal				
30 F	1.2			
ACTH	1.8			
Mety I	1.5			
Mety II	15.7			

Table III (cont.)

Subjects Disease Age, years Sex	Day of cycle			
	7	14	21	26
32 F	0.8			
ACTH	1.7			
Mety I	6.0			
Mety II	7.2			
26 M		0.3		
HCG		2.0		

ACTH = corticotrophin
Mety = metyrapone
F = female
M = male
HCG = human chorion gonadotrophin

Discussion

A simple thin-layer chromatographic method suitable for the routine estimation of pregnanediol in urine has been worked out. As to its specificity control experiments have shown that the 5β-pregnan-3α,20α-diol values may have been contaminated by a negligible amount of 5β-pregnan-3α,20β-diol only. The procedure was found to allow, in addition to the resolution of the naturally occurring C_{18}, C_{19} and C_{21} steroids, a sharp separation and the simultaneous determination of pregnanediol, allopregnanediol (5α-pregnan-3α,20α-diol) and pregnanetriol (5β-pregnan-3α,17α, 20α-triol) from the same urine specimen. According to comparative tests, after acid and enzymatic hydrolysis the pregnanediol values were still within the methodological variation even at high enzyme concentrations. As regards the pregnanetriol determination, we refer to an earlier paper [23].

Our normal pregnanediol values were found lower than those measured by the classical methods [2, 24, 25]; they were, however, in the range as determined by the newer thin-layer or gas-liquid chromatographic procedures [8—18]. This can be ascribed to the effective separation of pregnanediol from other steroid metabolites and non-steroidal contaminants by thin-layer chromatography, particularly with double development introduced in the present method.

Determinations in some typical endocrinopathies performed as an additional control of our method revealed aberrations in steroid metabolism observed by other authors [5, 16, 18, 24, 26]. The importance of pregnanediol determinations to control placentar and ovarian function is indisputable [2, 5, 16, 24]. However, it has already been suggested in an early work of KLOPPER et al. [26] that pregnanediol assay may be of value in evaluating adrenal function. An increased pregnanediol excretion in Cushing's syndrome

and the normal or subnormal amounts in simple obesity observed by us indicate the differential diagnostic value of pregnanediol estimation in these conditions. The insufficient enzyme function in the adrenogenital syndrome may result in an augmented secretion of the early precursors of adrenal hormones, a situation in which an increase in pregnanediol excretion may be expected. The elevation of the pregnanediol values after the administration of corticotrophin or of metyrapone pointed to an adrenocortical influence on the production of pregnanediol precursors progesterone and/or pregnenolone [16]; this, however, will have to be settled by future studies.

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Dr. Tibor FEHÉR
Lajos BODROGI

First Department of Medicine, Semmelweis University Medical School, Budapest VIII., Korányi S. u. 2/A, Hungary

Katalin G. FEHÉR

Second Department of Obstetrics and Gynaecology, Semmelweis University Medical School, Budapest VIII., Üllői u 78/A, Hungary

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EFFECT OF FLAVONE-FREE DIET AND OF ATHEROGENOUS TREATMENT ON LIPID CONTENT AND INTIMAL STRUCTURE OF RAT AORTA

By

R. Szabó, A. Antal, A. Rednik, K. Lipták, I. Győri, M. Gábor and S. Benkő

SECOND DEPARTMENT OF MEDICINE, UNIVERSITY MEDICAL SCHOOL, AND INSTITUTE OF PHARMACODYNAMIC RESEARCH, SZEGED

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The effects of the Sherman-LaMer-Campbell diet (Sh), cholesterol-feeding (Ch) and thyroidectomy (Th) and their combinations have been studied in the rat. Thyroidectomy and cholesterol feeding reduced capillary resistance, an effect further enhanced by flavone deficiency. The lowest limit of the increase in the cholesterol level at the end of a ten week period was found in the group fed a flavone-deficient diet but no cholesterol. In the groups subjected to atherogenous treatment, total lipids accounted for a relatively larger fraction of aortic wet weight than in the flavone-deficient groups. Cholesterol concentration in the aorta revealed only minor differences between the individual groups and even these were unrelated to the changes in serum cholesterol level or in capillary resistance. The intimal surface of the aorta displayed the most conspicuous changes in the case of flavone deficiency and of flavone deficiency combined with thyroidectomy (Th + Sh). The lesions thus produced were primarily attributed to an accumulation of mucoids and, in the second place, to a swelling of the ground substance. Flavonoid (hesperidine-methyl-chalcone) had an inhibitory effect on the elevation of serum cholesterol and on the reduction of capillary resistance but had no notable influence on the intimal changes in the aorta, nor on the increase in its lipid concentration resulting from the combined effect of atherogenous treatment and a flavone-deficient diet.

It has long been known that flavone-deficient diets affect capillary resistance in the skin of the rat [1, 2, 3]. Various atherogenous factors have also been found to reduce capillary resistance in laboratory animals [4—7]. Increased capillary fragility due to flavone-deficiency or to atherogenous factors proved to respond well to flavone treatment [4—9]. Vascular purpura as a sign of capillary fragility may accompany human atherosclerosis [10], and it has been shown that flavonoid treatment may have a beneficial influence on reduced capillary resistance associated with human atherosclerosis [11].

In view of the part played by vascular lesions in atherogenesis [12] it may be assumed that flavone deficiency promotes lipid deposition, in other words, the development of atheromatous lesions, by inducing functional (possibly structural) changes of the vascular wall. Certain flavonoids have been found to prolong the survival of rats subjected to atherogenous treatment [13]. In certain species (rabbit, chick) the changes of the arterial elastic structure associated with atherosclerosis proved to respond well to flavone therapy [14]. Administration of vitamin P was followed by a reduction of the hypercholesterolaemia and an improvement of the fatty degeneration of the liver induced by cholesterol feeding [15].

In view of these experimental facts it seemed of interest to compare the effects of a flavone-deficient diet and of atherogenous factors on capillary resistance and on certain other parameters closely related to the process of atherogenesis. It was also studied whether these two factors were synergistic, and whether the lesions induced by flavone deficiency combined with atherogenous factors would also respond to flavone treatment.

Material and methods

Eighty-nine male R-Amsterdam rats weighing between 160 and 230 g were divided into seven groups. Details of the interventions are shown in Table I.

A group fed the Sherman-LaMer-Campbell diet and a sham-operated group, served as controls. Cholesterol feeding, being incapable to induce atherosclerosis in the rat [16] was not used alone.

The surgical interventions were performed under ether anaesthesia three weeks before the dietary periods of 10 weeks. The sham-operation consisted in a horizontal incision of the cervical skin, slight separation of the connective tissue and suture of the wound. The normal (control) diet was standard compressed rat food. The vitamin C and flavone-deficient Sherman-LaMer-Campbell diet [17] is composed of oatmeal, heated milk powder, butter and salt. The animals were given food *ad libitum*. Cholesterol 1 g per body weight introduced by a gastric tube, was given for a period of 10 weeks. Hesperidine-methylchalcone (Gee-Lawson Chemical Ltd. London) was given in doses of 50 mg/kg in 1 ml saline subcutaneously, for 10 weeks.

Capillary resistance was assessed by the LAVOLLAY—NEUMANN method [18], by the aid of a glass cup 8 mm in diameter placed over a shaved area on the back, the values being expressed in cm Hg. The period of observation was 10 weeks. Serum cholesterol was estimated by the method of ZAK [19] at the start, mid-time and end of the period of study. Both parameters were assessed by comparison of the initial and final values.

At the end of the observation period the animals were killed by decapitation. After inspection of the organs for gross changes, the aorta was removed from its origin down to the bifurcation; after blunt separation of the periadventitial fatty tissue, it was slit in its entire length, unfolded and inspected under the dissection microscope. This was done by the blind method; the examiner knew the serial number of the animals but was ignorant of the method used. The intimal changes were graded as proposed by ZEMPLÉNYI et al. [20], viz.

grade 0: intact endothelial surface,

grade 1/2: one or two peri-orificial intimal pads (flat bulges in the vicinity of the orifices of the lateral branches),

Table I

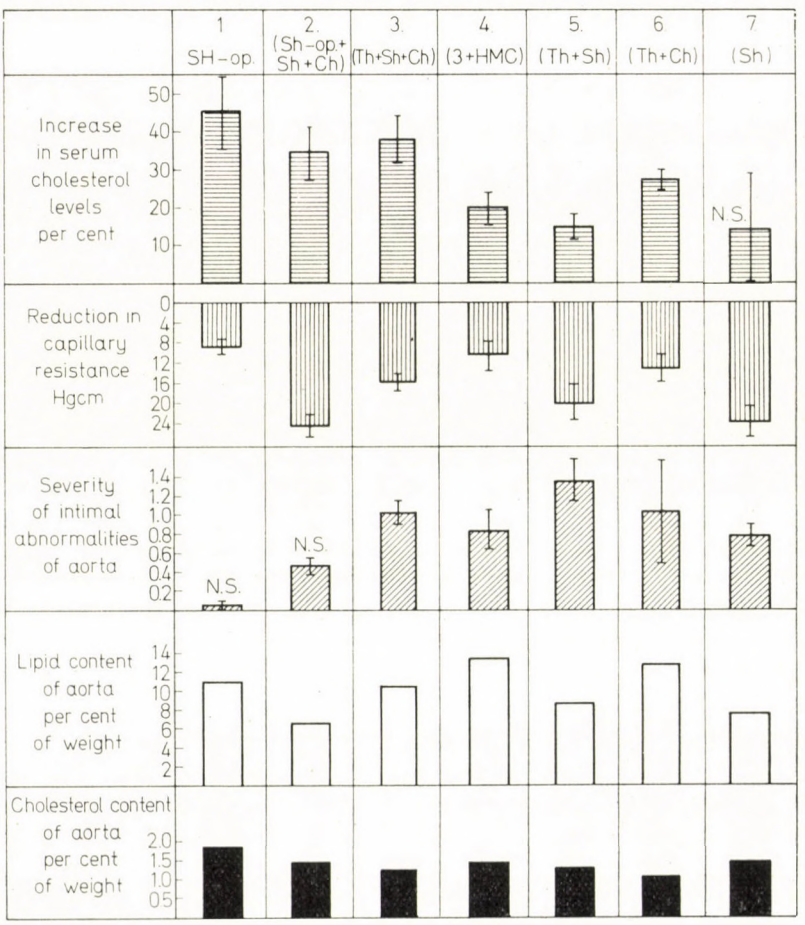
Group (serial number)	Type of treatment	Type of treatment abbreviated	Number of animals
1.	Sham-operation (normal diet)	SH-op	5
2.	Sham-operation + Sherman-LaMer-Campbell diet + Cholesterol-feeding	SH-op + Sh + Ch	8
3.	Thyroidectomy + Sherman diet + Cholesterol-feeding	Th + Sh + Ch	16
4.	As group 3 + hesperidine methylchalcone	Th+Sh+Ch+H	15
5.	Thyroidectomy + Sherman diet	Th + Sh	14
6.	Thyroidectomy + Cholesterol-feeding	Th + Ch	26
7.	Sherman diet	Sh	5

grade 1: occurrence of peri-orificial pads in the entire length of the aorta,
grade $1^1/_2$: involvement of the majority of orifices,
grade 2: involvement of all orifices, plus one or two extra-orificial focal lesions,
grade $2^1/_2$: involvement of all orifices with numerous extra-orificial lesions,
grade 3: as above, with the lesions confluent.

The aorta was halved lengthwise, one half serving for lipid determination and the other for histologic study together with the other organs.

The aortic specimens being too small for individual analysis, lipid determinations were done in the collected specimen of each group. After repeated acetonic extraction the collected extracts were evaporated to a determined volume, and the total lipids were estimated by the method of SWAHN [21], cholesterol by the method of ZAK [19], the values being expressed as percentage of wet weight [22].

The differences between the individual groups and the initial values were analysed by Student's *t*-test. For the total lipid and total cholesterol concentration in the aorta there was only a single value in each group, so that a statistical comparison of the individual groups could not be done. However, dividing the groups according to whether a given method (e.g. deficient diet) had been used, supplied some information concerning the effect of the individual interventions on the lipid concentration of the aortas.



Note N.S = not significant (against zero, i.e. the initial value)

Fig. 1

Table II

Group	1		2		3	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Capillary resistance, cm Hg, $\bar{x} \pm s\bar{x}$	31.67± 2.11	23.75± 2.39	32.14± 1.84	7.86± 1.01	23.5± 1.07	8.5± 1.07
Serum cholesterol, mg per 100 ml $\bar{x} \pm s\bar{x}$	139.0± 5.93	177.0± 9.0	135.5± 3.7	167.9± 3.87	137.13± 2.93	173.71± 5.06
Grade of aortic damage $\bar{x} \pm s\bar{x}$		0.125± 0.125		0.5± 0.164		1.03± 0.132
Total lipids of aorta, weight per cent		10.5		6.27		11.2
Cholesterol in aorta, weight per cent		1.85		1.44		1.24

Results

Results are summarized in Fig. 1 and Table II.

An increase in serum cholesterol was demonstrable at the end of the observation period in every group but No. 7 which had had an exclusively flavone-free diet. A relatively slight but statistically significant increase was demonstrable in groups 4 and 5. The results of comparative evaluation between the individual groups are seen in Table III. The figures 1—7 at the top and the right side represent the individual groups. The figures at the meeting points of the rows belonging to the respective groups express the p-values of statistical comparison of the two groups. On comparison of the individual groups (Table III) the increase in serum cholesterol was significantly less with thyroidectomy + deficient diet than with thyroidectomy + cholesterol feeding. The part of cholesterol feeding was shown by the significant differences between group 2 and 5, as well as between group 3 and 5. (Differences in respect of group 7 were not significant, probably because of the considerable scatter within this group.) The difference between group 3 and 4 indicated that atherogenous treatment combined with a flavone-deficient diet induced a significantly higher increase in serum cholesterol than the same treatment supplemented with flavonoid.

A reduction in capillary resistance was found in all groups. This was slightest in the shamoperated animals (group 1) but the mean for this and the atherogeneous treatment group (group 6) and that with flavonoid added to the flavone-deficient diet (group 4) smaller than the mean for the other groups ($p < 0.1\%$). A considerable reduction in capillary resistance was found

4		5		6		7	
Before	After	Before	After	Before	After	Before	After
treatment		treatment		treatment		treatment	
26.25± 1.55	16.1± 1.59	29.64± 3.08	7.3± 0.72	27.67± 2.00	17.0± 1.17	33.0± 2.00	10.0± 1.58
134.62± 2.01	155.5± 2.398	134.67± 2.92	149.5± 2.25	145.48± 1.75	172.39± 4.75	167.0± 9.47	180.25 4.66
	0.82± 0.178		1.39± 0.273		1.01± 0.174		0.80± 0.122
	13.3		8.81		12.8		7.5
	1.47		1.31		1.08		1.44

Table III

Statistical comparison of the increase in serum cholesterol level in the individual groups (p-values)

	1	2	3	4	5	6	7
1		<40	<60	<50	< 1	<20	<20
2			<40	<10	< 1	<40	<20
3				< 5	< 0.1	<20	<10
4					<40	<40	<40
5						< 5	<70
6							<30
7							

·n the flavone-deficient groups, particularly in group 2 where flavone deficiency had been combined with cholesterol-feeding. Correlations between the individual groups are shown in Table IV.

As to the intimal changes in the aorta, there were practically none in the sham-operated animals (groups 1 and 2). In all the thyroidectomized animals the intimal changes were significant, most markedly so in the case of thyroidectomy + flavone-deficient diet, and least so with substituted flavone deficiency (group 4), although the figures in Table V show no significant difference between groups 3, 4, 5 and 7. On the other hand, groups 3 and 5 deviate from groups 1 and 2 alike, and so does group 7 from group 1.

Table IV

Statistical comparison of the decrease in capillary resistance in the individual groups (p-values)

	1	2	3	4	5	6	7
1		<0.1	<1	<60	< 5	<40	< 1
2			<1	< 1	<80	< 1	80
3				<10	< 5	< 10	< 5
4					< 1	<100	< 5
5						< 1	<100
6							< 2
7							

Table V

Statistical comparison of intimal changes in the aorta in the individual groups (p-values)

	1	2	3	4	5	6	7
1		<20	<1	<10	< 5	< 10	< 1
2			<5	<30	< 5	< 20	< 30
3				<40	<30	<100	< 40
4					<10	< 50	<100
5						< 30	< 30
6							< 60
7							

The total lipid content of aorta was higher on thyroidectomy + cholesterol feeding (groups 3, 4, 6) ($p < 0.05$) and lower in flavone deficiency (groups 2, 3, 5, 7) ($p < 0.05$). Since it was likewise in the flavone-deficient groups where the lowest capillary resistance was found we may well associate a reduced capillary resistance with a low aortic lipid concentration. In the interest of a clear view of the correlations between total lipid concentration and changes of the intimal surface as well as the other points, the various groups have been arranged in a diminishing order of succession on the grounds of the parameters presented in Fig. 1. This is shown in Table VI. From Table VI it clearly emerges that each of the parameters determines a different order of succession of the individual groups. This is also valid for the intimal changes and for the lipid content of aorta. On these grounds, at least under the present experi-

Table VI

Increase in serum cholesterol	I>	III>	II>	VI>	IV>	V>	VII
Decrease in capillary resistance	II>	VII>	V>	III>	VI>	IV>	I
Gravity of intimal changes	V>	III>	VII>	IV>	VI>	II>	I
Total lipid content of aorta	IV>	VI>	III>	I>	V>	VII>	II
Cholesterol content of aorta	I>	IV>	II>	VII>	V>	III>	VII

mental conditions, there seems to be no close relationship between the severity of intimal changes and the total lipid and cholesterol contents of the aorta. This alone would suggest that the intimal pads do not consist of lipid deposits.

Cholesterol concentration in the aorta was fairly consistent, i.e. in the range of 1.3 to 1.4 per 100 g. It was surprising to find the lowest values in the group with atherogenous treatment (group 6).

Discussion

The observation that atherogenous treatment affects capillary resistance has been supported by the present findings. Damage to the vascular wall constitutes one of the main factors of the pathomechanism of experimental atherosclerosis [12]. The atherogenous factors by themselves are sufficient to bring about the well-known changes involving the enzymes [20] and morphology [12] of the vessel wall. On these grounds it seems reasonable to attribute the reduced capillary resistance in atherosclerosis to the same structural and metabolic changes.

The other essential factor in the pathomechanism of atherosclerosis is the accumulation of lipids in the vascular wall which may be regarded as an imbalance between supply or local production and elimination (breakdown and transport) of lipids. One of the possible causes of this imbalance may be an excessive supply of fats indicated by high serum cholesterol levels. Under the conditions of the present experiments the large difference in serum cholesterol between the individual groups are not reflected by the aortic cholesterol concentration. The slightest elevations of the serum cholesterol level were found in the case of flavone deficiency with no cholesterol feeding (groups 5, 7) as well as in that of flavonoid supplemented flavone deficiency (group 4);

on the other hand, the aortic cholesterol concentration in these groups was not the lowest in contrast to the total lipid concentration. In rabbits after cholesterol feeding for 18 weeks it was the total lipid concentration of the aorta rather than its cholesterol concentration which seemed to be related to the gravity of the intimal changes [22]. By analogy, on the basis of the aortic lipid concentration, we might expect the gravest changes to occur in the atherogenous groups 3, 4 and 6.

The changes though being in fact considerable were actually surpassed in severity by those found in group 5 where no cholesterol had been given but thyroidectomy combined with flavone deficiency. The present results thus suggest that the severity of intimal changes bears no relationship to the aortic lipid concentration and makes any connection between the intimal pads and increase in lipid concentration improbable, and that flavone deficiency and thyroidectomy seem to have a synergistic effect in promoting the development of these lesions.

As referred to in the introduction, a decrease in capillary resistance is associated both with flavone deficiency and with atherosclerosis and responds to flavonoid substitution in both cases. In certain species, vitamin B_6 and/or C deficiency results in atherosclerosis or in its deterioration [23, 24]. Apart from our own observations we lack evidence as regards the effect of flavone deficiency on the development of atherosclerosis. From the fact that experimental atherosclerosis induced in certain species has been found to respond to flavone administration, only indirect conclusions can be drawn as regards the possible atherogenous effects of flavone deficiency.

Under the present experimental conditions a flavone deficient diet by itself failed to produce an increase in the cholesterol level. Nor did the results reveal any relationship between the decrease in capillary resistance reflecting the degree of flavone deficiency and the increase in the serum cholesterol level. On the other hand, the data presented in Fig. 1 and Table VI seem to suggest an inverse relationship between the decrease in capillary resistance and the lipid contents of the aorta. (If group 1 is disregarded, all other groups are clearly opposed to one another in their order of succession in rows 2 and 4).

In the given conditions, comparison of groups 3 and 4 seemed best suited for the evaluation of the responsiveness to flavonoids. Small flavonoid doses (50 mg/kg) were found to inhibit the elevation of serum cholesterol and to counteract the fall in capillary resistance [8, 9, 15]. (The last-named effect was not significant (Table IV), probably because of the small dose.) On the other hand, the same flavonoid dose failed to reduce the changes in the lipid content and of the intimal changes due to the joint effect of atherogenous treatment and of a flavone-deficient diet.

Theoretically, the reduction in aortic lipid content found in the flavone

deficient group may have been due to an enhanced elimination of lipids. Still, it is more probable that it resulted from a local dilution, as suggested by the inverse relationship between the reduction in capillary resistance and total lipid content of the aorta, particularly in those groups where the former was the highest and the latter the lowest. This may have been due to an accumulation of water or mucoids, or both. On the evidence of our unpublished observations [25], flavone deficient diets may induce cerebral oedema in the rat. The possibility of mucoid accumulation would be in harmony with the synergistic effect of thyroidectomy and of flavone deficiency on the intimal changes, as suggested by the comparison of groups 2 and 3, and of 5 and 7; moreover, on the evidence of the histologic studies still in progress [26] the intimal pads, the flat prominences of the aortic intima correspond to deposits of a substance staining blue with Pas-Hale.

Accumulation of mucoids is a well-known feature of the early stage of atherosclerosis [27—30] where vascular damage and reparation [12, 31] with a nonspecific mesenchymal reaction [32] are predominant and lipid accumulation is still absent from the changes. The mucopolysaccharides may well be involved in the retention of lipids by the vascular wall [24, 33], thus also in the progression of the process. On these grounds the intimal changes in the rat aorta, resulting from flavone deficiency combined with thyroidectomy may well correspond to the initial, nonspecific stage of atherosclerosis where the lesions are still free from lipid deposits.

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Dr. Rezső SZABÓ
Dr. András REDNIK
Dr. Klára LIPTÁK
Dr. Sándor BENKŐ
II. Belklinika, Szeged
Korányi rkp. 8—10, Hungary

Dr. Attila ANTAL
Dr. Miklós GÁBOR
Gyógyszerhatástani Intézet
Szeged, Eötvös u. 2, Hungary

Dr. István GYŐRI
Szeged, Beloiannisz tér 10, Hungary

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UNTERSUCHUNG DER HUMORALEN UND ZELLULÄREN ABWEHR BEI LEUKÄMIE UND MYELOFIBROSE

Von

A. PATAKFALVI, Á. GÓGL, K. SIMON, Gabriella BÖHM
Zsuzsanna MISZLAY und Edit KAJTSA

I. MEDIZINISCHE KLINIK (DIREKTOR: PROF. DR. T. JÁVOR)
DER MEDIZINISCHEN UNIVERSITÄT PÉCS

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Bei chronischer lymphatischer und myeloischer Leukämie sowie bei Myelofibrose wurde die humorale Immunität durch gleichzeitiger Bestimmung der Isoagglutinine, der auf die Umgebungsantigene einwirkenden Antikörper und des quantitativen Immunglobulins untersucht. Zur Untersuchung der zellulären Immunität kamen analog zum Tuberkulintest, Kutanteste zur Anwendung. Bei lymphatischer Leukämie ließ sich eine mit der Progression der Krankheit in Korrelation stehende bedeutende Verminderung der Antikörper- bzw. Immunglobulinsynthese beobachten, die in erster Linie die Produktion der in die IgA und IgM-Gruppen gehörenden Immunglobuline betraf. Bei myeloischer Leukämie und Myelofibrose konnte nebst im wesentlichen normaler Antikörpersynthese eine dissoziierte Störung der Immunglobulinsynthese nachgewiesen werden. Anhand der Erscheinung, daß die Verminderung des Antikörpertiters im allgemeinen ausgeprägter war, als das aufgrund der Menge der Immunglobuline anzunehmen gewesen wäre, wird die Anwesenheit von über keine Antikörperwirkung verfügenden Immunglobuline angenommen. Wie darauf die Kutanteste hinwiesen, erleidet die zelluläre Immunreaktion bei myeloischer Leukämie eine bedeutende Schädigung, während bei lymphatischer Leukämie eine mäßige Kutanreaktion zutage tritt.

Zur Feststellung der humoralen und zellulären Immunität haben wir bei verschiedenen hämatologischen Krankheitsbildern Gesamteiweiß-Bestimmungen, papier- bzw. agarelektrophoretische Untersuchungen vorgenommen; außerdem wurde fallweise 3—4monatlich auch die Konzentration der zirkulierenden Antikörper bestimmt, welche Untersuchungen in den späteren Perioden durch quantitative Immunglobulinbestimmung und Untersuchung der zellulären Immunreaktion ergänzt wurden. In vorliegender Arbeit wollen wir über unsere bei Myelofibrose und bei Leukämien verschiedenen Typs ermittelten Ergebnisse berichten. Das Ziel unserer Arbeit war eine Antwort auf folgende Fragen zu erhalten:

1. Welchen Ausmaßes ist die Schädigung der Immunglobulin- bzw. Antikörpersynthese und der zellulären Immunreaktion in den einzelnen Krankheitsformen;

2. und auf welche Weise ändert sich die Antikörper- und Immunglobulinkonzentration während des Krankheitsverlaufs?

Material und Methodik

Die Antikörper- und Immunglobulinbestimmungen fanden bei folgende Gruppen statt:

1. Kontrollgruppe: erwachsene Normalpersonen. Antikörperbestimmung wurde in 114 Fällen (61 Frauen, 53 Männer, durchschnittliches Lebensalter 34,7 Jahre), Immunglobulinspiegel-Bestimmungen in 60 Fällen (32 Frauen, 28 Männer, durchschnittliches Lebensalter 32 Jahre) und Untersuchung der Allergie vom Tuberkulin-Typ mit verschiedenen Antigenen in 29 Fällen durchgeführt.

Gruppe 2. Lymphatische Leukämie. In 47 Fällen wurde die Konzentration der zirkulierenden Antikörper bestimmt, während in 25 quantitative Immunglobulinbestimmungen stattfanden. Kutanteste kamen in 23 Fällen zur Anwendung. Angesichts dessen, daß die Antikörpertiter bei den einzelnen Patienten mehrmals, in 3—4monatigen Intervallen bestimmt wurden, haben wir bei jedem einzelnen Patienten die Mittelwerte dieser mehrmaligen Bestimmungen bei der Ermittlung der Durchschnittswerte in Betracht gezogen. Die Mehrzahl der Patienten erhielt eine Chlorambucil + Prednisolon-Behandlung, in einigen Fällen kamen auch Röntgenbestrahlung sowie Procarbazin und Mannomustin zur Anwendung.

Gruppe 3. Myeloische Leukämie, 21 Fälle. Die Krankheitsdauer betrug in 16 Fällen 0—3 Jahre und in 5 Fällen 3—10 Jahre. Die angewandte Therapie war in der Mehrheit der Fälle Busulfan und in einigen Fällen Dibromomannitol + Tetramesyl-D-Mannit.

Gruppe 4. Myelofibrose. Antikörper-Bestimmungen fanden in 15 Fällen und Immunglobulinspiegel-Bestimmungen in 12 Fällen statt. Die Kutanreaktion wurde in 5 Fällen durchgeführt. Die palliative Behandlung bestand aus Verabreichung von Eisenpräparaten, Folsäure, Prednisolon, Metandienon bzw. in einigen Fällen von Busulfan und Dibromomannitol.

In den zur Gruppe 2 und 3 gehörenden frischen bzw. früher diagnostizierten Fällen wurden die Untersuchungen im allgemeinen vor dem Beginn der Behandlung durchgeführt.

Die Isoagglutinine wurden mit dem direkten Hämagglutinationsverfahren, die auf die Umgebungsantigene einwirkenden Antikörper (Salmonella typhi O., Vi., Shigella flexneri 1 b, 2 a, 3, Shigella Sonnei, Escherichia coli 026, 055, 086, 0111) mit der indirekten Hämagglu-

Tabelle I

Antigene zu den Kutantesten

A. Staphylococcus aureus haemolyticus

B. Staphylococcus albus
Micrococcus (Gram-pos.)

C. Streptococcus haemolyticus
Lancefield A, C, G

D. Streptococcus viridans
Streptococcus pneumoniae
Streptococcus haemolyticus LANCEFIELD D.

E. Neisseria pharyngis
Klebsiella pneumoniae
Haemophilus influenzae
Micrococcus (Gram-neg.)

F. Bordatella pertussis
Bordatella parapertussis
Bordatella bronchioseptica

G. E. coli
Pseudomonas aeruginosa
Bacillus proteus, Providentia

H. Brucella abortus

I. MANTOUXsche Probe 1 : 100 000

tinationsmethode und das Staphylokokkus-Antitoxin mit dem, auf Hämolyseinhibition beruhenden Verfahren nach BACKHAUSZ und Mitarb. [3], auf TAKÁTSYschen Platten durchgeführt. Die Antikörperwerte der Patienten wurden mit den entsprechenden Ergebnissen der Kontrollgruppe verglichen.

Die Bestimmung der 3 Haupt-Immunglobulinfraktionen erfolgte mit Radial-Immundiffusion [24]. Zu den Untersuchungen kamen die Immunseren Anti-IgG-25-B, Anti-IgA-30-K, Anti-IgM-1-L und Anti-IgM-110-L (Human, Budapest) zur Anwendung. Die zur Bestimmung der Immunglobulinwerte der Kontrollpersonen angewandte Standardlösung bestand aus dem Gemisch von Normalseren (Human, Budapest), dessen IgG-Gehalt 1 500 mg%, IgA-Gehalt 200 mg%, IgM-Gehalt 160 mg% betrug.

Bei der Untersuchung der Allergie vom Spättyp kamen die in Tabelle I angeführten Antigene (Human, Budapest) zur Anwendung; die intrakutan verabreichte Menge betrug 0,1 ml. Die Ablesung erfolgte nach 15 Minuten sowie nach 24 und 48 Stunden. Die Tuberkulinprobe wurde mit Verdünnungen von 1 : 100 000 vorgenommen.

Ergebnisse

1. Kontrollgruppe. Die durchschnittlichen Titerwerte sind in Tabelle II dargestellt. Im Einklang mit der Feststellung anderer Verfasser [25, 40] ließen sich die einzelnen Antikörper in Normalfällen sowohl qualitativ als auch quantitativ nachweisen. Die Titer der einzelnen Antikörper schwanken auch unter normalen Verhältnissen zwischen verhältnismäßig weiten Grenzen. Die anhand von 60 Kontrollfällen bestimmten normalen Immunglobulin-Mittelwerte veranschaulicht Tabelle III. Bei der Untersuchung der allergischen Reaktion vom Spättyp wurde die Positivität sämtlicher Kutanteste als 100% angenommen. In den Normalfällen betrug die Positivität 30,2% (Tab. IV).

2. Lymphatische Leukämie. In der 0—3jährigen Periode der Krankheit hat sich der Titer der einzelnen Antikörper — im Verhältnis zu den Normalkontrollen — wesentlich vermindert, während die Konzentration einiger Antikörper unterhalb der meßbaren Grenze lag. Ausgeprägter war die Verminderung in den 3—8jährigen Fällen, in denen etwa die Hälfte der Antikörper überhaupt nicht meßbar war (Abb. 1 und Tab. II). Auch in der Beziehung des Immunglobulins waren die auffallendsten Veränderungen in dieser Gruppe vorzufinden. In den Fraktionen IgA und IgM erreichte keiner der Werte den normalen Durchschnitt, häufig ließen sich ganz niedrige Werte registrieren und es kamen auch Mangelzustände vor. Der IgA-Spiegel war meistens mäßig vermindert, in einigen Fällen konnten aber auch den Normaldurchschnitt übertreffende Werte gemessen werden. In den Fällen, in denen die Leukämie seit mehr als 3 Jahren bestand, war die Verminderung noch ausgeprägter (Abb. 2). Die allergische Reaktion späten Typs zeigte im Vergleich zu den Normalwerten eine mäßige Verminderung (Tab. IV).

3. Myeloische Leukämie. In dieser Gruppe wiesen die Antikörpertiter im Verhältnis zu den normalen Mittelwerten keine wesentlichen Abweichungen auf; die einzelnen Antikörper wiesen mäßige Verminderungen und auch geringe Erhöhungen auf (Tab. II). Bei den Immunglobulinen übertraf der IgG-Spiegel in den ersten 3 Jahren der Krankheit den normalen Durchschnitt. Verminderun-

Tabelle II

Antikörper-Durchschnittswerte in den Kontroll-

Immunglobulingruppe	IgG + IgA + IgM			IgG+A	
Untersuchte Antikörper	Anti-A	Anti-B	Shigella Sonnei	Salmonella typhi O	Salmonella typhi Vi
Normaler Durchschnittstiter (n = 114)	32	75	22	12	8
Lymphatische 0—3 Jahre (n = 27)	18	29	7	5,7	3,5
Leukämie 3—8 Jahre (n = 20)	12	8	3,5	2	1,1
Myeloische 0—3 Jahre (n = 16)	45	61	18	14	5
Leukämie 3—10 Jahre (n = 5)	14	64	20	12	4
Myelofibrose (n = 15)	60	64	15,4	11,8	5

Tabelle III

Immunglobulinwerte in den Kontroll- und Krankheitsgruppen

Gruppen	Immunglobulinwerte (mg%)		
	IgG	IgA	IgM
Normalgruppe (n = 60)	1570± 52,3	220±12	193±15,5
Lymphatische Leukämie (n = 25)	1411±134	92±12	64± 8
Myeloische Leukämie (n = 21)	2070±138	176±21	186±34
Myelofibrose (n = 12)	2610±279	243±61,8	252±64,3

gen ließen sich am häufigsten in der IgA-Fraktion nachweisen, außerdem traten in der IgM-Fraktion auffallende Schwankungen in Erscheinung. Die Abnahme aller drei Immunglobulinfraktionen meldete sich bei ein und demselben Patienten nur selten, am häufigsten kam die Verminderung der isolierten IgA-Fraktion vor, während die anderen beiden Ig-Fraktionen entweder normal oder erhöht waren. Mit dem Fortschreiten der Krankheit konnte die allgemeine Verminderung der Ig-Fraktionen auch bei dieser Gruppe nachgewiesen werden (Abb. 3). Wie darauf auch die in Tabelle IV angeführten Angaben hinweisen,

und Krankheitsgruppen

IgA	IgA + IgM			IgM			IgG + IgM
Shigella flexneri		E. coli		Shigella flexneri 3	E. coli		Staphylococcus α-Antitoxin (I. E.)
2a.	1b.	055	026		086	0111	
11	41	10	14	56	14	12	0,75
7,8	13	3,7	5,4	18	4,6	3,4	0,64
4	6	1,7	2,75	7,5	2,3	1,1	0,46
16	46	13	11	49	16	12	0,47
7	57	6	10	49	8	7	0,64
15,4	30	9,2	11	35	12,8	9,4	1,06

Tabelle IV

Untersuchungsergebnisse der allergischen Reaktionen vom Spättyp

Gruppe	Anzahl der Fälle	Ablesungszeit der Reaktion		
		15 Min.	24 St.	48 St.
Kontrollgruppe	29	29,0%	30,2%	30,2%
Lymphatische Leukämie	23	11,1%	31,4%	25,4%
Myeloische Leukämie	10	4,4%	19,9%	14,4%
Myelofibrose	5	4,4%	24,4%	26,6%

war eine verringerte Intensität der Tuberkulinreaktion ebenfalls in den Fällen mit myeloischer Leukämie am ausgeprägtesten (Tab. IV).

4. Myelofibrose. Die Antikörpertiter gestalteten sich ähnlich, wie bei myeloider Leukämie (Tab. II). Die bedeutendsten Streuungen wiesen die Immunglobulinwerte auf. In einem Fall trat — besonders im polyzythämischen Stadium der Myelofibrose eine wesentliche Vermehrung aller drei Immunglobulin-Fraktionen in Erscheinung (Abb. 4). Kutanteste wurden in 5 Fällen ausgeführt; nennenswerte Abweichungen ließen sich nicht verzeichnen (Tab. IV).

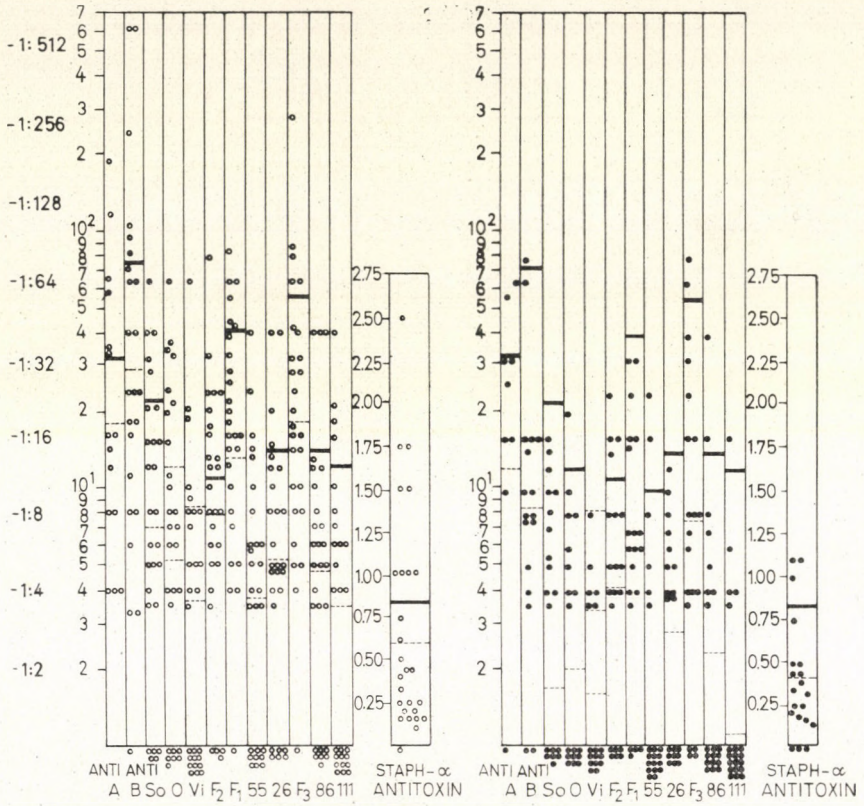

Abb. 1. Antikörpertiterwerte lymphatischer Leukämiekranken, in einer der Krankheitsdauer entsprechenden Aufschlüsselung. Abbildung 1/A etnthält die Angaben von 27 Patienten (0—3 Jahre) und Abbildung 1/B die von 20 Patienten (3—8 Jahre). (Die auf der linken Seite des senkrechten Schenkels befindlichen Nummer veranschaulichen die Verdünnungen der Seren, während die rechtsseitigen Nummer den Log-Titerwerten entsprechen.) Die dicken Linien veranschaulichen die Durchschnittswerte der Kontrollgruppe, die gestrichelten Linien die entsprechenden Werte der Patienten

Besprechung

Den bei lymphatischer Leukämie zwischen den rezidivierenden Infektionen und dem Defekt der Antikörperbildung bestehenden kausalen Zusammenhang hat als erster ROTKY [35] erkannt, doch wurde den immunologischen Beziehungen der lymphatischen Leukämie lange Zeit hindurch keine besondere Aufmerksamkeit gewidmet. ARENDS [2] ist es zu verdanken, daß das Interesse der Kliniker für die Erscheinung erneut wach gerufen wurde, indem er über einen mit häufigen, schweren bakteriellen Infektionen und totaler Agammaglobulinämie einhergehenden Fall berichtete. Seit Einführung der elektrophoretischen Verfahren vermochten zahlreiche Verfasser bei lymphatischer Leukämie

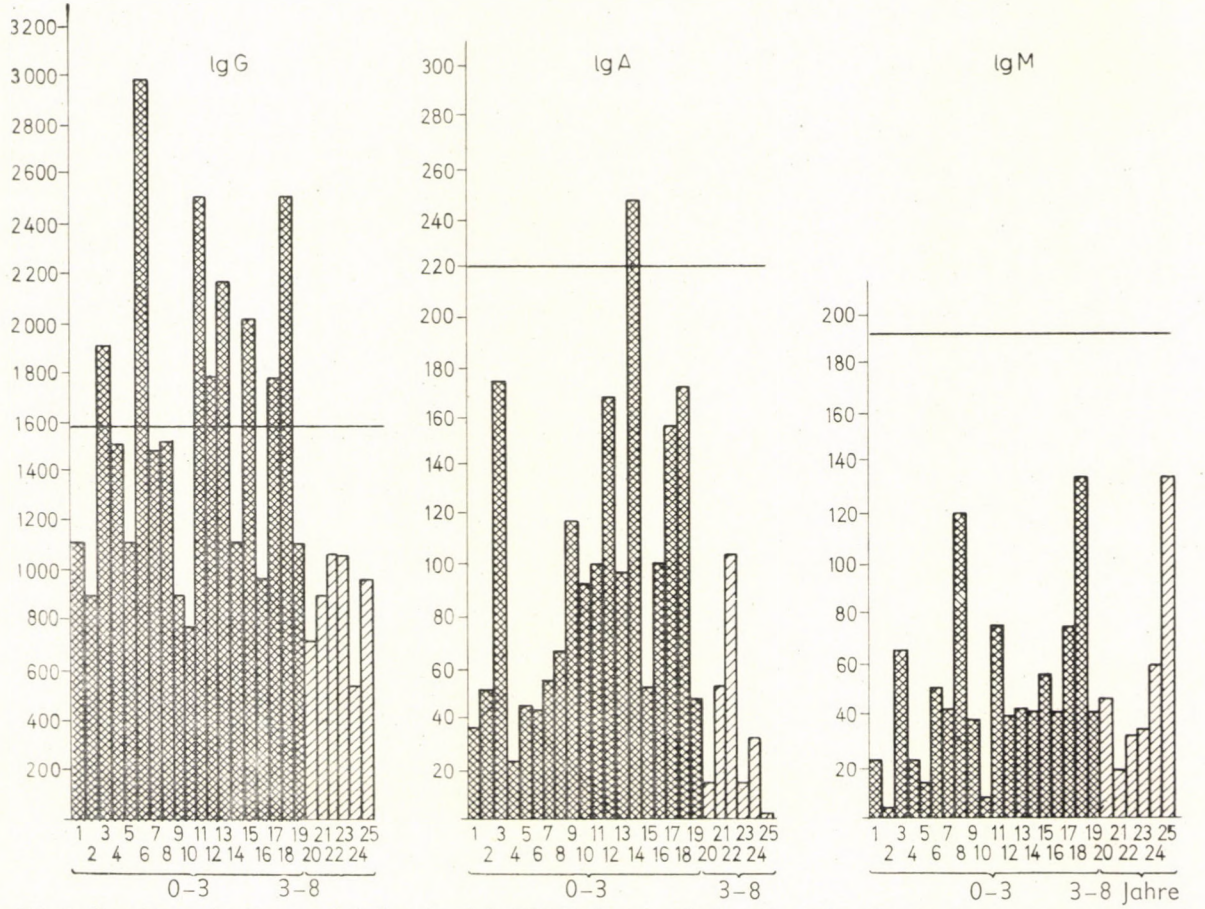

Abb. 2. Gestaltung des Immunglobulinspiegels bei 25 Patienten mit lymphatischer Leukämie, in einer der Krankheitsdauer entsprechenden Aufschlüsselung. Die waagerechte Linie entspricht dem normalen Immunglobulinspiegel

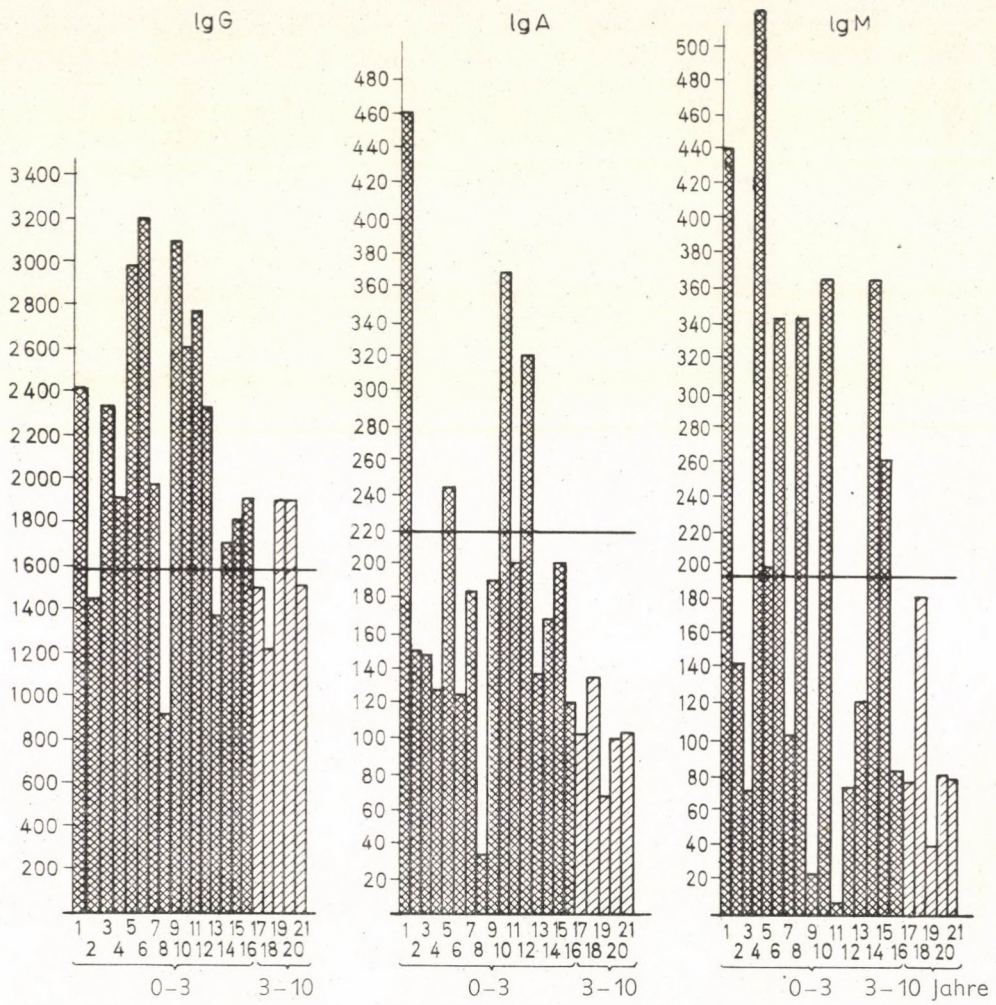

Abb. 3. Immunglobulinspiegel von 21 Patienten mit myeloischer Leukämie, in einer der Krankheitsdauer entsprechenden Aufschlüsselung

Hypogammaglobulinämie zu beobachten; die Prozentzahl dieser Fälle betrug im Material von JIM [19] 50%, in dem von WUHRMANN und WUNDERLY [41] 25%, im Krankengut von CREYSSEL und Mitarb. [10] 50% und bei den Patienten von HUDSON und WILSON [18] 70%. Die Untersuchungen von CREYSSEL und Mitarb. [10] sowie SHAW und Mitarb. [37] ergaben, daß zwischen Hypogammaglobulinämie und der Häufigkeit der Infektionen eine Korrelation besteht. Laut ULTMANN und Mitarb. [39] sind die Überlebensziffern der normogammaglobulinämischen Patienten doppelt so hoch als die der hypo-

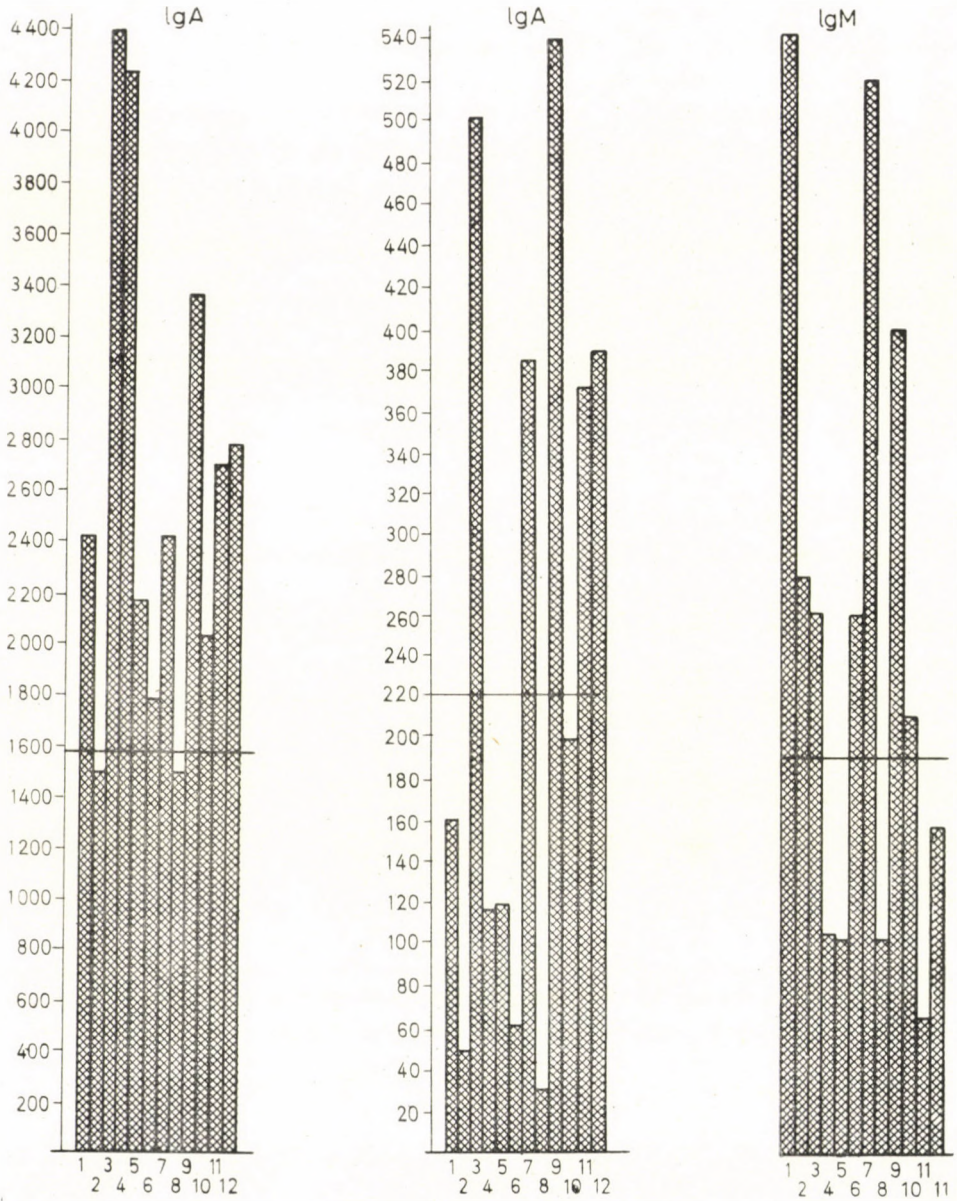

Abb. 4. Immunglobulinspiegel von 12 Patienten mit Myelofibrose

gammaglobulinämischen Kranken. Einige Verfasser beschrieben auch mit Hypergammaglobulinämie und sogar mit pathologischer Eiweißbildung einhergehende Fälle [5, 26, 34].

Die einzelnen Immunglobuline wurden — im Mangel monospezifischer Seren — mittels Immunelektrophorese untersucht. In mit Gammaglobulinverminderung einhergehenden Fällen von lymphatischer Leukämie konnte die Abnahme des einen oder mehrerer Immunglobuline beobachtet werden [4, 16, 27, 42]. Eine quantitative Bestimmung ist aber mit der erwähnten Methode mit nur annähernder Genauigkeit möglich.

Mit der Untersuchung der zirkulierenden Antikörper bei lymphatischer Leukämie haben sich mehrere Forscher befaßt. Die Untersuchungen beschränkten sich im allgemeinen auf die Isoagglutinine [4, 36] und auf die Analyse der durch verschiedene Antigenreize ausgelösten Antikörperreaktionen. Diese letzterwähnte Methode ist aber nicht ungefährlich, außerdem wird die aktuelle Antikörperproduktion durch manche endogene und exogene Faktoren beeinflußt. SHAW und Mitarb. [37] erhielten auf Wirkung von Mumps-, Diphtherie- und Influenza-Vakzination in einem Teil ihrer Fälle herabgesetzte Antikörperreaktionen. Andere Verfasser [6, 11, 37] beobachteten nach Typhus-, Paratyphus A—B- und Tularämie-Vakzination bzw. nach Verabreichung von Pneumokokkus-Polysaccharidenantigen verminderte Immunreaktionen [12, 15]. Laut HARTEL und Mitarb. [14] bleibt bei lymphatischer Leukämie gleichzeitig auch die sekundäre Immunantwort weg. Die Tuberkulinreaktion wurde früher bei Lymphogranulomatose untersucht. In bezug auf die allergische Reaktion vom Spättyp bei lymphatischer Leukämie finden sich in der Literatur widersprechende Daten. Während bei den Untersuchungen von HARTEL und HAYDUK [14] in manchen Fällen nicht nur die MANTOUXsche Reaktion sondern auch die nach der Brucella-Vakzination vorgenommenen Brucellinteste ein negatives Resultat lieferten, vermochten andere Verfasser keine Verminderung der Hautreaktion zu beobachten [18, 36].

Die Untersuchung der Isoagglutinine und der auf die bakteriellen Antigene einwirkenden Agglutinine liefert sich auf die Synthese der in die verschiedenen Immunglobulin-Fraktionen gehörenden Antikörper beziehende wesentliche Aufklärungen. Angesichts dessen, daß die Träger ein und desselben Antikörpers fallweise immer andere Immunglobuline sein können, läßt sich ein genaues Bild über den Zustand des humoralen Immunsystems nur anhand der gleichzeitigen Untersuchung einer größeren Anzahl von Antikörpern und quantitativen Immunglobulinbestimmungen gewinnen. Aufgrund unserer parallel vorgenommenen Untersuchungen konnte eindeutig festgestellt werden, daß die Antikörper- bzw. Immunglobulinsynthese bereits im Initialstadium der lymphatischen Leukämie vermindert ist. Mit dem Fortschreiten der Krankheit wird die Schädigung ausgeprägter, besonders was die Synthese

der Immunglobuline bzw. Antikörper IgA-und IgM-Typs anbelangt. In besonders fortgeschrittenen Fällen, z. B. bei 3—8jährigem Überleben ist es augenfällig, daß sich der Titer der einzelnen Antikörper in bedeutenderem Maße vermindert, als das anhand der Immunglobulinkonzentration anzunehmen wäre. Diese Beobachtung spricht ebenfalls dafür — obwohl die diesbezüglichen direkten Beweise noch fehlen — daß in diesen Fällen auch mit der Anwesenheit funktionell geschädigter, über keine Antikörperwirkung verfügender Immunglobuline gerechnet werden kann. Die Ergebnisse der Kutanteste ließen auf keine wesentliche Schädigung der zellulären Immunität schließen.

Bei myeloischer Leukämie konnte kein häufigeres Auftreten von Infektionen und dementsprechend auch keine Schädiung der Gammaglobulin- bzw. Antikörpersynthese beobachtet werden [4, 22]. CAPONE und Mitarb. [9] beschrieben einen myeloischen Leukämie-Fall, zu dem sich ein mit Hypogammaglobulinämie einhergehendes Antikörpermangelsyndrom gesellte; ihres Erachtens besteht auch zum gemeinsamen Vorkommen der beiden Krankheitsbilder eine Möglichkeit. Wir beobachteten im Laufe unserer Immundiffusionsuntersuchungen die dissoziierte Störung der Immunglobulinsynthese, d. h., daß die verminderte Produktion der einen Immunglobulin-Fraktion durch die gesteigerte Produktion der anderen Fraktion ausgeglichen wurde. Der dissoziierte Defekt der Immunglobulinproduktion widerspiegelte sich in den Ergebnissen der Antikörperuntersuchungen nicht. Die Erkennung eines Immundefekts zellulären Typs ist ein schwierigeres Problem. Allein anhand einer im Vergleich zu den Kontrollwerten häufigeren Negativität kann die Schädigung des zellulären Immunsystems noch nicht festgestellt werden. Zu diesem Zweck eignet sich die — auch von uns durchgeführte — Blast-Transformationsuntersuchung der Lymphozyten; über unsere diesbezüglichen Ergebnisse wollen wir zunächst berichten.

Unseres Wissens wurden bei Myelofibrose keine derartigen Untersuchungen vorgenommen, was wahrscheinlich auch mit dem Umstand zusammenhängt, daß die Myelofibrose auch heute noch unter die seltenen Diagnosen gehört. HITTMAIR [16] fand bei Myelofibrose eine ausgeprägtere Infektionsneigung. Die bei unseren Patienten vorgefundene hochgradige individuelle Immunglobulinschwankung dürfte dem Umstand zugeschrieben werden, daß die Krankheitsdauer bei unserem Material unterschiedlich (1—14 Jahre) war. Als beachtenswert halten wir indessen die Tatsache, daß sich im polyzythämischen Stadium der Myelofibrose alle drei Immunglobulin-Fraktionen wesentlich vermehrten (z. B. Abb. 5 Fall Nr. 3 und 11).

Eine besondere Frage bildet bei einer jeden Gruppe die Rolle der, im Tierexperiment eingehend analysierten, immunsuppressiven Behandlung [33]. Wie wir darüber bereits berichteten, haben wir bei einem großzahligen Krankenmaterial vor und nach der Behandlung Antikörper- und Immunglobulinbestimmungen durchgeführt [32]. Bei der malignen Wucherung des lympho-

retikulären Systems ist entschieden die Grundkrankheit von immunsuppressiver Wirkung, nach der Behandlung kann auch ein paradoxer Effekt, namentlich eine Resuppression eintreten.

*

Herrn Dr. R. BACKHAUSZ und Frl. J. LAJOS, die bei unseren Patienten mit lymphatischer Leukämie die ersten Antikörperuntersuchungen vornahmen, sind wir für ihre liebenswürdige Mitwirkung zu besonderem Dank verpflichtet.

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Dr. Albert PATAKFALVI
Dr. Árpád GÓGL
Dr. Kornél SIMON
Assist. Gabriella BŐHM
Dr. Zsuzsanna MISZLAY
Dr. Edit KAJTSA

I. sz. Belklinika, Pécs, Ungarn

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CIRCULATORY REGULATION AFTER EXPERIMENTAL TRICUSPID LESION

HAEMODYNAMICS DURING THE DEVELOPMENT OF CONGESTIVE HEART FAILURE

By

F. Solti, Júlia Nagy, M. Iskum and Anna Hartai

FIRST DEPARTMENT OF MEDICINE, SEMMELWEIS UNIVERSITY MEDICAL SCHOOL, BUDAPEST

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The haemodynamic changes associated with the development of congestive heart failure have been studied on an experimental model of combined tricuspid failure and stenosis, induced in 20 dogs and 40 controls. Cardiac output, circulating blood volume and mean transit time were estimated by the dye dilution method, and blood flow in the individual organs by a dual isotope fractionation method. The haemodynamic parameters were measured prior to and 11 to 16 weeks after the intervention. According to the findings, the tricuspid lesion induced an increase in venous and right atrial pressure, with a reduction in cardiac output and in the mean transit time, and an increase in circulating blood volume as well as in total peripheral vascular resistance. Major changes were demonstrable in circulatory regulation: cerebral and cardiac blood flow were unaffected in spite of the reduction in cardiac output; this was made possible by the increase in the cardiac and cerebral fractions of cardiac output. This was associated with an increase in renal, hepatic muscular and cutaneous vascular resistance and a reduction of these organ fractions of cardiac output.

The behaviour of blood circulation in congestive heart failure has been widely studied. It is well known that congestive failure is associated with a reduction of myocardial contractility which results in a reduction of cardiac output even at rest, parallel with a slowing of circulation and an increase in circulating blood volume. On the other hand, little is known about the manner in which circulatory regulation changes parallel with the development of congestive failure, in particular, how vascular resistance behaves in the important areas of blood supply and how the individual organ flows participate in cardiac output. We have studied these questions in animal experiments since their investigation in humans lies beyond the present possibilities.

Material and methods

Preliminary experiment. Various methods have been recommended for the production of valvular lesions or of other conditions giving rise to congestive heart failure. We decided for combined artificial mitral and tricuspid lesions in view of their similarity to human valvular disease.

Dogs of either sex weighing between 15 and 25 kg were studied. Anaesthesia was induced with 0.20 g/kg of sodium pentobarbital intravenously and continued with ether-oxygen narcosis under positive pressure breathing through an intratracheal cannula.

Combined tricuspid lesion was produced in 11 animals by the following method. The chest was opened by an incision in the 4th right intercostal space. A small incision was made in the pericardium and 10 ml of a 2% solution of procaine amide was injected into the peri-

cardial sac. The right auricle was clamped with a flat vascular clamp at its atrial junction, a purse string suture was placed over the site of constriction and the auricle was excised. The right index was introduced into the opening thus caused, the purse string suture was tightened, the clamp removed, the index was now advanced into the right atrium and into the upper part of the right ventricle. The ventral and septal cusps of the tricuspid valve were ruptured digitally. The finger was now withdrawn while checking the success of the intervention. Production of tricuspid regurgitation was conveyed to the finger by a thrill which was also palpable through the right atrial wall. Now the index finger was reintroduced through

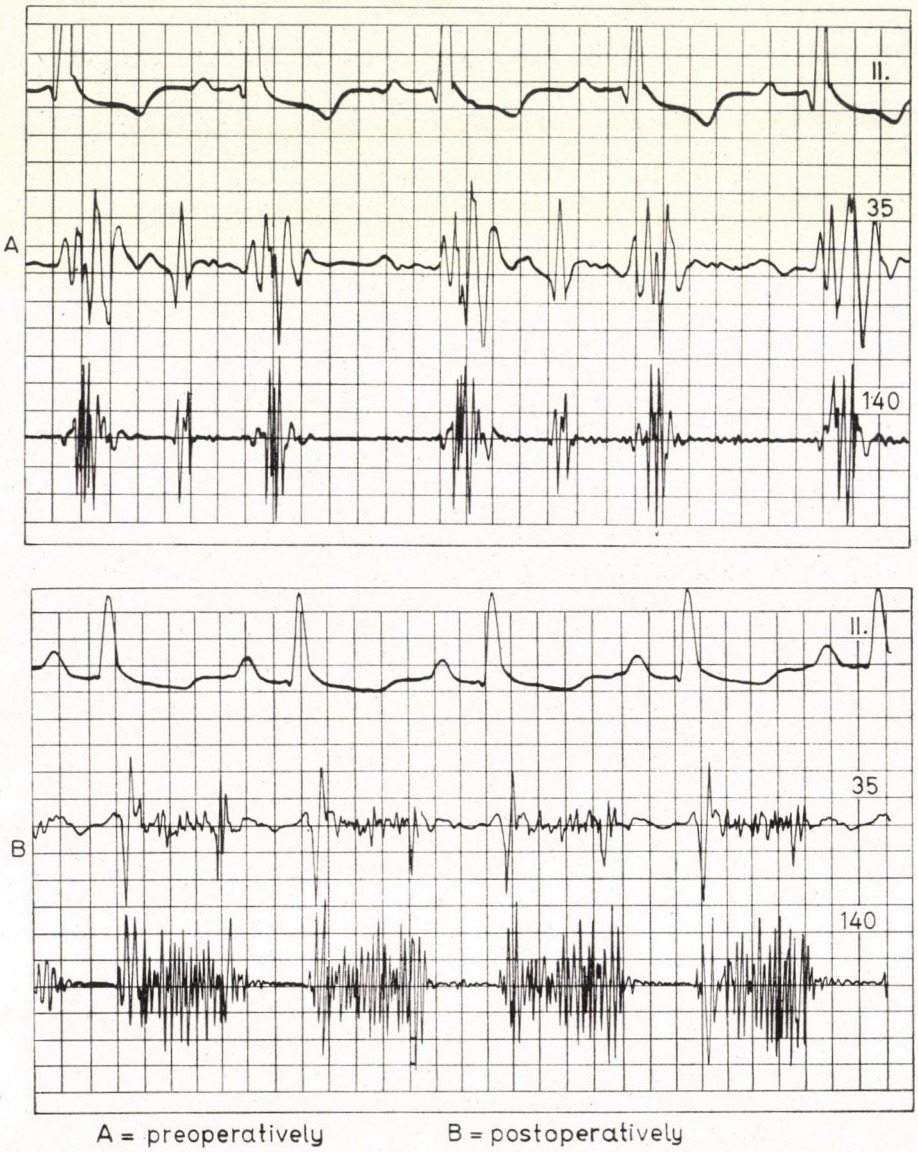

Fig. 1

the atrio-ventricular orifice, the base of the ventral cusp was pressed against the anterior wall of the right ventricle and sutured to its wall with a needle passing through its base, under the control of the introduced finger. The atrial wound was closed by the purse string suture. Penicillin was applied locally and the chest was closed. 0.5 g streptomycin and 1.000.000 U penicillin were administered daily during the first postoperative days.

Combined mitral lesion was induced in 17 animals. The site of approach was the 4th left intercostal space, otherwise the operation was described above.

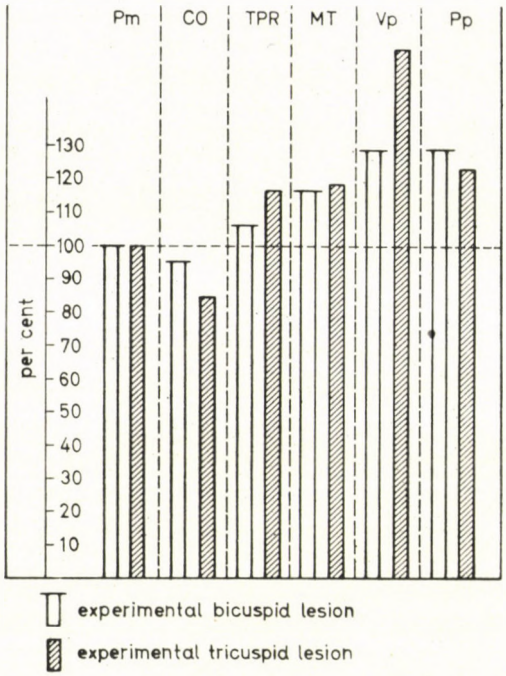

Fig. 2

The following investigations were performed before and after operation.

1. ECG (Hellige 3-channel direct-recorder) and PCG (frequency filters Nos 35 and 140).

2. Arterial blood pressure by the aid of a catheter inserted into the external iliac artery, using an electromanometer.

3. Estimation of cardiac output and of circulating blood volume, by Evans blue dilution.

4. Recording of venous pressure by the aid of a catheter inserted into the v. cava inferior via the femoral vein, using a Moritz—Tabora apparatus.

5. Electromanometric measurement of right atrial and ventricular pressures by the aid of a catheter inserted via the femoral vein under X-ray control.

The animals were sacrificed between the 8th and 20th week after the intervention. Valvular lesion and condition of the heart were checked. During the observation period ranging from 8 to 20 weeks in the animals developed oedema or ascites only in response to exercise (running) or saline infusion.

PCG revealed systolic and diastolic murmurs in all of the cases and a presystolic murmur in a number of animals. A typical PCG-pattern of tricuspid lesion is shown in Fig. 1. A holosystolic murmur has appeared after the tricuspid lesion.

The changes induced included an increase in venous pressure, in mean transit time and in right atrial pressure, and generally a reduction in cardiac output.

A graphic representation of the influence of the tricuspid and mitral lesion on the parameters examined is seen in Fig. 2. The changes are expressed in per cent of preoperative values, these being taken as 100%.

It can be seen that combined tricuspid or mitral lesions induced the same haemodynamic changes. The reason why we adopted the combined tricuspid lesion for the further studies was that its technique was simple and its lethality low.

Tricuspid lesion was thus induced by the foregoing procedure in 20 dogs and the haemodynamic parameters were studied 12 to 16 weeks after its establishment; 40 dogs served as controls.

Methods. Intravenous anaesthesia was induced with 0.20 mg/kg of sodium pentobarbital. The external iliac arteries were prepared on both sides and cannulated, one cannula serving for the measurement of blood pressure, and the other for collecting blood samples. The femoral vein was prepared and a short catheter was passed up through it into the v. cava inferior. Venous pressure was measured by a Moritz—Tabora apparatus, and 2 mg/kg of Evans blue was injected for the estimation of circulating blood volume, cardiac output and circulation time. Samples were taken at one-second intervals. Circulating blood volume was determined from samples obtained 5 min after the injection of Evans blue. Cardiac output was estimated from dye-dilution [1, 2]. Regional blood flow was estimated by the indicator fractionation method [3]. For the measurement of cerebral blood flow, cerebral vascular resistance and cerebral fraction of cardiac output a double isotope fractionation was used [4, 5]. The animals were given 30 μC ^{86}Rb and 5 μC ^{131}I antipyrine in 5 ml physiologcial saline intravenously, and 2 min later they were killed by a rapid intracardial injection of

Table I

Haemodynamic changes associated

		P_m	CO l/min	BV l	MT sec	TVR dyne. sec. cm^{-5}	Brain	
							BF	VR
n	A	20	20	20	20	20	20	20
	B	40	40	40	40	40	40	40
$\bar{x}$	A	*113* (15)	*1.9* (0.4)	*1.2* (0.1)	*17* (2.01)	*3.83* (2.04)	*52* (5)	*131* (26)
	B	*111* (11)	*2.4* (0.5)	*1.0* (0.1)	*13* (1.8)	*2.91* (0.65)	*54* (13)	*128* (17)
diff	A—B	2	—0.5	0.2	4	0.92	—2	3
s diff	A—B	3.38	0.13	0.04	0.51	0.19	3.03	7.37
p		>50	*<001*	*<001*	*<001*	*<001*	>40	>70

Abbreviations
P_m = mean arterial pressure
CO = cardiac output
BV = circulating blood volume
MT = mean transit time
TVR = total vascular resistance
BF = blood flow individual organs
VR = vascular resistance in individual organs

2 g sodium barbiturate. The organs to be studied were weighed in toto and boiled three times in $3 \times N$ NaOH. Skin and muscle samples were taken from the lower extremities. ^{86}Rb and ^{131}I activities in the investigated organs were measured by a spectrometer focussed on the photo-peaks of ^{86}Rb and ^{131}I. The values for cardiac output, circulating blood volume and total vascular resistance were referred to 10 kg weight, while, blood flow and vascular resistance in the individual organs to 100 g tissue. The results were evaluated statistically by Student's two-sample *t*-test.

Results

Results are presented in Table I. It can be seen that in the dogs with tricuspid lesion, cardiac output was reduced, while mean transit time total peripheral resistance and circulating blood volume were increased. At the same time, renal, hepatic, muscle and skin flow were markedly reduced together with a significant increase in the vascular resistance of these organs,

with experimental tricuspid lesion

Heart		Kidney		Liver		Muscle		Skin	
VF	*VR*	*BF*	*VR*	*BF*	*VR*	*BF*	*VR*	*BF*	*VR*
20	20	20	20	15	15	20	20	20	20
40	40	20	20	20	20	40	40	40	40
119	*57*	*320*	*23*	*52*	*133*	*9*	*865*	*8*	*902*
(13)	(10)	(83)	(8)	(7)	(32)	(3)	(428)	(2.5)	(423)
112	*62*	*523*	*13*	*78*	*88*	*14*	*524*	*13*	*543*
(23)	(17)	(67)	(2)	(10)	(34)	(4)	(186)	(4)	(172)
7	−5	202	10	−26	45	5	340	5	359
5.61	4.15	23.77	1.78	3.00	7.97	1.07	79.05	0.98	76.80
>20	>20	*<001*	*<001*	*<001*	*<001*	*<001*	*<001*	*<001*	*<001*

A = dogs with experimental tricuspid lesion
B = control (normal) dogs
n = number of experiments
$\bar{x}$ = mean values and scatter (sd)
diff = difference between the means of the two groups
s diff = scatter of difference between the means of the two groups
p = probability

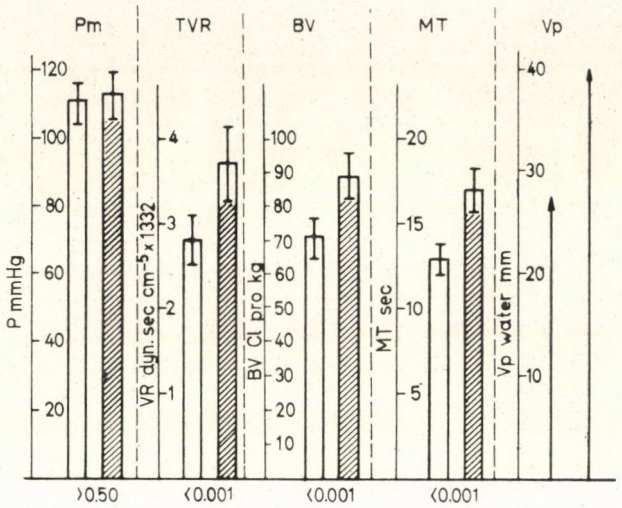

Fig. 3

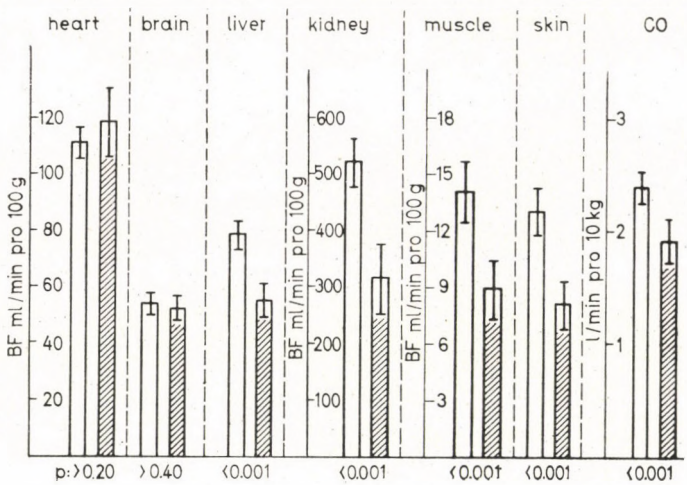

Fig. 4

but cardiac as well as cerebral blood flow and vascular resistance remained largely unchanged.

Fig. 3 illustrates the changes in the circulatory parameters; the empty columns show the values of the control group, while the hatched columns, those of the group with valvular lesion.

Fig. 4 shows the values for blood flow in the individual organs and of cardiac output.

In the knowledge of cardiac output and of the flow in the individual organs

it was possible to assess the cardiac output's participation of the organs. Fig. 5 illustrates the shifting of cardiac output in fraction after tricuspid insufficiency in per cents of the mean control values.

As Fig. 5 shows, the tricuspid lesion was followed by a significant increase in the cerebral and coronary fractions of cardiac output. This accounted for the fact that, despite the overall reduction in cardiac output, cerebral and coronary flow remained normal. On the other hand, there was a significant

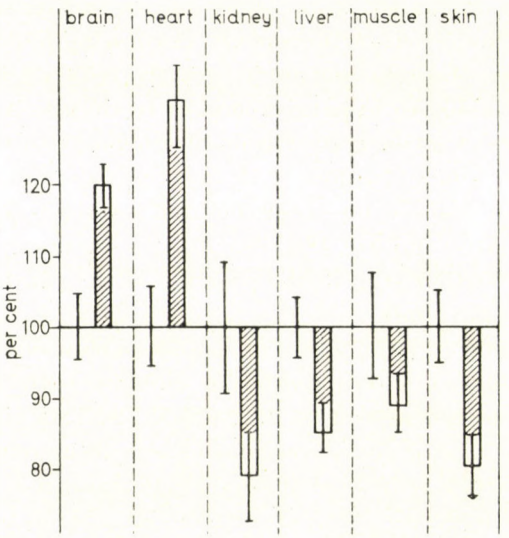

Fig. 5

reduction in the renal, hepatic, muscle and skin fractions of cardiac output; in other words, it is the blood supply of these organs which bears the brunt of reduced cardiac output.

Discussion

For the study of haemodynamic changes in congestive cardiac failure, induced valvular lesions are generally used as experimental models. As one of the earliest models of this kind, experimental bicuspid regurgitation was used by CUSHING and BRANCH [6], later by BERNHEIM [7] and by others [8—11, etc.]. Experimental pulmonary stenosis and failure [12—15] as well as aortic stenosis [16—21] have also been applied in similar studies. Tricuspid lesion is the most widely used experimental procedure of this kind, either by itself [2, 6] or in combination with pulmonary stenosis and or failure [22—28]. However, the normal signs of congestive heart failure, thus

oedema, ascites, congestive bronchitis, etc. were confined to a small number of cases; in the majority, congestive failure remained latent, only physical exertion or saline infusions brought about a transitory oedema. The results of the present study are consistent with these observations; the heart failure was latent congestive, in other words, circulation was seemingly compensated at rest and oedema was absent, but the presence of incipient failure was indicated by an elevation of venous pressure.

On the evidence of the present findings, the development of congestive heart failure is accompanied by a reduction of cardiac output and of the velocity of blood flow with an increase in circulating blood volume and total peripheral resistance. Cardiac output is usually reduced in congestive heart failure, unless this is due to hypervolaemia or to shunts. A slowing of circulation, a prolongation of mean transit time also belongs to the general features of congestive heart failure. In dogs with induced valvular lesions, an increase in circulating blood volume has been pointed out by several authors as an early sign of congestive failure [28, 29, 30]. An increase in total peripheral resistance marks a further step in the progress of the condition, when circulatory regulation already fails.

Data on vascular resistance and on the blood flow of the individual organ reflecting the regulation of circulation in animals with valvular lesion are scarce. The results of BARGER et al. [22, 31, 32] and of CONN and BOYS [33] suggest that in dogs with induced valvular lesions an increase in renal vascular resistance parallel with a reduction of renal blood flow are early signs of congestive heart failure. RICHARDS [34] connects the increase in renal and peripheral resistance with stagnation hypoxia; in fact, an improvement of the circulatory state results in a relief of renal and peripheral vasoconstriction. On the evidence of the studies of HALMÁGYI et al. [35] and of BROD [36], the development of congestive heart failure goes hand in hand with an increase in sympathetic vasoconstrictor tone causing an increase in pulmonary as well as systemic vascular resistance and an especially great increase in renal and cutaneous vascular resistance. WÉGRIA et al. [37] and WEST et al. [38] measured an increased blood flow from the coronary sinus in dogs with induced valvular lesion. WERKŐ [39] associates the production of congestive heart failure with a significant reduction in renal, splanchnic and extremital blood flow.

The present results suggest that congestive heart failure not only affects the circulation (cardiac output, transit time, circulating blood volume, venous pressure) but also modifies its regulation. While cardiac and cerebral flow remains unaffected despite the reduction in cardiac output, vascular resistance is greatly increased in the kidney, liver, muscles and skin, reducing thereby their flow as well as their organ cardiac output's fractions. Our results thus support the view of WERKŐ [39] that in congestive heart failure, vascular resistance in the peripheral and splanchnic areas is excessively high, and are

also consistent with the findings of WÉGRIA et al. [37] and WEST et al. [38] in that congestive heart failure leaves coronary blood flow unaffected.

Neither the data in the literature, nor the present results have thrown sufficient light on the mechanism accounting for the changed haemodynamic regulation in congestive heart failure. After acute coronary ligation [40] and in experimental atrioventricular block [5], the haemodynamic changes follow a similar pattern as after valvular lesions, as reflected by an increased cardiac and cerebral fraction of cardiac output at the expense of the muscular and cutaneous fractions. Both experimental models involve a significant reduction in cardiac output. This would suggest that in congestive cardiac failure it is the reduction in cardiac output which constitutes the primary factor initiating the haemodynamic changes and accounting for the shifting of the cardiac output's fractions.

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Dr. Ferenc SOLTI
Dr. Júlia NAGY
Dr. Miklós ISKUM
Dr. Anna HARTAI

Budapesti Orvostudományi Egyetem I. sz. Belklinikája
Budapest VIII., Korányi S. u. 2/a.

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INTRA-ACINAR LIPOLYSIS, AN EARLY SIGN OF INTRAVITAL PANCREATIC AUTODIGESTION

By

E. BÁCSY, Z. NAGY and M. PAPP

INSTITUTE OF EXPERIMENTAL MEDICINE, HUNGARIAN ACADEMY OF SCIENCES, AND SECOND INSTITUTE OF PATHOLOGY, SEMMELWEIS UNIVERSITY MEDICAL SCHOOL, BUDAPEST

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Fluorescent and electron microscopic studies showed that sunflower oil injected into the pancreas of dogs and pigeons finds its way into acinar cells. By the calcium-lead sulphide method the presence of fatty acids can be demonstrated. Electron micrographs exhibit autophageous vacuoles with zymogen granule content within cells still intact in structure.

More than a century ago, CLAUDE BERNARD (1856) induced pancreatitis in dogs by injecting olive oil into the gland through the efferent duct. In man, the reflux of chyme from the duodenum into the pancreas has been reported to give rise to acute pancreatitis. Vegetable oil and chyme are substrates for lipase. The present paper describes experiments showing that sunflower oil injected into the pancreas gains access to the acinar cells where it breaks down to fatty acids and autolysis may set in.

Materials and methods

Thirteen dogs of either sex and 14 kg average body weight, fasted for 16 hours after a meal of raw meat, were laparotomized under chloralose anaesthesia, and 6—8 ml of sunflower oil were injected into the pancreas through the duct of Santorini to induce pancreatitis. Biopsy was undertaken before and immediately, and 15 minutes after the injection and then at 15-minute intervals up to the 150th minute. The tissue fragments were fixed overnight in 4% cold formalin containing calcium, pH 7.2. After keeping them for 24 hours in cold 7.5% saccharose and for the same time, in 30% saccharose, the samples were frozen by carbon dioxide snow and 6 μ thick sections were cut in a cryostat.

Fatty acids were demonstrated with the calcium lead-sulphide method of GOMORI (1945). The sections were treated in the refrigerator for 10 minutes with 0.1% calcium chloride and for another 10 minutes with 2% lead citrate. After several washings, the preparations were allowed to stand for 3 minutes in diluted ammonium sulphide, washed thoroughly with water and, finally, mounted in a concentrated sugar solution.

Lipids were stained either with chelidonium extract for 5 minutes, phosphine 3R for 3 minutes, or Nile blue according to KLEEBERG (cit. CLARA, 1965).

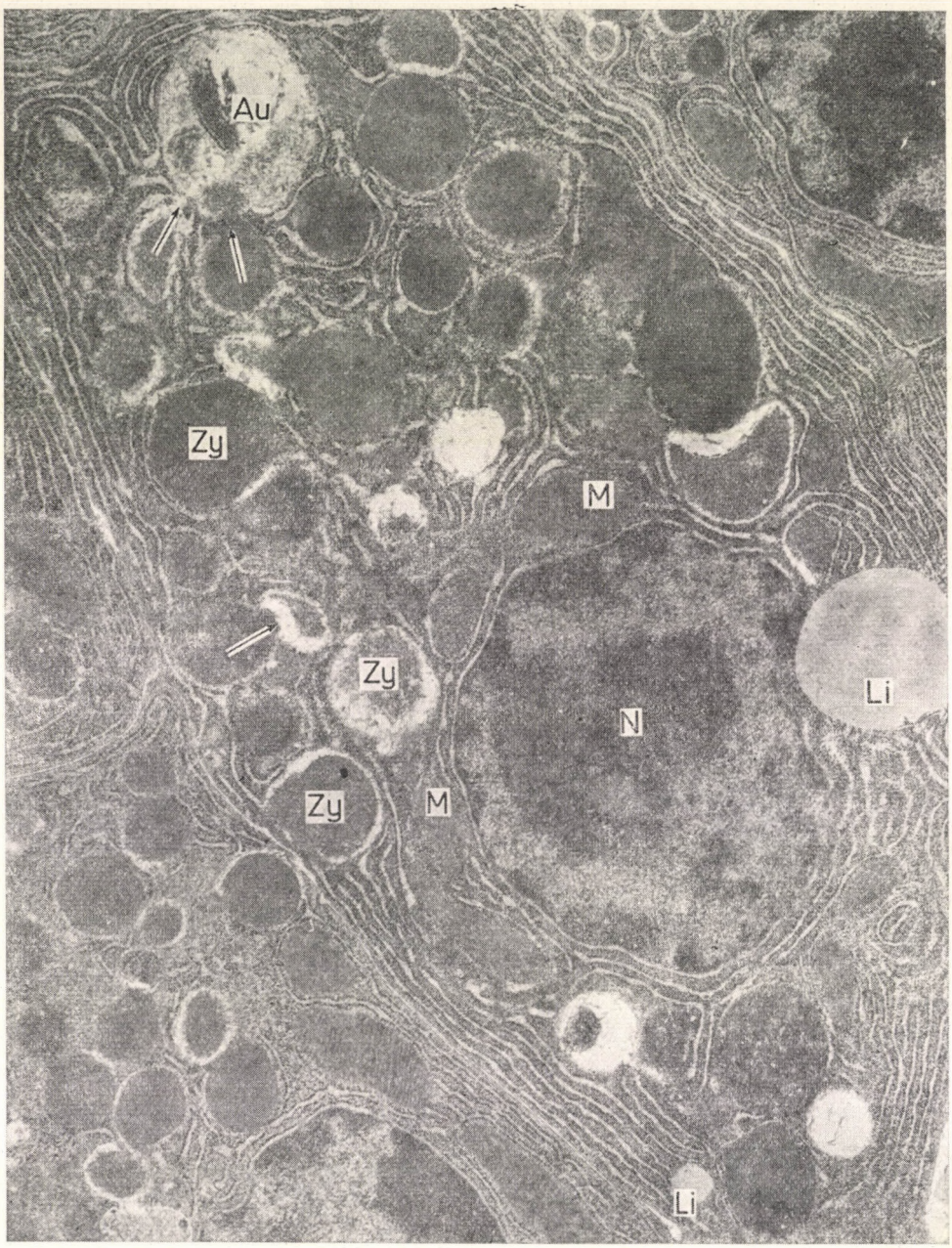

Fig. 3. (×19 200) and *Fig. 4.* (×43 200) Pigeon. Lipid droplets (Li), zymogen granules (Zy) and autophagous vacuole (Au) containing fragments of endoplasmic reticulum (Er) and zymogen granule's content (↑)

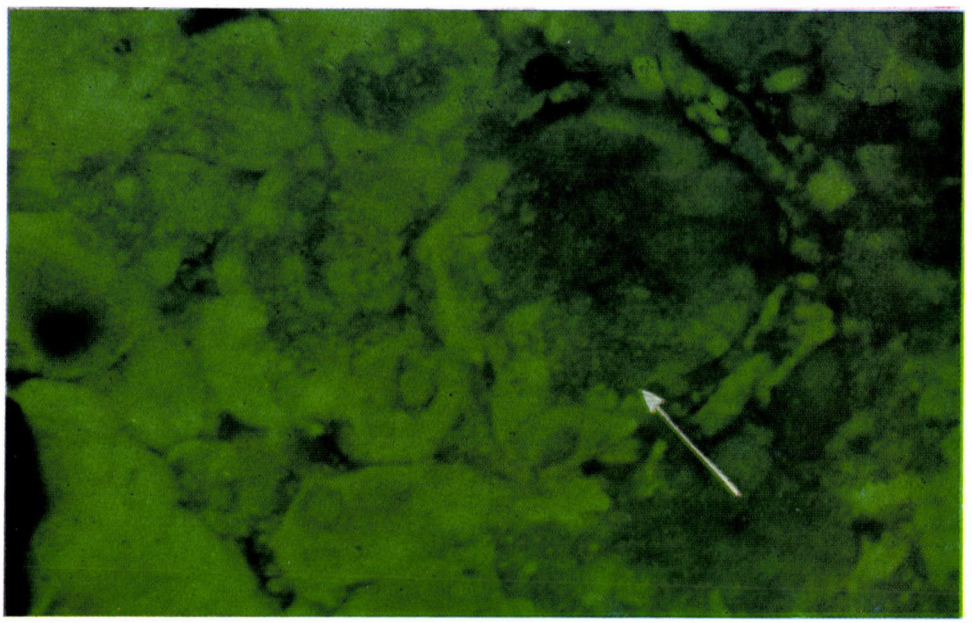

Fig. 1. Dog. Lipid droplets in pancreatic acinar cells (↑) and oil in the efferent ducts. Phosphine 3 R staining, ultraviolet light-induced fluorescence. × 200

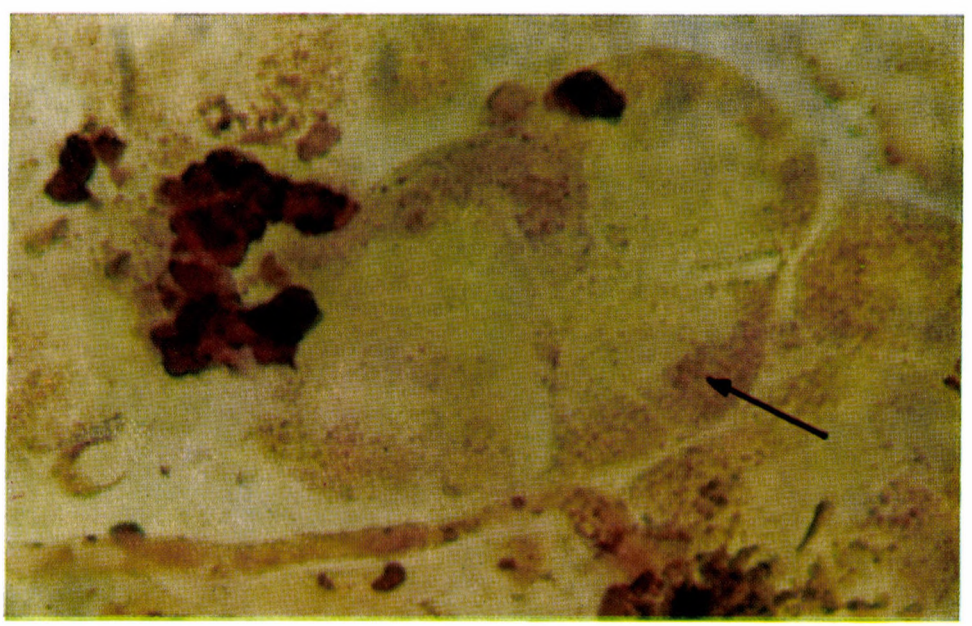

Fig. 2. Dog. Fatty acids as brown lead sulphide deposits in pancreatic acinar cells (↑). × 300

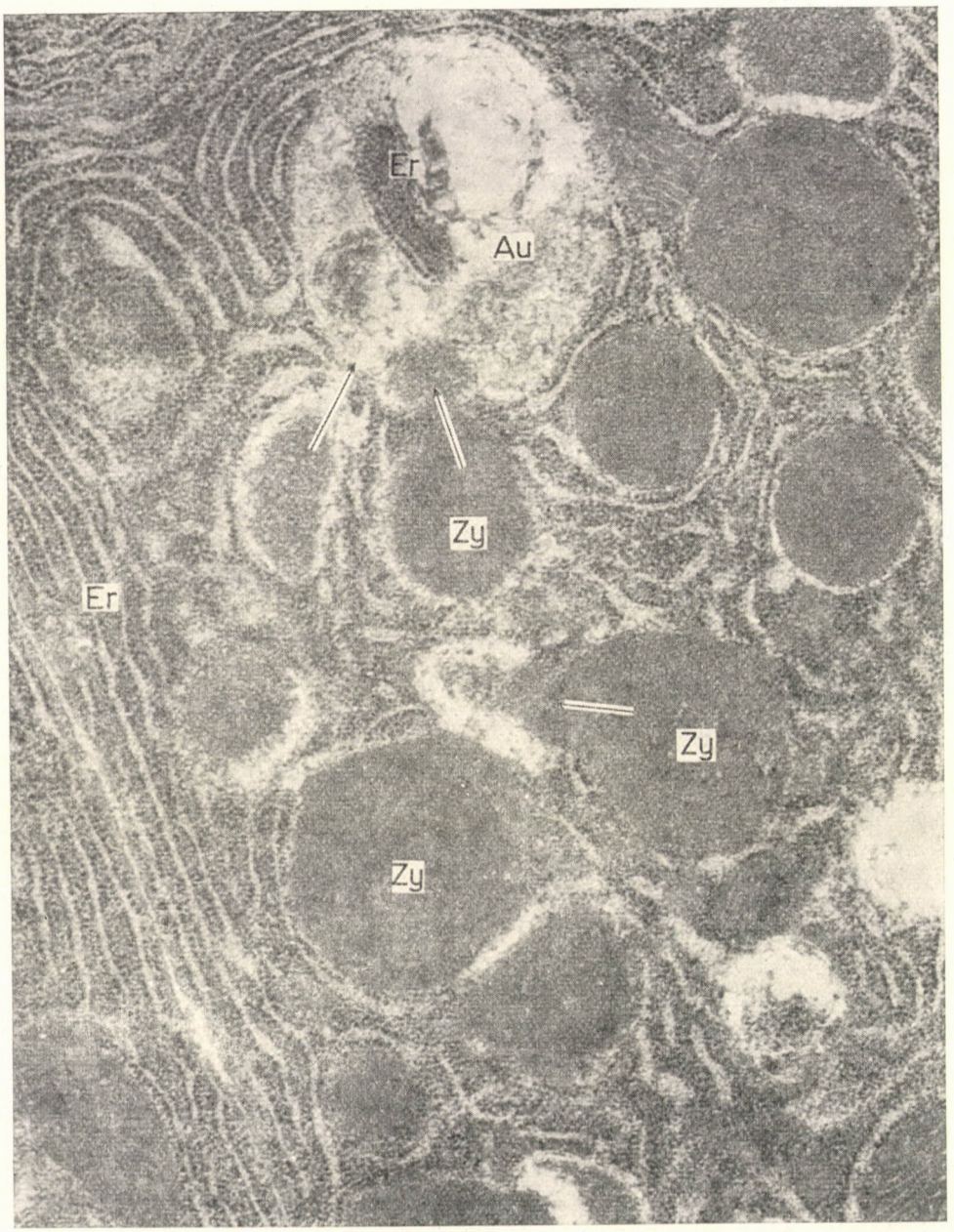

Fig. 4

For electron microscopy, anaesthetized pigeons were given each an injection of 0.2 ml of sunflower oil into the ventral pancreatic lobes through the efferent duct. Three hours later, the lobes were perfused through their artery first with Holt's fixative and then with cold 2% glutaraldehyde. Pieces of pancreatic tissue were allowed to stand in cold 2% glutaraldehyde for 2 hours, then in 1% osmium tetroxide, and embedded in Durcupan-Araldite. Sections cut in an LKB III microtome were counterstained with uranyl acetate and lead citrate, and examined in a JEM 6AS electron microscope.

Results and discussion

Immediately after its injection, the oil showed a bluish-white fluorescence in the dilated efferent ducts and interstitial spaces when stained with chelidonium extract, and had a bright yellow fluorescence after staining with phosphine 3R. In the biopsy specimens taken thirty minutes later, the oil appeared with the same stains as droplets in acinar cells (Fig. 1). Between the 90th and 120th minute, such droplets occupied a large part of the cytoplasm in nearly all these cells. Incidentally, earlier authors demonstrated that trypsin infused into the efferent duct secured the entry of India-ink particles into acinar cells (NUDELMAN et al., 1965). With Nile blue, 15 minutes after the injection of oil blue-coloured, non-fluorescent droplets were visible in the acinar cells. In the efferent ductules, the acinar lumina, and the interstitial spaces, the oil assumed the same blue colour, indicating that it contained free fatty acids (CLARA, 1965).

At 15 minutes after the oil injection, the fatty acids appeared in the acinar cells first as a delicate, afterwards as a rather coarse brown precipitate (Fig. 2). The oil in the efferent ducts and in the interstitium likewise assumed a brown colour, pointing to increased fatty acid content. The erythrocytes also became brown.

Electron microscopy revealed the presence of lipid droplets in apparently intact acinar cells. In circumscribed areas of these cells, autophagous vacuoles were seen to contain disintegrating endoplasmic reticulum, and some of the zymogen granule content (Figs 3, 4); in the intact pancreas this phenomenon could not be observed. The conclusion is that the acid radicals set free during lipolysis, produce locally an acid milieu which favours proteolysis (BLEYL et al., 1966), phospholipolysis (SCHMIDT et al., 1969), elastolysis (GEOKAS et al., 1968) or all three resulting in autolysis.

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Dr. Miklós PAPP, Budapest IX, PoBox 67, Hungary

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THERAPEUTIC ABORTION AS A POSSIBLE SOURCE OF RH IMMUNIZATION

By

Susan R. Hollán, Judith G. Szelényi, Vera N. Sőtér and Mária Hasitz

NATIONAL INSTITUTE OF HAEMATOLOGY AND BLOOD TRANSFUSION, BUDAPEST

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In a strictly controlled trial foetal cells were detected by both, the acid elution and the urea method in the circulation of 13 (16.9%) out of 77 mothers within 12 hours after delivery, and in 18 (16.1%) out of 112 women within 12 hours after curettage. The anti-D prevention programme launched in Hungary gives second priority to all Rh-negative women who had not been immunized and are subjected to artificial abortion.

Previous works have drawn attention to therapeutic abortion as a possible source of Rh-immunization [1—3]. The most disputed problem is still the occurrence of transplacental haemorrhage (TPH) as a consequence of early artificial termination of pregnancy. The problem is of great importance in decisions of national programmes for the prevention of Rh-immunization.

In a strictly controlled trial, foetal cell scores were determined within 12 hours after delivery in 77, and after early artificial termination of pregnancy in 112 women. The foetal/adult erythrocyte ratio was estimated in each blood sample by both the acid elution [4] and urea elution methods [5]. Each foetal cell score was counted by two experts independently. The final counts are results of the scanning of 80 high power and 200 low power microscopic fields from each preparation. In order to check the error of the foetal cell counts, parallel estimation of foetal/adult red cell ratio from known dilutions of cord blood/adult blood was carried out by the acid elution and the urea elution methods. Results are summarized in Table I.

It is obvious from Table I that up to a dilution of 1 to 10 000 the results of the foetal cell scores estimated by the two methods, corresponded well. Above this dilution both methods yielded unreliable results.

In contrast to the scanning of normal foetal/adult cell ratios, the two methods gave different results in various types of haemolytic anaemia with reactivated foetal type erythropoiesis [5, 6]. In these cases it was always the acid elution technique which gave the false positive results, while the results of the urea elution technique agreed well with those of the alkali denaturation test for foetal haemoglobin.

A similar discrepancy was noted in 4 of the pregnant women under study. Foetal cells were detected in their blood with the acid elution method, while the urea method revealed no foetal cells. Based on earlier results [5, 6], these false positive cases have not been included into the group diagnosed as having TPH.

Table I

Estimation of foetal/adult red cell ratios from known dilutions of cord blood/adult blood

Actual dilution	Estimate of foetal/adult red cell ratio	
	acid elution method*	urea elution method*
1 : 250	1 : 214	1 : 254
1 : 1 000	1 : 916	1 : 1 144
1 : 2 000	1 : 2 217	1 : 2 287
1 : 4 000	1 : 3 842	1 : 4 089
1 : 10 000	1 : 10 793	1 : 10 264
1 : 20 000	1 : 12 928	1 : 16 122
1 : 50 000	1 : 18 364	1 : 26 561

* Total number of fields scanned: 80 high power fields (magn. 1000×)
200 low power fields (magn. 40×)

Table II

Presence of foetal cells in maternal blood samples taken within 12 hours after delivery, and after early artificial termination of pregnancy

	Total	Foetal cell present	
		No. of cases	Per cent
Delivery	77	13	16.9
Curettage	112	18	16.1
Total	189	31	100.0

Foetal cells were detected by both methods in the circulation of 13 out of 77 mothers after delivery, which corresponds to a 16.9% frequency. Foetal cells were present in the same percentage (16.1%) in the blood of women after curettage (Table II).

Large transplacental haemorrhage occurred in one case after delivery; 1 : 5000 foetal cells corresponding to about 1 ml of TPH, and foetal/adult cell ratios ranging from 1 : 10 000 to 1 : 30 000 occurred with the same frequency in both groups under study (Table III).

Studies for the detection of anti-D antibodies after early artificial termination of pregnancy are in progress. MATTHEWS [7] found that about a quarter to a third of the terminations seem to have TPH. CLARKE [8] quoting the results of MATTHEWS [9] stated that none out of 97 Rh-negative mothers

Table III

Foetal cell scores in blood samples taken within 12 hours after delivery, and after early artificial termination of pregnancy

	Total No.	No. of cases with a foetal/adult cell ratio of		
		1 : 1000	1 : 5000	1 : 10 000–1 : 30 000
Delivery	13	1	2	10
Curettage	18	0	2	16

* Estimated by the urea elution method

observed for six months after their abortion has produced an antibody. Based on these observations, CLARKE [8] expressed the view that the risk of immunization after abortion probably is not as great as it might be expected.

We are fully aware that final conclusions can be based only on the results of antibody determination during subsequent pregnancies following abortions. In spite of the problem not being fully clarified, according to the suggestions of Dr. I. Simonovits our national prevention programme gives second priority to all Rh-negative women who had not been immunized and are subjected to artificial abortion. This decision was motivated by the fact that a high percentage of abortions are being carried out in young girls who may have to face eventual tragic consequences of Rh-immunization.

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Addendum

Subsequent to submitting this manuscript for publication one of the authors (S. R. HOLLÁN) had the opportunity to read the galley proof of a paper prepared by Professor C. A. CLARKE on "Prevention of rhesus isoimmunization. J. clin. Genet." (in press) which gives a detailed review of the literature on immunization following abortion.

The Memorandum of the *WHO Scientific Group on the Prevention of RH Sensitization, Geneva, 19—23 October, 1970* included in the recommendations, that D-negative women following abortion should receive a dose of anti-D, unless it is shown that the conceptus is D-negative.

Dr. Susan R. HOLLÁN
Dr. Judith G. SZELÉNYI
Dr. Vera N. SŐTÉR†
Mária HASITZ

National Institute of Haematology and Blood Transfusion
Budapest XI., Daróczi út 24, Hungary

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The new Executive Committee of the **Italian Society of Pathology** has been elected on 15th July, 1970.

The new officers are:

President: Prof. Luigi Califano

Vice-Presidents: Prof. Paolo Craxì and Prof. Luigi Leinati

Secretary: Prof. Alfonso Giordano

Vice-Secretary: Prof. Giuseppe Pontieri

Counsellors: Prof. Elio Baldacci, Prof. Antonio Giampalmo and Prof. Dino Merlini.

The new Executive Committee, according to the wishes of the great majority of the members of the Italian Society of Pathology, and following the suggestions of the Executive Committee of the European Society of Pathology, expressed during the meeting held in Paris on July, 4th, 1970, has invited the European Society of Pathology to held its 3rd Congress in Italy, on September 14—16 1971, on occasion of the 2nd centenary of G. B. Morgagni's death.

Meanwhile, the Committee has decided to postpone to 1972 the 12th Congress of the Italian Society of Pathology.

*

XI INTERNATIONAL CONGRESS OF INTERNAL MEDICINE

is being held in Delhi under the aegis of the Association of Physicians of India from October 25—30, 1970. Excellent scientific and social programmes are being arranged. Further particulars can be obtained from the Congress Secretariat, V. P. Chest Institute, Delhi University, Delhi-7.

*

JOURNÉES EUROPÉENNES DES CENTRES D'AUDIOPHONOLOGIE

Thème: PROGRÈS EN AUDIOPHONOLOGIE

LYON, 29-30 Novembre, 1-2-3 Décembre 1970

DIMANCHE 29 NOVEMBRE 1970

Amphithéâtre de la Clinique
d'Oto-rhino-laryngologie et d'Audiophonologie
Pavillon U, Hôpital Edouard-Herriot

AVANT - PROGRAMME

Langues officielles: Français, Anglais

DIMANCHE 29 NOVEMBRE 1970

14 h 00: Accueil des Congressistes.

15 h 00—15 h 30: Prof. D. HENNEBERT (Bruxelles)
Contribution belge à l'Audiophonologie.

15 h 30—16 h 00: Prof. E. FORTUNATO (Rome)
La création de l'enseignement officiel de l'Audiophonologie en Italie

16 h 00—16 h 30: M. P. VEIT (Paris)
Evolution en Europe de la profession d'audio-prothésiste.

16 h 30: P A U S E

17 h 00—17 h 30: Mme S. BOREL-MAISONNY (Paris)
La profession d'orthophoniste.

17 h 30—18 h 00: M. L. ZIEGLER (Paris)
La profession de professeur de sourds.

LUNDI 30 NOVEMBRE 1970

(matin)

9 h 00— 9 h 45: Introduction: Professeur P. Mounier-Kuhn.

9 h 45—10 h 45: Prof. C. Kaphan (Marseille)
Place de l'informatique en Audiophonologie.

10 h 45—11 h 45: Dr. P. Hennebert (Bruxelles)
Année 60: un tournant en vestibulométrie.

11 h 45: P A U S E

12 h 00—13 h 00: Prof. L. B. W. Jongkees (Amsterdam)
Aspects expérimentaux de la physiologie du vestibule.

(après-midi)

14 h 30—15 h 00: Prof. V. Pfaltz (Bâle)
Progrès techniques en électronystagmographie.

15 h 00—15 h 30: Prof. A. Montandon (Genève)
Progrès en électronystagmographie.

15 h 30: P A U S E.

16 h 00—17 h 00: Prof. G. Greiner et Prof. C. Conraux (Strasbourg)
Progrès cliniques en vestibulométrie.

17 h 00—17 h 30: Prof. M. Arslan (Padoue)
Progrès en thérapeutique des vertiges.

17 h 30—18 h 30: Prof. C. Chouard (Paris)
Physiologie du nerf facial.

MARDI 1er DÉCEMBRE 1970

(matin)

8 h 30— 9 h 30: Prof. B. Vallancien (Paris)
Progrès en exploration fonctionnelle du larynx.

9 h 30—10 h 00: Prof. K. Vrticka (Lucerne)
L'analyse acoustique.

10 h 00—11 h 00: Mme S. Borel-Maisonny (Paris)
La tomoanalyse de la parole.

11 h 00: P A U S E.

11 h 30—12 h 00: Prof. Molinari (Padoue)
L'innervation laryngée.

12 h 00—12 h 30: Prof. L. Croatto (Padoue)
Traitement des insuffisances vélaires.

12 h 30—13 h 00: Dr G. Cornut (Lyon)
Hormonothérapie et voix.

(après-midi)

14 h 30—15 h 00: Pr. J.P. Haguenauer (Lyon)
Microchirurgie laryngée.

15 h 00—16 h 00: Prof. A. Appaix (Marseille)
Progrès dans la connaissance des retards de langage chez l'enfant.

16 h 00: P A U S E.

16 h 30—17 h 00: Prof. F. Giaccai (Florence)
Discrimination fréquentielle.

17 h 00—17 h 30: Prof. M. Del Bo (Milan)
Les progrès en audiométrie tonale supraliminaire.

17 h 30—18 h 00: Prof. L. Bernabei (Sienne)
Le temps et la sensation sonore.

18 h 00—18 h 30: Dr J.P. Rebattu (Lyon)
Réparation des lésions cochléo-vestibulaires: acquisitions médicales et médico-légales.

MERCREDI 2 DÉCEMBRE 1970

(matin)

8 h 30— 9 h 00: Prof. J. Van Den Eeckhaut (Leuven)
La régulation vasculo-tensionnelle de l'oreille interne.

9 h 00— 9 h 30: Prof. M. Burgeat (Paris)
Progrès en physiologie de l'audition.

9 h 30—10 h 00: Prof. S. Rauch (Olten)
Progrès en biochimie de l'oreille interne.

10 h 00: P A U S E.

10 h 30—11 h 00: Prof. H. Spoendlin (Zürich)
Bruit et cochlée.

11 h 00—11 h 30: Prof. R. Hahn (Turin)
La voie osseuse.

11 h 30—12 h 30: Prof. M. Portmann (Bordeaux)
L'électrocochléogramme.

12 h 30—13 h 00: Prof. K. TERKILDSEN (Copenhague)
Impedance measurements.

(après-midi)

14 h 30—15 h 00: Prof. H. A. BEAGLEY (Londres)
Methods of investigating non organic hearing loss.

15 h 00—15 h 30: Prof. W. KEIDEL (Erlangen)
D. C. potential in the human cortical evoked response, a new contribution to the objective audiometry.

15 h 30—16 h 00: Prof. C. HOLM et Prof. G. STANGE (Fribourg en Brisgau)
Importance des lignes de stimulation — excitations évoquées acoustiques pour la discrimination de la parole chez l'enfant.

16 h 00: P A U S E

16 h 30—17 h 00: Prof. P. PIALOUX (Paris)
Potentiels évoqués chez les aphasiques.

17 h 00—18 h 00: Prof. E. BOCCA (Ferrare)
Tests d'atteinte centrale de l'audition.

18 h 00—18 h 30: Prof. J. C. LAFON (Besançon)
Le test phonétique.

18 h 30—19 h 00: Prof. O. SCHINDLER (Turin)
Progrès en audiométrie vocale.

JEUDI 3 DÉCEMBRE 1970

(matin)

8 h 30— 9 h 00: Prof. P. BIESALSKI (Mayence)
Progrès dans les procédés d'exploration clinique chez l'enfant sourd.

9 h 00— 9 h 30: Prof. G. PERELLO (Barcelone)
Audiométrie infantile.

9 h 30—10 h 00: Dr Danièle CHARACHON (Lyon)
Dépistage de la surdité chez l'enfant.

10 h 00: P A U S E

10 h 30—11 h 00: Prof. H. EWERTSEN (Copenhague)
Ear level hearing aids for both ears.

11 h 00—11 h 30: Prof. J.P. DE REYNIER (Lausanne)
Appareillage acoustique de l'enfant.

11 h 30—12 h 00: Prof. J. BOURGUET (Rennes)
Education auditive des enfants sourds.

(après-midi)

14 h 30—15 h 30: Prof. G. Decroix (Lille)
L'appareillage acoustique de l'adulte.

15 h 30—16 h 00: Prof. L. Fisch (Zürich)
Les progrès en thérapeutique chirurgicale oto-neurologique.

16 h 00: P A U S E

16 h 30—17 h 00: Prof. Ag. R. Charachon (Grenoble)
Progrès en chirurgie de l'oreille.

17 h 00—18 h 00: Prof. Ag. A. Morgon (Lyon)
Exposé de synthèse.

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Prof. Escher

Prof. Menzio

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II. INTERNATIONAL CONGRESS OF PSYCHO-NEUROENDOCRINOLOGY

BUDAPEST, JULY 1—3, 1971

The Congress will be held in Budapest, and the program will consider the following topics;

1. Developmental neuroendocrinology
2. Biosynthesis and release of pituitary trop-hormones
3. Drug actions on neuroendocrine and brain mechanisms
4. Hormonal effects and brain mechanisms
5. Developments in clinical neuroendocrinology

Each of the 5 major topics will be introduced by review lectures of 20 to 30-minute duration, which will be given by invited speakers. Each session will be followed by a free discussion of 6 minute duration and presentation of new unpublished data by registered participants.

A registration fee of U. S. $ 20 or of 20 rubels (for participants from socialistic countries) will be requested from participants till March 31, at the address as follows: Hungarian National Bank in favour of the IBUSZ account — RCS 505019.

Further information may be obtained from the Secretariate of the Congress of International Society of Psycho-Neuroendocrinology, MOTESZ, Budapest I., Apród u. 1—3.

PROF. K. LISSÁK
Chairman of the International Congress
of Psycho-Neuroendocrinological Society

2nd CONGRESS OF THE EUROPEAN ASSOCIATION OF RADIOLOGY

International Congress Centre RAI Amsterdam, the Netherlands, 14—18 June, 1971

Secretariat before and after the Congress	c/o Holland Organizing Centre 16 Lange Voorhout The Hague, the Netherlands Telephone: (070) 65.78.50 Telex: 33111 Cables: Hocland — The Hague
Secretariat during the Congress	International Congress Centre RAI Europaplein Amsterdam, the Netherlands Telephone: (020) 42.51.51

Any additional information on the Congress will gladly be given by the Congress secretariat, c/o Holland Organizing Centre, 16 Lange Voorhout, the Hague, the Netherlands.

The Association of Scientists in Roumania is organizing in Bucharest, November 12th and 13th, 1970, a Symposium on the topic

"CYBERNETICS IN BIOLOGY AND MEDICINE"

The proceedings of the Symposium will take place in plenary sessions and the following specialized sections:

1. Cybernetics, a tool for biological phenomena research, Modelation of biological regulator phenomena.
2. Cybernetical interpretation of human pathological states.

For further information about the Symposium please, write to:

THE ASSOCIATION OF SCIENTISTS IN ROUMANIA

Secretariate of the Symposium

"CYBERNETICS IN BIOLOGY AND MEDICINE"

Bucharest — Str. Progresului 10 — ROMANIA
Căsuta Poștală nr. 90
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ACTA MEDICA

ТОМ 27 — ВЫП. 3

РЕЗЮМЕ

ФУНКЦИОНАЛЬНОЕ ИССЛЕДОВАНИЕ КРОВООБРАЩЕНИЯ КОЖИ У БОЛЬНЫХ С ИСТИННОЙ ПОЛИЦИТЕМИЕЙ

I. Действие экспозиции холоду и вазодилятаторов

Д. НАДЬ, П. ДАРОЦИ и Ч. БАЛАЖ

На основе обследования значительного числа больных с истинной полицитемией авторы выявили гемодинамические изменения во всей сосудистой системе кожи. Эти изменения наблюдались независимо от стадии заболевания, как при обострении так и в период ремиссии.

ИЗУЧЕНИЕ ВОЗБУДИМОСТИ И СКОРОСТИ ПРОВЕДЕНИЯ ИМПУЛЬСА ПЕРИФЕРИЧЕСКИХ НЕРВОВ ЧЕЛОВЕКА ПРИ НАРКОЗЕ ЭВИПАНОМ

Ф. МЕХЛЕР, Ф. КАЙТОР, и И. ХИДАШИ

Авторы у 20 лиц, страдающих нервномышечными заболеваниями, изучали изменения возбудимости (порог раздражения), скорости проведения импульса и рефрактерных периодов двигательных волокон локтевого нерва под влиянием внутривенного введения гексобарбитала натрия (Эвипан). Было установлено, что порог возбудимости, дистальный латентный период и скорость проведения импульсов двигательных волокон локтевого нерва изменяются в зависимости от концентрации гексобарбитала натрия.

В связи с этим обсуждаются литературные данные и предполагаемый механизм действия Эвипана.

ДЕЙСТВИЕ СИНТЕТИЧЕСКОГО ЧЕЛОВЕЧЕСКОГО АКТГ НА ФУНКЦИЮ КОРЫ НАДПОЧЕЧНИКОВ У ЧЕЛОВЕКА И У КРЫСЫ

Ф. А. ЛАСЛО, К. КОВАЧ, И. ФАРЕДИН, Л. ЦАКО, Ф. ДУНСТ, И. СИЙЙ, И. ТОТ, А. БИРО и М. ЮЛЕС

Авторы доказали, что гуманный кортикотропин, синтезированный *Баюсом* и сотрудниками (1, 2), содержащий 39 аминокислот, обладает как у крысы, так и у человека биологической активностью: в значительной мере стимулирует кору надпочечников. В связи с применением препарата не наблюдалось вредных побочных явлений. В некоторых случаях синтетическим АКТГ лечились также больные, которые были чрезмерно чувствительны в отношении нативных препаратов гормона.

КЛИНИЧЕСКИЕ И ЭКСПЕРИМЕНТАЛЬНЫЕ ДАННЫЕ О ВЗАИМОДЕЙСТВИИ МЕЖДУ ДИЭНЦЕФАЛЬНЫМИ ОБЛАСТЯМИ ЦЕНТРАЛЬНОЙ НЕРВНОЙ СИСТЕМЫ И ФУНКЦИЕЙ ЩИТОВИДНОЙ ЖЕЛЕЗЫ

Л. САНТО, Б. МЕШЕ и А. Л. РЕВИЦКИ

Авторы анализируют отдельные клинические данные и свои собственные наблюдения о связи между промежуточным мозгом и щитовидной железой. То наблюдение, что для заместительного лечения больных с врожденной микседемой требуется существенно больше гормона щитовидной железы чем обычно, причем известные побочные действия не появляются, позволяет заключить о том, что центральная нервная система или соответствующие ядерные области промежуточного мозга больных, страдающих врожденной микседемой, претерпевают аномалию развития. В опытах на крысах было доказано, что в промежуточном мозге животных, у которых дачей метилтиоурацила была вызвана микседема, закономерно возникающее варьирующее изменение ядра в области вентромедуллярного ядра и nucleus habenulae можно восстановить адекватным заместительным лечением. В противоположность этому варьирующие изменения ядер промежуточного мозга животных с экспериментальной врожденной микседемой, не удалось восстановить дачей гормона щитовидной железы.

ИЗМЕНЕНИЯ ЛИМФАТИЧЕСКИХ УЗЛОВ, РАЗВИВАЮЩИЕСЯ ПОД ВЛИЯНИЕМ ДЛИТЕЛЬНОЙ ДАЧИ АНТИЭПИЛЕПТИЧЕСКОГО ПРЕПАРАТА ГИДАНТОИНА

ДЬ. СЕГЕДИ, Г. КРАСНАИ, П. ШОРСЕГИ и Г. САБО

В связи с регулярным обследованием 170 больных эпилепсией авторы изучали частоту и гистологические особенности лимфаденопатий, развивающихся под влиянием длительного лечения антиэпилептическим препаратом гидантоина. В 15% больных авторы наблюдали набухание лимфатических узлов многолетней давности. Авторы называют это изменение хронической гидантоиновой лимфаденопатией. В 10 случаях была проведена биопсия лимфатических узлов. Гистологическая картина показала атрофию лимфоретикулярной ткани, ангиофиброзную тканевую пролиферацию и подчас узловатую перестройку лимфатических узлов (нодулярный фиброз). Злокачественного перерождения не наблюдалось ни в одном случае. Авторы анализируют связи между изменениями лимфатических узлов и вызывающими автоиммунологические заболевания свойствами препаратов гидантоина.

ДЕЙСТВИЕ СВИЩА ГРУДНОГО ПРОТОКА И ЛИМФАТИКО-ВЕНОЗНОГО АНАСТОМОЗА НА АСЦИТ

Д. САБО, Ж. МАДЬЯР и П. ШЕРЕНЬИ

У нормальных собак и у собак с повышением венозного давления в большом кругу кровообращения (животные с перикардитом сжатия) между давлением в грудном протоке и шейным венозным давлением не наблюдается постоянного градиента давления. При асците, вызванном путем суживания нижней полой вены, давление в грудном протоке в среднем на 6,9 $\pm$ 0,7 мм рт. ст. выше, чем давление в яремной вене. Следовательно, у устья грудного протока имеется функциональное сужение.

Под влиянием свища грудного протока, у этих животных уменьшается асцит и давление в воротной вене (нижней полой вене). Это действие только временное, и после прекращения свободного оттока лимфы, асцит и застой вновь повышаются.

Созданием шейного лимфатико-венозного анастомоза в половине подопытных животных можно уменьшить асцит, вызванный суживанием нижней полой вены. Однако, у части животных со сжатием полой вены, асцит и венозный застой могут уменьшаться и самопроизмольно. Вопреки этому кажется, что у части животных анастомоз оказывает благоприятное влияние.

У одного больного с циррозом печени шейный лимфатико-венозный анастомоз прочно прекратил асцит, до тех пор не поддающийся лечению.

На основе наблюдений, сделанных в опытах на животных и у одного больного, авторы считают, что при соответствующем выборе подходящих случаев, создание шейного лимфатико-венозного анастомоза у больных с циррозом, сопровождающимся значительным асцитом, вполне обосновано.

СТЕРОИДЫ В КОЖЕ И ВОЛОСЯНОМ ПОКРОВЕ ТЕЛА ЧЕЛОВЕКА

V. Методика количественного определения и количество свободных холестеринов и их эфиров в человеческой коже

И. ТОТ, И. ФАРЕДИН и М. ЮЛЕС

В коже женщин, не страдающих эндокринными заболеваниями, и женщин с гирсутизмом, а также в коже мужчин с акнэ авторы изучали количество свободных холестеринов и их эфиров.

Из 80—90 мг цельной кожи они изготовляли срезы на замораживающем микротоме и экстрагировали из срезов кожи при помощи смеси этанол-эфира (1 : 1) свободные холестерины и их эфиры. Затем методом двойной хроматографии на тонком слое силикогеля Г авторы отделяли свободные холестерины эфиров и определили методом цветной реакции по Златкис, Зак и Бойле количество отдельных фракций.

Было установлено, что только 2/3 части холестерина, определенного в коже живота женщин (не покрытой волосами) являются свободным холестерином и почти одна треть — эфирами холестерина. В волосатой коже лобка обнаруживается больше свободного и эфирного холестерина, чем в брюшной коже. Содержание свободного холестерина и его эфиров в коже женщин с гирсутизмом на фоне заболевания коры надпочечников, не отличалось от величин, определенных в коже контрольных женщин. В коже спины мужчин с акнэ количество холестерина существенно больше, чем в коже спины здоровых мужчин подобного возраста.

ФИЗИКАЛЬНЫЕ ОСНОВЫ ЭЛЕКТРОЭНЦЕФАЛОГРАММЫ

Т. ГЕРЕБ

Автор исчислял потенциал двойного слоя, помещенного во внутреннем пространстве замкнутого шара, на поверхности шара. Этим предоставил основу для научного анализа колебаний потенциала, сопровождающих эпилептический приступ. На основе полученных формул можно объяснить несколько особенно трудно толкуемых особенностей ЭЭГ.

ОПРЕДЕЛЕНИЕ ПРЕГНАНДИОЛА В МОЧЕ ПРИ ПОМОЩИ ХРОМАТОГРАФИИ НА ТОНКОМ СЛОЕ

Т. ФЕХЕР, Г. К. ФЕХЕР и Л. БОДРОГИ

Дается описание простого специфического метода для определения прегнандиола в моче при помощи хроматографии на тонком слое. Метод применим для измерения уровня стероидов в моче у небеременных и беременных женщин, а также у мужчин и детей, предоставляя также возможность для симультанного определения прегнандиола и прегнантриола в одном и том же образце. Подытоживаются результаты контрольных исследований, величины в норме и наблюдения, сделанные при типичных эндокринных заболеваниях.

ИЗМЕНЕНИЯ СОДЕРЖАНИЯ ЛИПОИДОВ И ИНТИМЫ АОРТЫ КРЫС, ПОЛУЧАВШИХ ДИЕТУ С НЕДОСТАТКОМ ФЛАВОНА, А ТАКЖЕ ПРИ ДАЧЕ ХОЛЕСТЕРИНА

А. АНТАЛ, А. РЕДНИК, К. ЛИПТАК, И. ДЬЁРИ, М. ГАБОР и Ш. БЕНКЁ

Авторами было изучено на крысах действие диеты по Шерман—Ламер—Камбеллу (Ш), дачи холестерина (Х) и тиреоидектомии (Т). Для уяснения взаимодействия между этими вмешательствами, авторы применяли их также одновременно в различных комбинациях. Было установлено, что тиреоидектомия и дача холестерина понижают сопротивление капилляров, причем недостаток флавона вызывает усиление этого эффекта. К концу 10-недельного периода диеты, наименьшее повышение уровня холестерина наблюдалось в группах, получавших диету с недостатком флавона, без дачи холестерина. В влажном весе

аорты доля общих липоидов была сравнительно большей в группах, получавших холестерин, в то же время аорта животных групп с недостатком флавона содержал сравнительно меньше липоидов. В отношении содержания холестерина аорты, между отдельными группами были выявлены только незначительные разницы, которые не отражали ни отклонения содержания общих липоидов, ни изменения сывороточного холестерина, ни капиллярной резистентности. Изменения поверхности аорты были наиболее выраженными у животных, получавших диету с недостатком флавона или при диете с недостатком флавона + тиреоидектомии (Т + Ш). Авторы объясняют эти изменения отчасти отечным набуханием основного вещества, но главным образом с накоплением мукоидных веществ. Дача флавоноида гесперидин-метил-халкон) тормозила повышение уровня холестерина у уменьшение капиллярной резистентности, но, повидимому, не оказала достоверного эффекта на изменения поверхности и содержания липоидов аорты, вызванные с совместной дачей холестерина и диеты с недостатком флавона.

ИЗУЧЕНИЕ ГУМОРАЛЬНОЙ И КЛЕТОЧНОЙ ЗАЩИТЫ ПРИ ЛЕЙКАМИЯХ И МИЭЛОФИБРОЗЕ

А. ПАТАКФАЛЬВИ, А. ГОГЛЬ, К. ШИМОН, Г. БЁМ, Ж. МИСЛАИ и Э. КАЙТША

Авторы изучали гуморальный иммунитет у больных с хронической и миэлоидной лейкемией и с миэлофиброзом с помощью метода одновременного определения изоагглютининов, противотел против антигенов среды и количественных иммуноглобулинов. Для изучения клеточного иммунитета они проводили — по аналогии туберкулиновой пробы — кожные пробы. При лимфоидной лейкемии наблюдается выраженное уменьшение производства противотел т. е. иммуноглобулинов, показывающее корреляцию с прогрессированием болезни и прежде всего касающееся понижения иммуноглобулинов групп IgA и IgM. — При миэлоидной лейкемии и миэлофиброзе, наряду с по существу нормальным синтезом противотел, было выявлено диссоциированное расстройство синтеза иммуноглобулинов. Понижение титра противотел было в общем более выраженными, чем это можно было ожидать на основе количества иммуноглобулинов. На основе этого наблюдения авторы полагают наличие иммуноглобулинов, не обладающим действиями антител. — На основе результата кожных проб при миэлоидной лейкемии нарушение иммунологической клеточной реакции более значительное; при лимфоидной лейкемии наблюдается умеренное понижение кожной реакции.

ИЗМЕНЕНИЕ РЕГУЛИРОВАНИЯ КРОВООБРАЩЕНИЯ ПОСЛЕ СОЗДАНИЯ ИСКУССТВЕННОГО ПОРОКА ТРЕХСТВОРЧАТОГО КЛАПАНА

Ф. ШОЛЬТИ, Й. НАДЬ, М. ИШКУМ и А. ХАРТАИ

При помощи искусственного порока трехстворчатого клапана (недостаточность трехстворчатого клапана, стеноз трехстворчатого клапана) авторы изучали изменения кровообращения, сопровождающие развитие сердечной декомпенсации. У 20 собак былсоздан искусственный комбинированный порок трехстворчатого клапана, а 40 собак служили контролями. Для определения минутного объема, количества циркулирующей крови и времени кругового крови использовали метод дилюции красителя (2 мг звенсблау на кг веса тела), а для измерения кровотока в сердце, мозгу, почках, печени, мышцах и коже — методику фракционированного измерения перфузии органов при мечении двумя изотопами (меченный 30 μC 86 Rb + 5 μC 131 I антипирин). Анализы кровообращения проводились через 11—16 недель после создания порока. Соответственно результатам исследований, под влиянием искусственного порока трехстворчатого клапана одновременно с повышением венозного давления уменьшается минутный объем, кровообращение замедляется, количество циркулирующей крови увеличивается и общее сосудистое сопротивление в большом кругу кровообращения усиливается. В значительной мере изменяется также регулирование кровообращения. Вопреки уменьшению минутного объема, кровоснабжение мозга и сердца остается нормальным, фракция мозга и сердца в минутном объеме увеличивается. В то же время в значительной мере повышается сопротивление сосудов почек, печени, мышц и кожи, а фракции этих органов в минутном объеме уменьшаются.

ВНУТРИАЦИНОЗНЫЙ ЛИПОЛИЗ, РАННИЙ ПРИЗНАК ПРИЖИЗНЕННОГО ПАНКРЕАТИЧЕСКОГО САМОПЕРЕВАРИВАНИЯ

Э. БАЧИ, З. НАДЬ и М. ПАПП

Флюоресцентно- и электронномикроскопические исследования показали, что подсолнечное масло, введенное в поджелудочную железу собаки и голубя, проникает в ацинозные клетки. Методом кальция-сульфида свинца можно выявить наличие жирных кислот. При электронномикрографии наблюдаются автофаговые вакуоли, содержащие зимогенные зернышки внутри клетки, обладающей интактной еще структурой.

ИСКУССТВЕННЫЙ АБОРТ КАК ВОЗМОЖНАЯ ПРИЧИНА РЕЗУС-ИММУНИЗАЦИИ

Ш. Р. ХОЛЛАН, Й. Г. СЕЛЕНЬИ, В. Н. ШЁТЕР и М. ХАШИЦ

В ходе одновременных исследований, проводившихся методом элюции кислотой и методом собственной уреи + элюции, авторы наблюдали после раннего прервания беременности подобную частоту появления фетальных клеток в кровообращении матери, как и после родов.

Охватывающую всю страну программу по профилактическому применению анти-D-иммуноглобулина для предупреждения резус-изоиммунизации авторы распространяют также на искусственное прервание беременности резусотрицательных, еще не иммунизировавшихся беременных женщин.

AUDIOMETRY AND HEARING AID

The importance of hearing sounds through the perception of audiophenomena emitted by the external world is vital. This function is indispensable for the creation of human speech and thereby of conscious existence, as well as for the transformation of man to a member of society. Hearing means the conversion of mechanical energy of air vibrations, produced by the sound source, into a stimulus to the nervous system: actually into electric waves, followed by the complex process of the development of audio-emotions.

Hearing and its testing

The hearing centre appreciates sound intensity on the basis of the number or "amount" of the arriving stimuli. Sound means the vibrations of the particles of a body (the sound source), which arrive at the hearing organ through a conductive (gaseous, liquid or solid) medium. The sound frequency perceptible for the human ear is between 16 and 20 cps. Above these frequencies, that is, the so-called infrasounds, and below this range or those called ultrasounds cannot be heard by the human ear. Sounds of excessive intensity bring about a feeling of pain. The pain threshold within the full frequency range is represented by the sounds of 10^{-3} watt/m^2 intensity.

The sound intensity range is, accordingly, between 10^{-16} and 10^{-3} watt/m^2, that is, the human ear is capable to perceive a sound energy 10 milliard-times that of the threshold value mentioned above.

The top limit of audible sounds is decreasing with age, thus the hearing threshold will increase. Its determination, that is, the test of reduced hearing can be performed by using an audiometer which is an electronic valve operated sound generator whereby sounds of different frequencies can be produced. The sound intensity may be varied between the hearing threshold and the feeling of pain. The curve representing reduced hearing in the function of frequency is called audiogram. The curve of reduced hearing thus obtained reveals the frequency range where the subject needs hearing improvement. The maximum amplification of the various transistorized hearing aids can be adjusted to match the sounds hardly heard by the ear.

Noise damages

After an extended period of time spent in noisy job, the hearing nerve may be permanently damaged which, in turn, might lead to a similarly permanent reduction of hearing. It is best to prevent any such noise effects. Excessive reduction of hearing will unfavourably influence the subject both in doing his job and participating in social life. It is, therefore,

necessary to improve this condition by increasing the ability of hearing as much as possible, which is readily feasible by the application of a hearing aid. Formerly, poor hearing was improved by making use of the "hearing tube" which facilitated the collection of sound waves, and directed them to the ear. Development in electroacoustics has rendered possible the manufacture of high-output hearing aid types. In the following paragraphs some audio-examination devices, modern hearing aids, and test lamps will be described.

Audiometer for clinics

The audiometer constructed by Medicor Works for clinical use meets the highest level requirements of clinics and audiological testing stations. It is a fully transistorized apparatus, equipped with a recording unit.

The double-channel system of the instrument makes possible the performance of all the audiological examinations generally adopted in clinical practice, being suitable for hearing threshold measurements by using either air or bone conductivity, then for the determination of the Békésy differential threshold, and the implementation of both the Luscher and noise audiometry type examinations, such as the Langenbeck test.

With a tape recorder connected, the apparatus may be used for audiometric speech testing purposes. These examinations have been made much faster and accurate than before, by the continuous sound and noise level adjustment, recording unit, built-in mike, and simple operation.

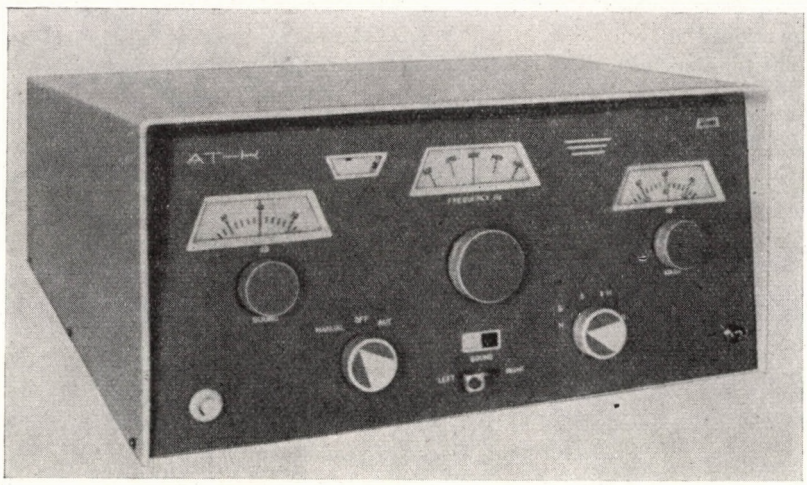

Fig. 1. Audiometer for clinics

Békésy audiometer

With this audiometer, the audiogram is plotted automatically after appropriate setting and start. Sound level modification is by a servomotor, directed in rotation by the patient, who exerts control by a pushbutton held in his hand, depending on if he has heard the sound in the earpiece or not. Operation is automatic, according to the variation of the test frequencies in preset intervals.

As an advantage of the automatic construction, the speed of sound intensity and frequency variations is changed electrically, instead of a mechanical gear. In addition, the construction permits manual intervention or control.

The Békésy audiometer produced by Medicor is used for the following audiological examinations:

- Audiothreshold curve plotting,
- Determination of the Békésy threshold difference,
- Carhart fatigue threshold measurements,
- Sound intensity equilibrium test,
- Audiotest by bone conductivity.

Measurement accuracy is greatly increased by the as narrow as 0.3 dB stages of sound intensity adjustment.

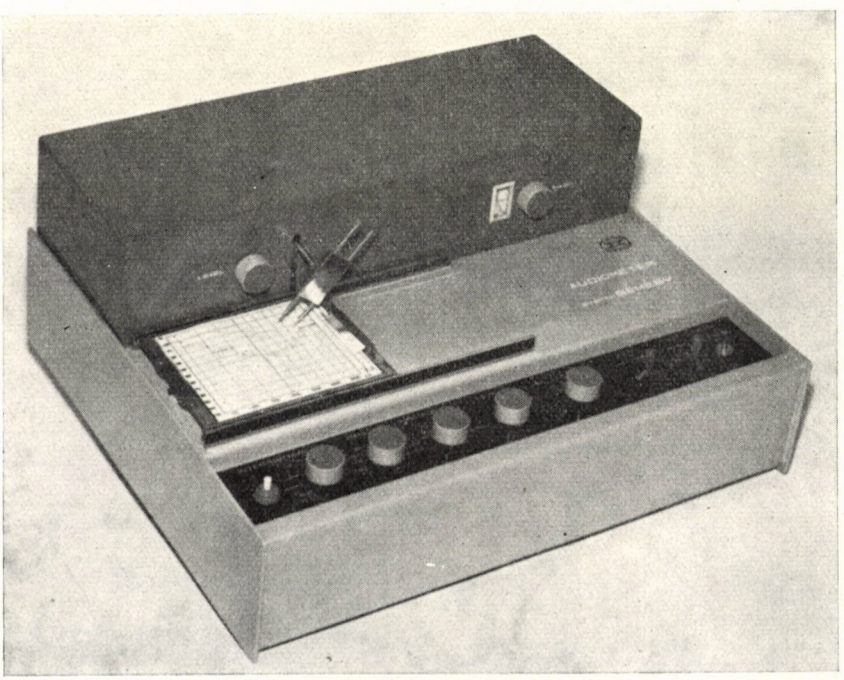

Fig. 2. Békésy audiometer

Hearing deficiencies of this and other types can be discovered in due time by in-site screening performed in industrial plants, schools, and by municipal authorities, if an adequate portable equipment is available. To meet this requirement, Medicor Works have developed two transistorized small-size screening audiometers of different output figures, whereby extensive screenings can be readily carried out at a rapid rate. Due to their small size and light weight, these instruments can be transported without difficulty. They operate on the basis of the audiothreshold principle, where the variation of the intensity of clear sounds through the earpiece will determine the audiolimit of the patient.

Type-A screening audiometer

Owing to its small size and light weight, the screening audiometer type-A might be called a "pocket audiometer" instrument. The circuits enclosed in a light-weight plastic housing are operated by a pair of two-way switches and a pushbutton. One of the switches selects the frequency, while the other directs the sound to the right or left ear. The sound signal is started by the pushbutton, and the apparatus is equipped with two miniature batteries.

Experiences collected through mass examinations confirm that for fast informatory screening purposes, two typical audiofrequencies and a preset sound pressure level are entirely sufficient to detect most hearing deficiencies.

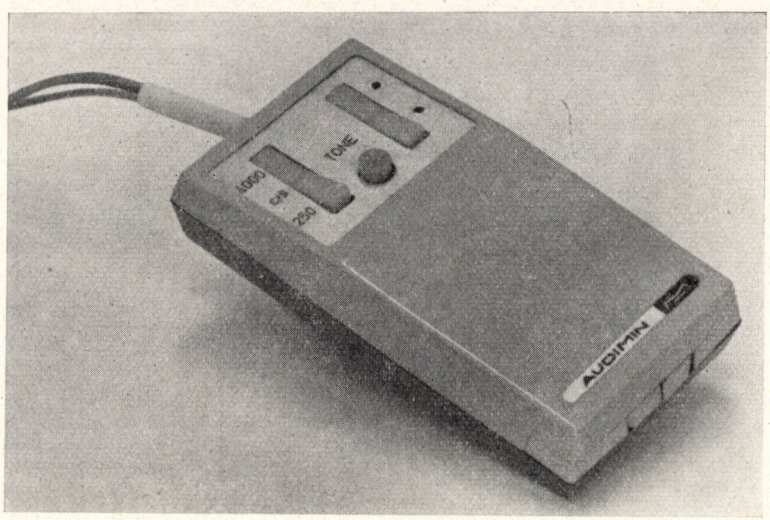

Fig. 3. Type-A screening audiometer

Type-B screening audiometer

This instrument is suitable to satisfy much higher requirements than those met by Type-A. Its four frequencies and six sound pressures provide for the determination of a total of 24 measurement points in the audiotest. The limited dimensions and light weight make possible the in-site performance of refined examinations. The transistorized circuits are supplied by two standard 4.5 flat batteries and, therefore, the apparatus can be used without any difficulties even where no mains are accessible.

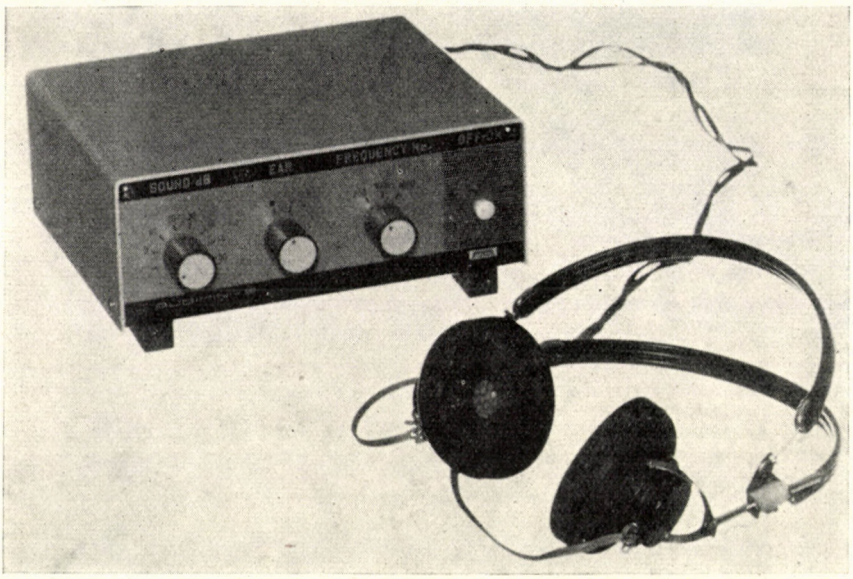

Fig. 4. Type-B screening audiometer

New single and double-bulb examination lamps

These can be used as universal independent light sources for dental treatment on surgery purposes, in oto-rhino-laryngotomical operations and examinations, cosmetic treatments, or as auxiliary light sources to increase light intensity and reduce the shadow effect during major surgical operations.

Common characteristics of both types are:

— Increased illumination intensity (adjustable in 2 stages),
— Uniformly illuminated area,
— Low-heat, no-glare, colour corrected light,
— Reduced weight and floor space requirement,
— Operational safety, easy operation and cleaning,
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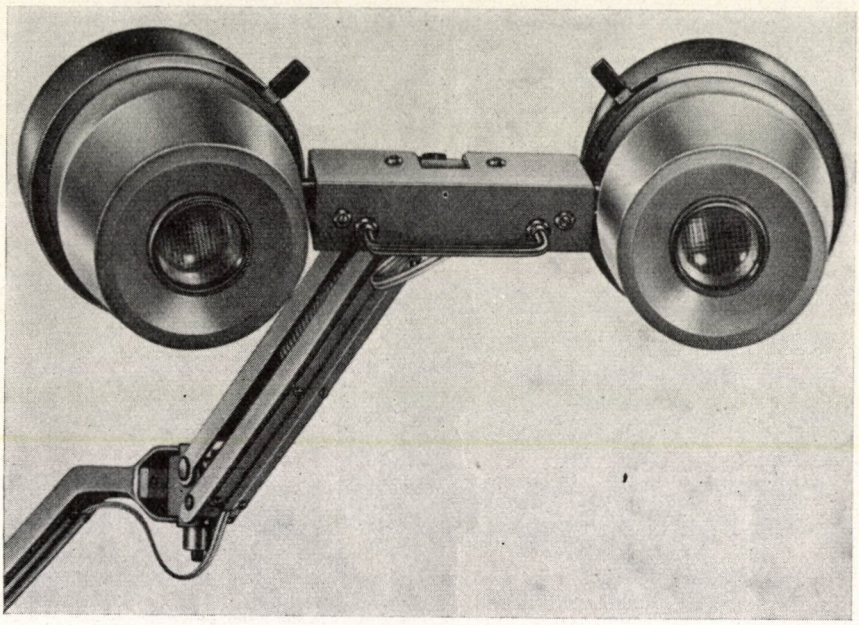

Fig. 5. Double-bulb test lamp

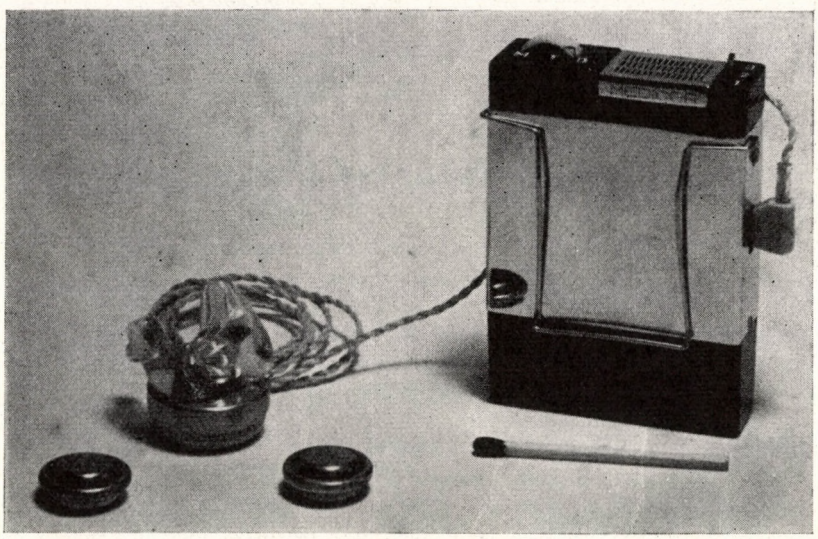

Fig. 6. Box-type hearing aid

Box-type hearing aid

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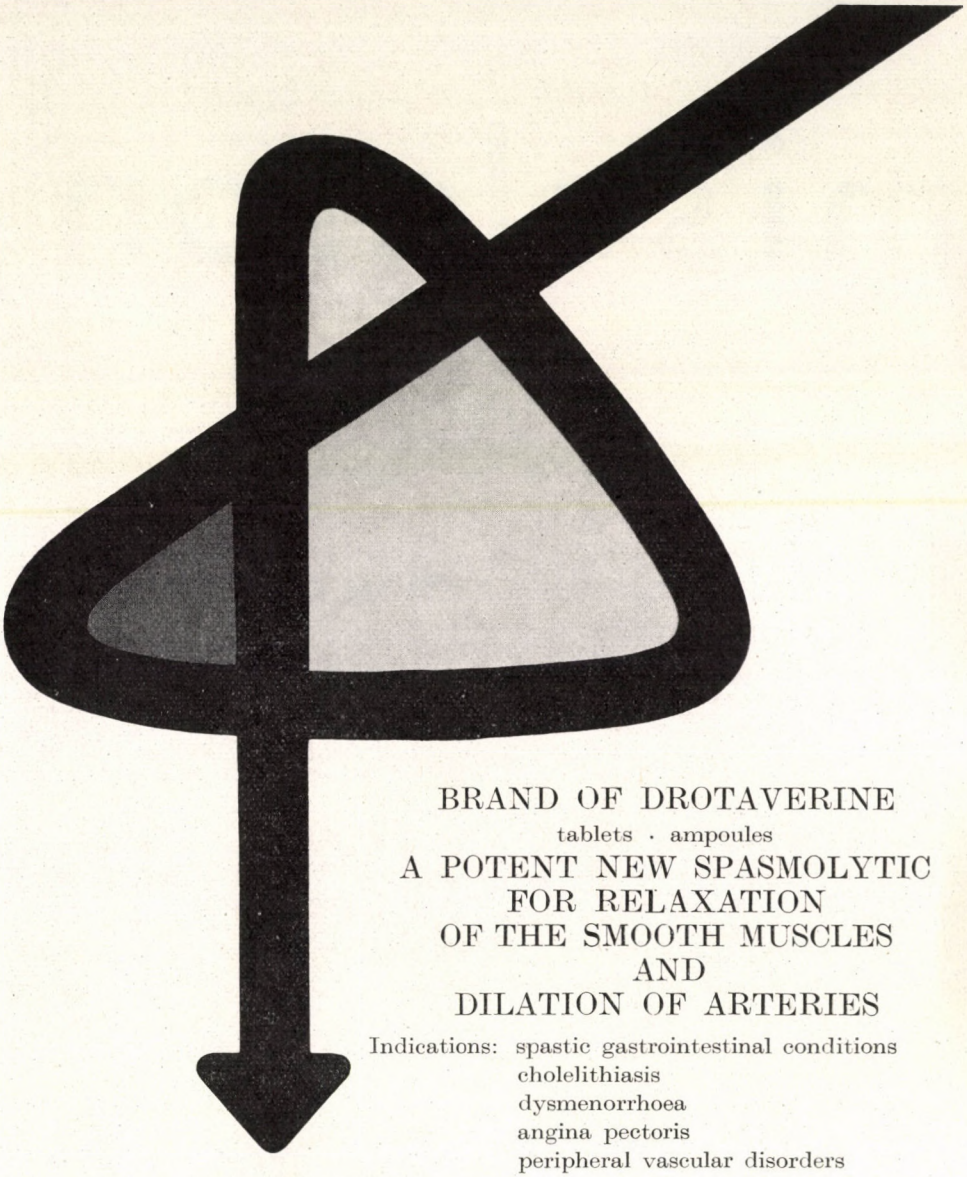

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EFFECTS OF A CYTOSTATIC AGENT AND OF A FIBRIN-STABILIZING FACTOR ON ATHEROSCLEROSIS IN THE RABBIT

By

S. BENKŐ, KATALIN BENKŐ and L. TÖRÖK

SECOND DEPARTMENT OF MEDICINE, UNIVERSITY MEDICAL SCHOOL, SZEGED

Received October 24, 1969

The influence of thrombopenia on the evolution of cholesterol-induced atherosclerosis and the behaviour of the fibrin-stabilizing enzyme in this process have been studied from the angle of the thrombogenic theory of atherosclerosis in two independent series of experiments. Thrombocytopenia induced by busulfan mannitol failed to affect the severity of cholesterol-atherosclerosis. A significant accumulation of the fibrin-stabilizing enzyme in the altered vessel wall was demonstrable in cholesterol-induced atherosclerosis.

Studies concerned with the pathogenesis of atherosclerosis are mostly based on the lipid theory. In recent years there has, however, appeared evidence in favour of altered blood clotting as a significant pathogenetic factor reviving thus ROKITANSKY's thrombogenic theory (1852). It is the merit of DUGUID (1949, 1959) to have recognized the similarity between the microscopic features of arterial thrombosis and those of the atheromatous plaque which led him to the conclusion that the plaques typical of atherosclerosis were actually organized, endothelium-coated, parietal clots. Some authors regard the agglomeration and agglutination of platelets, while others the deposition of fibrin, as the primary factor of arterial blood clotting.

MUSTARD, the prime proponent of the "platelet-centric" theory (1964), demonstrated in serial electron microscopic studies that the platelet-conglomerates enlarge by further aggregation of platelets; this is followed by the appearance of fibrin in larger and larger amounts. The subsequent stage of organization is marked by the appearance of smooth muscle and connective tissue cells. Finally, the lesion receives an endothelial lining. On the other hand, in the view of ASTRUP (1959), the first representative of the "fibrinocentric" theory, the fibrinous deposits involved in the process of tissue reparation accompanying recurrent intimal lesions may be too excessive to be disposed of by the fibrinolytic mechanisms of the organism; as a result, the fibrin mass fails to resolve and attracts platelets which attach to its surface. The white clot thus formed on the damaged intima becomes organized and forms the basis of the fibrous plaque.

The thrombogenic theory has given rise to extensive investigations into the haemostatic aspects of atherosclerosis. Evidence derived from these studies closely connects atherosclerosis with an increased coagulability, i.e. with an

enhanced clot-forming tendency of the blood. Moreover, it has been demonstrated that hyperlipaemia also favours blood-clotting, a finding which provides a connecting link between the two theories.

The present report deals with two independent experiments connected with the thrombogenic theory of atherosclerosis.

1. Development of atherosclerosis in thrombopenia induced by cytostatic agents in the rabbit

These experiments were prompted by the working hypothesis that a substantial reduction in the number of platelets might have a marked preventive effect on platelet aggregation and on the production of microthrombi and, by this fact, also on plaque formation.

The atherogenous factor was provided by cholesterol. Cholesterol-feeding to rabbits is known to result in hyperlipaemia, hypercholesterolaemia and beta$_2$-lipoproteinaemia. These abnormally high blood plasma levels affect the endothelium adversely and initiate the primary intimal lesions. Cholesterol also serves the purpose of an indicator substance, it being deposited in the altered areas thus indicating the site, number and extent of lesions.

Thrombopenia was induced by the cytostatic, busulfan mannitol (Mannogranol, Chinoin, Budapest), which has an inhibitory effect on medullary thrombopoiesis. The RES is also affected by the substance.

Material and methods

Male rabbits weighing 3 to 3.5 kg were used. Cholesterol was administered in daily doses of 1 g in semolina dumplings. The first series comprized eight animals. Four animals were administered 200 mg busulfan mannitol in saline into the ear vein three times weekly over five weeks; this was continued for another six weeks parallel with cholesterol feeding. The other four animals received cholesterol alone for six weeks.

The other series comprized four animals; they received busulfan mannitol over three months and cholesterol in daily doses of 2 g for the following three weeks. The five animals of the control group received cholesterol alone (daily dose 2 g) for three weeks.

Bleeding time (by the method of IVY), platelet count (Buerker's chamber) and platelet adhesiveness (Borchgrevink's method by phase contrast microscopy and double platelet count in venous blood and capillary blood sample) were estimated weekly. The degree of adhesiveness was inferred from BORCHGREVINK's index [5]:

$$\text{Index} = \frac{\text{platelet count (2')}}{\text{platelet count (0')}}$$

The index is a fraction; the smaller its value the higher the degree of adhesiveness. Platelet count (0') represents the figure at the start of bleeding and platelet count (2') that after an interval of two minutes.

The animals were killed by bleeding. The degree of atherosclerosis was expressed in terms of the grading scheme proposed by FODOR and ZEMPLÉNYI (1958). The viscera were examined for gross changes, the liver and aorta were also subjected to histological study.

Results

The results in group 1 are summed up in Table I. A comparison of the busulfan mannitol + cholesterol group with the group which fed cholesterol only, showed definite thrombopenia, a higher Borchgrevink index and a protracted bleeding-time in the treated animals. The aortic surface corresponded to an average grading of $++\pm$ in both groups (Table I).

Table I

Combined effect of busulfan mannitol and cholesterol on platelet count, platelet-adhesiveness and on the production of aortic atherosclerosis. Each figure represents mean values derived from four animals

Busulfan mannitol + cholesterol group (4 animals)				
	Platelet count (0′)	Platelet count (2′)	Index $\frac{\text{(th 2′)}}{\text{th 0′}}$	Bleeding time
Before treatment	304,500 ± 27,801.4	260,000 ± 24,832.7	0.86 ± 0.02	4.65 ± 0.24
At 5 weeks busulfan mannitol	145,000 ± 6,454.97	126,250 ± 7,465.2	0.85 ± 0.02	6.13 ± 0.24
At 6 weeks busulfan mannitol + cholesterol	130,000 ± 9,128.71	97,500 ± 7,500	0.73 ± 0.02	6.75 ± 0.32

Cholesterol group (4 animals)				
	Platelet count (0′)	Platelet count (2′)	Index $\frac{\text{(th 2′)}}{\text{th 0′}}$	Bleeding time
Before treatment	302,500 ± 13,149.7	270,000 ± 12,247.4	0.89 ± 0.02	5.13 ± 0.31
At 6 weeks: cholesterol	270,000 ± 12,909.9	165,000 ± 15,545.6	0.61 ± 0.04	4.38 ± 0.13

Mean severity of aortic atherosclerosis $++\pm$

The results obtained in group 2 are summed up in Table II. At the end of a three-month period of busulfan mannitol treatment, the original platelet count of 482,000 had fallen to an average of 250,000. The Borchgrevink index was 0.66 during busulfan treatment and 0.72 during the cholesterol period. In the cholesterol-fed control group, thrombopenia was absent and platelet agglomeration was also stronger than in the group with additional cytostatic treatment. The extent of atherogenesis corresponded to an average grading of + in both groups (Table II).

Table II

Influence of three weeks cholesterol-feeding after three months busulfan mannitol pretreatment on number and adhesiveness of platelets and on evolution of atheromatous plaques. Figures represent mean values

Busulfan mannitol + cholesterol group (4 animals)				
	Platelet count (0′)	Platelet count (2′)	Index $\frac{\text{(th 2′)}}{\text{th 0′}}$	Bleeding time
Original values	482,499.9 ± 11,814.55			
At 3 months busulfan mannitol 2 ml.	167,499.9 ± 75,00	109,999.9 ± 5,775.5	0.66 ± 0.07	6.75 ± 0.78
At 3 weeks cholesterol 2 g daily	250,000 ± 19,578.9	179,999.9 ± 14,719.6	0.72 ± 0.03	3.881 ± 0.43

Cholesterol group (5 animals)				
	Platelet count (0′)	Platelet count (2′)	Index $\frac{\text{(th 2′)}}{\text{th 0′}}$	Bleeding time
Original values	257,999.9 ± 16,852.3			4.71 ± 0.85
At 3 weeks cholesterol 2 g daily	287,999.9 ± 11,135.5	123,999.9 ± 23,151.6	0.44 ± 0.07	4.60 ± 0.43

Mean grade of atherosclerosis +

Necropsy revealed atherosclerosis involving the aortic intima of medium severity in the first and of slight degree in the second group. However, no differences were demonstrable between the busulfan mannitol and the control group. Fatty degeneration of the liver was confined to the cholesterol-fed group and was never found in the busulfan mannitol treated animals. Atherosclerotic plaques were demonstrable in both groups.

Discussion

A substantial fall in the platelet count was induced by busulfan mannitol. Platelet adhesiveness, in other words, the retention by adhesion of platelets to the wound surface, diminished. Cholesterol feeding applied under such conditions failed to affect the number or extent of atherosclerotic plaques as compared to the control group. This observation is at variance with those reported by COHEN, COOMBS et al. (1967) who found that in thrombocytaemia induced by repeated small blood losses the atherogenic effect of cholesterol was definitely increased while in experimental thrombopenia it decreased. The inconsistency of these results may be explained by our having used a

cytostatic agent for the induction of thrombocytopenia the adverse effects of which might have counterbalanced the favourable effect expected from the reduction in platelet count. The absence of fatty degeneration of the liver fits in with the possibility that the amount of lipids available to the vascular wall has remained as high as before. The cytotoxic effects of busulfan mannitol also involve the activity of the RES, including the incorporation of lipids, by which it protects the vessel wall from lipid deposition under normal conditions (PICK et al. 1962, 1966). The leukopenia may represent a loss of a further protective factor of the vessel wall. Thrombopenia induced by busulfan mannitol has thus failed to exert any protective effect in cholesterol-induced atherosclerosis. Incidentally, it is by no means certain that the platelets play any significant part in the production of atherosclerotic plaques.

2. Study of the fibrin-stabilizing factor in aorta of normal and cholesterol-fed rabbits

This experiment had the aim of studying the alterations of the fibrin-stabilizing factor (LAKI and LORÁND, 1948) in the aorta of cholesterol-fed rabbits. This provided an indirect approach to the possible part played by fibrinogen and was also expected to furnish additional evidence to the fibrinocentric concept from the angle of the thrombogenic theory.

According to present knowledge, the conversion of fibrinogen to fibrin requires two enzymes: the proteolytic thrombin and the fibrin-stabilizing factor (LAKI and LORÁND, 1948), a transamidase capable of producing cross-links between the contiguous monomers of the fibrin molecule. In the presence of thrombin and calcium, the fibrin-stabilizing enzyme converts coagulated labile fibrin to stable fibrin resistant to an 8 *M* solution of urea (LAKI, 1968).

Material and methods

Rabbits of the New-Zealand strain weighing between 1500 and 2000 g were used. A cholesterol-enriched atherogenous diet (Nutritional-Biochemical Co.) was given in amounts of 30 to 40 g daily for 10 weeks. Six animals kept on a normal diet served as controls. The animals were killed by bleeding. Transamidase activity in liver and aorta was estimated by the method of TYLER and LAKI (1966). This procedure is based on the catalytic effect of the enzyme on the incorporation of various amines including glycine ethylester into fibrinogen and casein. Homogenized aortic and liver tissue was incubated with ^{14}C-labelled glycine ethylester in the presence of ionized calcium and of glutathione. From samples taken at determined intervals and subjected to three trichloracetic acid precipitations and centrifuging the uncombined labelled ester was removed. The residual precipitate was collected in 0.1 ml of diluted NaOH, made up to 10 ml with Bray's solution (1960) and its activity was measured in a Nuclear Chicago liquid scintillation detector. For details, see BENKŐ and LAKI (1968). The number of pulses per minute found in the samples expressed the amount of glycine ethylester incorporated into casein, and thus also the activity of the enzyme. The diagrams represent the scintiscan calculated for 1.0 ml of the 90-min. sample.

Results

Fig. 1 demonstrates mean hepatic transamidase activity under normal conditions and after cholesterol feeding. The difference between the two groups was not significant. Fig. 2 shows the figures for the aorta. In the rabbits fed an atherogenous diet the sclerotic portions of the aorta displayed a three

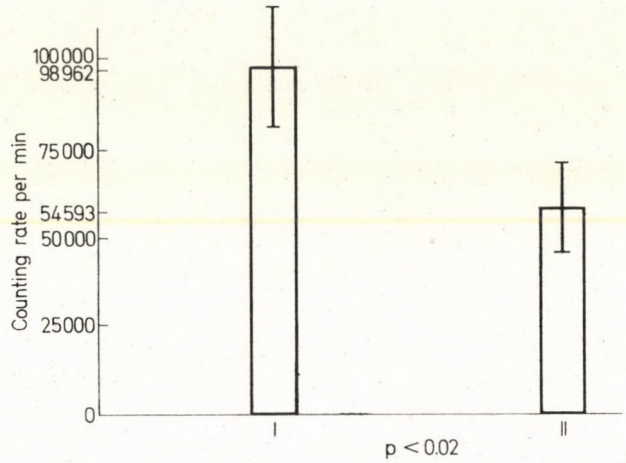

Fig. 1. Transamidase activity of liver tissue of normal and cholesterol-fed rabbits. Column I represents the transamidase activity of livers of cholesterol-fed rabbits, column II that of normal liver tissue. The activity is measured by the incorporation into casein of labelled glycine ethylester catalyzed by the enzyme after 90-min incubation. Incorporation is expressed by the scintiscan per minute. The columns represent mean values

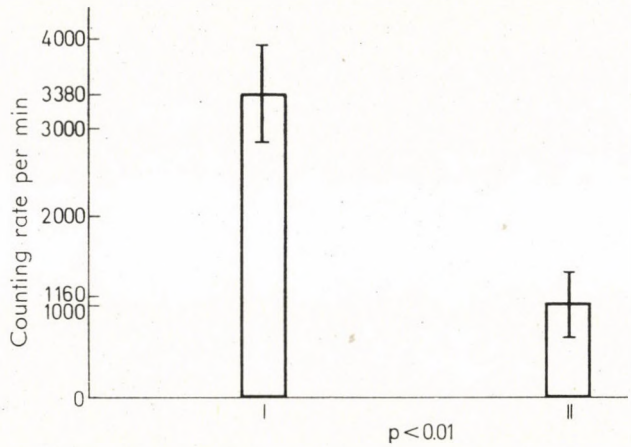

Fig. 2. Transamidase activity in the aorta of normal and cholesterol fed rabbits. Column I demonstrates mean transamidase activity in the atheromatous portions of the aorta in cholesterol-fed rabbits. Activity is measured by the scintiscan per minute obtained after 90-min. incubation. Column II demonstrates transamidase activity in the aorta of normal rabbits

times higher transamidase activity than those of the control group. Fig. 3 gives results of the self-control studies. It shows that the transamidase activity in the atherosclerotic portions was at least threefold of that in the unaffected portions.

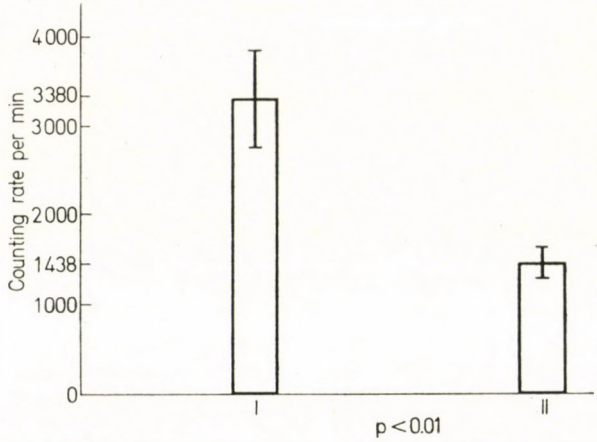

Fig. 3. Transamidase activity in normal and atheromatous aortic portions of cholesterol-fed rabbits. Column I represents transamidase activity in the atheromatous, column II those of the intact aortic portions, on the basis of the scintiscan per minute

Discussion

The above results revealed an enhanced activity of the fibrin-stabilizing transamidase in the aorta affected by cholesterol-induced atherosclerosis. This finding is consistent with the results of SHAINOFF et al. (1968) who found a threefold increase in the fibrinogen content of the sclerotic portions of aortas in cholesterol-fed rabbits. The fact that transamidase is produced in large amounts by the liver and that it is contained in blood plasma as well, raises the question whether the enzyme activity found in the present case had originated from the blood plasma or from the aortic wall. Considering the fact that the atheromatous plaques barely account for 10 or 20% of the affected vessel wall, they must represent elective sites of five or tenfold enzyme concentrations which are in the neighbourhood of those found in the liver. The considerable cell proliferation in the atherosclerotic plaques fits in with the interpretation that the excess enzyme may well be produced in the affected arterial wall.

General conclusions

The experiments reported above furnish evidence to the thrombogenic theory, the first in support of the platelet-centric, and the second, of the fibrinocentric concept.

In the first series it has been shown that a reduction in the platelet count even though significant, fails to affect the degree of cholesterol-induced atherosclerosis in the aorta. The accumulation of fibrinogen (SHAINOFF et al. 1968) and of the fibrin-stabilizing enzyme (BENKŐ and LAKI, 1968) in the sclerotic portion of the aorta of cholesterol-fed rabbits supports the fibrinocentric concept.

In our view, the primary effect of the atherogenic factors, such as hyperlipaemia and a haemodynamic lesion of the endothelial cell, is usually a combined one. The substances released from the damaged endothelium find their way to the blood plasma and react there with the clotting factors and fibrinogen molecules. The fibrin is precipitated and platelets adhere to the altered surface. The subintimal layer bordering on the affected endothelium becomes a site of reaction of mucopolysaccharides and of collagen elements. The chain of biochemical events occurring here may be attributed to a nonspecific mesenchymal reaction (HAUSS et al. 1968) which also accounts for the accumulation of acid mucopolysaccharides. The permeability of the altered endothelial membrane increases, as a result, certain plasma constituents such as fibrinogen filter through in together with other proteins and lipoproteins in increased amounts and find access to the subintimal layer (CONSTANTINIDES 1965). The biochemical consequence of the appearance of fibrinogen in the vessel wall is an enhanced production of fibrin-stabilizing enzyme of the mesenchymal cells at this site (BENKŐ and LAKI, 1968). Moreover, fibrinogen and the soluble collagen precursors meet and take the form of a gel which results in further biochemical alterations of the subintimal area.

Accumulation of lipoproteins in the vessel wall is bound to have its consequences. One of the signs of enhanced lipid metabolism is an increase in lipase activity of the vessel wall, as reflected by the increased lipolytic activity in the aorta after cholesterol feeding (SZABÓ et al. 1968). Accumulation of lipase as well as of the fibrin-stabilizing enzyme, represents an adaptation reaction. The part played by lipase may lie in a breakdown of the increasing amounts of fat. This leads to the obvious question whether the accumulation of fibrin-stabilizing enzyme is beneficial to the vessel wall. Stabilized fibrin by itself adds to the resistance of the vessel wall. Atherosclerosis is associated with a reparative fibroplasia of the affected arterial wall (CONSTANTINIDES, 1965). The process is basically the same as that of wound healing where the network of stabilized fibrin forms the framework of the granulation tissue (GERENDÁS, 1968). Tissue proliferation narrows the lumen, particularly

of minor vessels, which has adverse haemodynamic effects. Accumulation of the fibrin-stabilizing enzyme beyond a certain level involves a thickening of the vascular wall which is obviously harmful. In our view, it should be possible to prevent this by a specific transamidase inhibitor. DJALDTTI et al. (1965) demonstrated an active substance in the venom of the snake Echis colorata which has the capacity of inactivating the fibrin-stabilizing enzyme in vitro as well as in vivo. This finding might offer a promising approach to the inhibition of excessive production of stable fibrin in the vessel wall.

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Dr. Sándor BENKŐ, II. Belklinika, Szeged, Hungary
Dr. Katalin BENKŐ, Ideggyógyászati Klinika, Szeged, Hungary
Dr. László TÖRÖK, Bőrgyógyászati Klinika, Szeged, Hungary

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R-74 TREATMENT OF AUTOIMMUNE MYOSITIS

By

GY. PETRÁNYI and GY. SZEGEDI

FIRST DEPARTMENT OF MEDICINE, UNIVERSITY MEDICAL SCHOOL, DEBRECEN

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R-74, a new cytostatic agent with a potent immunosuppressive and a fair antileukaemic effect has been administered in autoimmune diseases. The action was a selective one, being largely confined to myositis. Administered to 23 patients over a five-year period of observation, either alone or in combination with steroids, R-74 was found to bring about a remission in a short time, without, however, affecting secondary muscular distrophy or atrophy. The drug's activity on muscular tissue was fairly selective; the skin manifestations of dermatomyositis and (in cases of myositis associated with SLE) other than muscular manifestations of SLE were poorly or not at all affected. In a number of cases, definite improvement of the myositis began in three or seven days. If the intravenous route of administration is strictly observed and a close check is kept on the blood picture, the doses which have proved effective in the course of this study, are entirely safe. The possible mechanisms of action of the drug are discussed.

Cytostatic agents have been used in the therapy of autoimmune diseases since a few years. They may affect the immune responsiveness at various levels but their action most probably lies in their inhibitory effect on the division of immunocompetent lymphoid cells challenged into excessive proliferation by the specific antigen. Remarkable is the result of 6-mercaptopurin (6MP, Purinethol) treatment in otherwise lethal steroid resistant lupus nephropathy and in autoimmune nephrosis [11, 12]. The present report publishes the good therapeutic results obtained in polymyositis and dermatomyositis with the new cytostatic agent, R-74.

Patients

R-74 (1,4-di-mesyloxyethyl-amino-1,4-didesoxy-m-erythrite) was given in the course of the last five years (1964—70) to a total of 23 patients, 20 females and 3 males, with the diagnosis polymyositis in 11, dermatomyositis in 7, and polymyositis associated with systemic lupus erythematosus in 5 cases (Table I). Polymyositis associated with malignant disease has not been observed during this period. Diagnosis was based apart from the clinical features on electromyography, enzyme (aldolase, creatinine-phosphokinase) studies, creatinine excretion, demonstration of autoantibodies to muscle and other

autoantigens, and LE-cell phenomenon and antinuclear antibody in the cases of SLE.

R-74 treatment was started directly after the abrupt onset in three cases, in the others it was given in acute flare-ups of the already long-standing process.

Table I

	Males	Females	Acute	Chronic
Polymyositis	1	10	3	8
Dermatomyositis	2	5	—	7
SLE + Myositis	—	5	—	5
Total	3	20	3	20

Method of treatment

All patients with the exception of the three acute cases, had had steroid therapy before. At first, R-74 treatment was prescribed in cases which had failed to respond to larger steroid doses (60 mg or more, daily). Later, after having gained convincing results with R-74, this treatment was applied in all exacerbations of autoimmune myositis. Administration of 10 to 20 doses of 30 to 60 mg daily over a period of 10 to 20 days (total dose 300—600 mg) was found to be the most appropriate schedule. The 23 patients under study had a total of 30 similar courses.

The dosage schedule has to be more cautious than in leukaemia (13) and a close haematologic supervision is imperative. No adverse effect involving the haemopoietic system has been observed with this dosage, in any of the cases. Treatment was, however, discontinued if the WBC count had dropped below 4000.

Results

Administration of R-74 resulted in full control of the acute clinical signs and symptoms of polymyositis in all but two cases. In seven cases, full remission was attained without additional steroids. On 21 occasions, previous maintenance therapy with steroids had not been discontinued when the process was flaring up, however, R-74 not only made it unnecessary to increase the former doses but actually permitted their reduction in 9 cases (Table II). No sign of a response was seen in two cases, but in one of them the dosage was inadequate. On the other hand, full remission ensued in both cases as a result of massive-dose corticoid therapy administered over long periods. In 6 out of the 7 patients where R-74 alone had resulted in complete remission, the process remained inactive without the need for any medication. The other patients continued on maintenance doses of steroids usually 15 mg of prednisolone daily. The duration of remissions varied between 3 and 30 months, in other words, some of the patients have been symptom-free as

Table II

Response	Polymyositis	Dermatomyositis	SLE + Myositis
Excellent	7	—	—
Good	4	6	4
None	—	1	1
Total	11	7	5

Excellent = remission by R-74 alone
Good = remission by R-74 + steroid

long as for 2.5 years. The average symptom-free period now attains one year, in contrast to the six months of steroid treatment. We had only one fatal case in the course of the last five years, but the cause of death was unrelated to the original disease, dermatomyositis. Four patients had repeated courses of R-74. One of them had as many as four; activity of the drug did not seem to decrease in these.

The acute form of polymyositis showed the best response with the slightest possible need for additional steroid treatment. In dermatomyositic polymyositis with systemic lupus erythematosus earlier steroid therapy which had failed to prevent recurrences of polymyositis, was not discontinued, but instead of being obliged to increase the doses we were actually able to reduce them in a few cases, without recrudescence of the SLE.

The first signs of improvement were noted as early as after the 3rd to 6th injection, i.e. usually within the first week of R-74 treatment. Symptoms of SLE remained unaffected by R-74. Even where the drug could have had no effect on the reduced activity due to a definite destruction and atrophy of the muscles, it helped to restore the activity of still functioning muscular elements by the control of inflammation. Long standing contractures of course failed to improve.

Discussion

The prognosis of polymyositis has been fundamentally changed by the use of steroids [1, 2, 7, 8, 9, 16, 17]. Our general experience with the steroids may be summed up as follows. *a)* Treatment should be started early with adequate doses and continued for periods of sufficient length; *b*) some cases are refractory to steroids, the symptoms persist and the process continues to progress; *c)* the long-term massive steroid treatment involves certain well-known hazards such as peptic ulcer and osteoporosis; items *b)* and

c) constitute the main reasons for prompting a search for other therapeutic possibilities.

R-74, a new cytostatic agent synthetized by a Hungarian team [3] belongs to the sugar-alcohols. It has a fairly selective lymphocytotoxic effect [13] and has been shown to represent a potent suppressor of the immune system. In the polymyositis patients under study its effect was manifest by the end of the first week, with relief of pain, improvement of mobility, a decline of the increased serum level of muscle enzymes and creatinin excretion.

The response to R-74 seems to be of a fairly selective nature. Apart from myositis, none of the other autoimmune organ manifestations including lupus nephropathy or rheumatoid arthritis as well as other systemic involvements in SLE, showed any noticeable response.

Owing to the adopted dosage scheme the only side effect of the drug was a transitory leukopenia, particularly lymphopenia. Haematological complications of any importance have not been noted. There was invariable a transitory elevation in the absolute blood lymphocyte count before its decline. This corresponded to the therapeutic stage of rapid shrinking of lymph nodes in lymphoid leukaemia associated with an initial depletion of the lymphoid system owing to the release of lymphoid cells [12]. Local complications were confined to venous thrombosis at the site of injection in two cases and to local sloughing consecutive to paravenous injection in one case. One of the patients developed a morbilliform, another a scarlatiniform rash at the start of treatment which did not reappear subsequently (release of reagins from the disrupted lymphoid elements?). These secondary effects were rare and, considering the benefits of the therapy, of little significance. All that has to be emphasized is the necessity of strictly intravenous injection and of interrupting treatment, should the leukocyte count drop under 4000 per ml.

On the evidence of the present observations the merits of R-74 therapy are as follows. 1) It may bring about remission in steroid-resistant cases; 2) it is steroid-saving, a point of particular importance in view of the hazards of long-term steroid therapy; 3) it may bring acute recurrences under control without the need for additional steroids; 4) in combination with steroids, it provides for a fuller suppression of autoimmune activity and adds to the length of the periods of remission.

The immunosuppressive properties of R-74 have been borne out by animal experiments [10]. In our laboratory the drug together with antilymphocytic serum now serves as a reference substance in the search for new immunosuppressing agents. It acts as a suppressor of cellular hypersensitivity also in humans.

The mechanism of the therapeutic effect of R-74 in polymyositis has yet to be clarified. In many cases, the response is rapid, taking sometimes not more than three days to appear. This would suggest that the drug's action

lies not in the suppression of antibody production, the less so as the halving time of antibodies is nearly tenfold this value. Moreover, the titre of anti-muscle antibody revealed no significant change in the course of treatment. The rapidity of improvement indicates that the activity of R-74 is directed at the aggressive immunocompetent lymphoid cells or, else, that its mechanism may possibly involve other factors in addition to immunosuppression. If we accept the first possibility, this would clearly indicate that the autoimmune type of myositis is due to a cellular autoimmune mechanism, and that the part played by antimuscular antibodies may be merely a secondary one.

MALAVIYA [6] also made therapeutic attempts with cytostatic agents using methotrexate for immunosuppressive purposes in four cases of dermatomyositis, with satisfactory results. From the fact that clinical cases of myositis respond to chemotherapeutic agents of the most different kinds of activity and that the immunosuppressive capacity is the only property common to all of them, it may safely be concluded that it is this very immunosuppressive character shared by all chemotherapeutic agents which accounts for the beneficial effect. At any rate, the presence of various immunological abnormalities in myositis [1, 4, 5] amply warrants the therapeutic use of immunosuppressive agents.

The same doses of R-74 which brought about a remission in nearly every case of autoimmune myositis within 7 to 14 days, failed to ensure a benefit in five cases of autoimmune renal disease. Conversely, 6-mercaptopurine the effect of which on autoimmune renal disease of the nephrosis type had been excellent [11, 12], we found valueless in the seven cases of myositis. This would suggest that various cytostatic agents have different ranges of activity within the field of autoimmune diseases, as do the antibiotics in respect of infective agents [14].

The selective response of myositis to R-74 prompted us to test the drug in myasthenia gravis, a condition which at least partly is also ascribed to an autoimmune mechanism. In a few cases the response to R-74 was as prompt as in myositis [13]. Autoimmune diseases of other kinds (SLE, rheumatic arthritis) could, however, not be influenced.

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Prof. Dr. Gyula PETRÁNYI
Dr. Gyula SZEGEDI
I. Belklinika, Debrecen, Hungary

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THE IMMUNOGRAM IN DIFFERENT TYPES OF HYPERMETABOLISM

By

L. MOSONYI, GY. DÓBIÁS, J. GÖNCZI and É. PERÉNYI

FOURTH DEPT. OF MEDICINE AND DEPT. OF CLINICAL LABORATORY, POSTGRADUATE MEDICAL SCHOOL BUDAPEST

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1. The results of immunograms based on the titres of antibodies to 14 environmental antigens have supported the earlier view of the authors in that antibody production tends to increase in hypermetabolic states.

2. Antibody production is stimulated by hyperthyroxinaemia until protein synthesis is depressed by the feed-back mechanism inversely influencing the hypothalamo-diencephalic functions.

Enhanced protein synthesis is one of the essential features of hypermetabolism, whether of the central (hypothalamo-diencephalic) or of the peripheral (hyperthyroxinaemic) type [7]. The experimental studies concerned with this subject seem from immunobiological point of view, however, inconsistent. SZABADOS et al. [10] were able to enhance antibody production to staphylococcus, diphtheria and tetanus toxin by feeding iodocasein to sheep, horses and oxen. Other research teams noted a reduction in antibody production [2] in experimental hypometabolism induced by cervical transection of the sympathetic pathways, i.e. interruption of the central impulses and the consequential depression of cellular metabolism. On the evidence of other studies the outcome of these interventions is however greatly influenced by external factors, for instance nutrition and care [4]. BACKHAUSZ et al. [1] noted an enhanced antibody production in certain cases of hyperthyroidism and of vegetative dystonia.

In an experimental study, MESS [6] succeeded in preventing anaphylaxis, particularly its lethal outcome, by producing a bilateral anterobasal hypothalamic lesion. The mechanism of this effect was attributed to two factors, viz. central hypothyroidism and adrenocortical hypertrophy. These facts support the view that hypothyroxinaemia interferes with sensitization and confirm the results of our earlier experiments according to which the titre of antibodies against various microorganisms are increased in states of hypermetabolism. These changes were particularly marked if the hypermetabolism was due to vegetative dystonia, in other words, to a central (hypothalamo-diencephalic) mechanism, whereas in the case of hyperthyroxinaemic protein synthesis it was only before specific treatment that consistently higher antibody titres were demonstrable. In fact, it was in the course of treatment,

that difference of the two types of hypermetabolism had become demonstrable: while in the case of antithyroid treatment the antibody titres were scarcely affected, and occasionally even tended to increase, sedatives with a central action induced a substantial reduction in the titre of certain antibodies and serum factors (anti-streptolysin O, typhoid-H, sheep red cell agglutinins, C-reactive protein).

These considerations have led us to study on large material the behaviour of antibodies against 14 environmental antigens.

Material and method

The studies were performed on 169 adult inpatients, 45 males and 124 females. They were followed up continuously during two years, in view of the possibility of seasonal variations in immune responsiveness. It was however found that seasonal grouping was unnecessary, since distribution of the titres was practically uniform all over the year, moreover, the rate of admission of patients with hyperthyroidism as well as of sympathicotonia was also equal all over the four seasons of the year. The diagnosis of hyperthyroidism was based on routine clinical studies and a typical hyperfunctional iodine uptake, while that of sympathicotonia on an enhanced metabolism in the absence of hyperthyroxinaemia and a ^{131}I uptake characteristic of thyroid iodine avidity in vegetative dystonia. No sex-related feature was noted in the patients' condition which would have made a grouping according to sex necessary. Although the rise or fall in the immune body titres tends to be more marked in females, this seems to be due to the lability of the vegetative sphere being more common in women. However, male patients with variations in their antibody titre were few for comparative studies of this kind.

Fifty-nine subjects served as controls. None of them had any demonstrable disease or endocrine disorder which might have interfered in any way with antibody synthesis [7]. Earlier infections were disregarded, these being as a rule uniformly distributed among test subjects and controls.

It was sought to test the immune responsiveness of the subjects under study on the evidence of the immunogram by determining the titre of antibodies to 14 environmental antigens against which, according to BACKHAUSZ et al. [1] 80 to 100 per cent of the adult population possesses antibodies.

The antigens used were: trichloroacetic acid extracts of the bacterial strains *Sh. flexneri* 1/b, *Sh. flexneri* 2/a, *Sh. flexneri* 3, *Sh. sonnei*, *E. coli* 0:26, *E. coli* 0:55, *E. coli* 0:86, *E. coli* 0:111; furthermore, rabbit, sheep and human (A and B) erythrocytes, staphylococcus alpha toxin and streptolysin 0.

The antibacterial antibodies were studied by the indirect haemagglutination procedure in the following manner. One ml of the bacterial extract was added to double strength phosphate buffer pH 7.2 in 1 ml saline and 0.25 ml of a suspension of Rh (D) positive, group O human erythrocytes which had been washed three times. After incubation at 37 °C of 2 hrs the erythrocytes were washed thrice and a 1.5 per cent suspension was prepared.

Iso- and heteroagglutinins against erythrocytes were demonstrated by direct haemagglutination, using a 1.5 per cent suspension of erythrocytes which had been washed three times. Direct as well as indirect haemagglutination tests were performed in Takátsy's microtitrator at serial dilutions 2:1. The titre was expressed by the natural logarithm of the highest serum dilution still giving positive haemagglutination. A phosphate buffer of pH 7.2 in saline served for washing, suspension and dilution of the erythrocytes.

Staphylococcus alpha antitoxin and antistreptolysin titres were estimated by the toxin neutralisation test [1] and expressed in antitoxin units on the basis of international serum standards.

Composition of the phosphate buffer was, NaCl, 8.5 g; Na_2HOP_4, 0.565 g; KH_2PO_4, 0.135 g; made up with distilled water to 1000 ml. To obtain double strength buffer, the double amounts of the salts were made up to 1000 ml.

The immunograms were evaluated on the following principle: the antibody titres were estimated in the sera of 104 normal blood donors presenting during the period of study and the values thus obtained were used as reference standards.

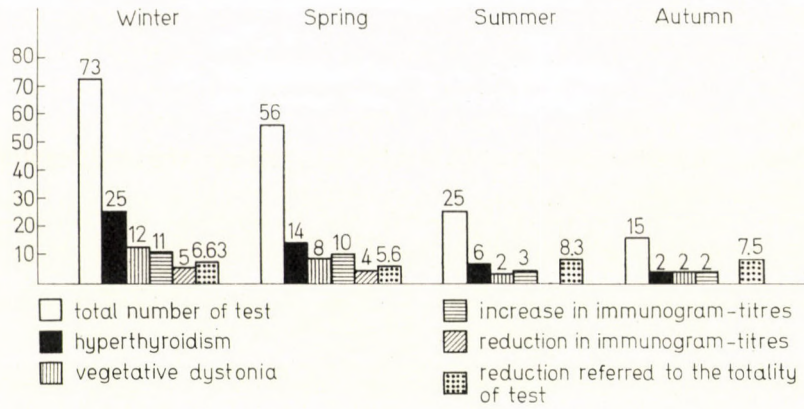

Fig. 1. Seasonal variations of immunogram

The immune response to environmental antigens depends, in addition to the individual responsiveness, also on earlier exposure to the given antigen. This results in a wide individual variety of the specific antibody titres and makes statistical evaluation possible. While for instance one person has high titres against E. coli 0:111 and low titres against Sh. sonnei, in another the situation may be the opposite. If the responsiveness of a given individual is not enhanced, the rise of antibody titres resulting from exposure to an antigen will not exceed a certain moderate limit. The range titre which may possibly occur in normal, i.e. immuno-

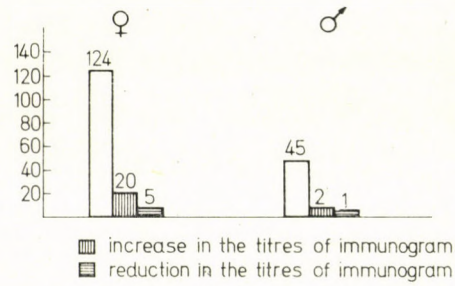

Fig. 2. Sex distribution of patients

logically not over-responsive individuals, has been determined in 104 cases. A basically similar immunological constellation was found in 59 inpatients with a normal metabolism. On these grounds, the individual antibody titres were regarded as high if they attained or exceeded the upper limit of the range found in 163 donors and control subjects, and low if they remained under the lowest limit of this range. The critical levels below which the titres were regarded as low were 1:2 for agglutinins, 30 AU/ml for AST, and 0.10 AU/ml for staphylococcus alpha toxin.

In 19 subjects the responses of the immunogram to antithyroid treatment and to drugs of diencephalic activity were studied. Variations were disregarded unless exceeding the original titre by at least two dilution steps in either direction. Rauwolfia preparations were generally used until clinical equilibrium had been restored, in a dose of 0.1 mg T. I. D. for 5 to 8 weeks. Of iodine in the form of Plummer's solution 15 drops were given T. I. D. to four patients. Belloid pills (hyoscyamine sulphate, 0.1 mg; total ergot alkaloids (expressed in ergotoxin),

0.3 mg; butobarbital, 30 mg) were prescribed T. I. D. for four weeks to two patients. Treatment usually lasted several months extending over the individual seasons of the year. In this way, a grouping according to seasons was unnecessary (Fig. 3). The antibodies against the individual antigens are presented on the circumference of a circle and their titres on the radius belonging to the corresponding antibody. In the interest of simplicity, the agglutinins against the bacterial extracts are given in an abbreviated form (Flexner 1b, Coli 26, etc.).

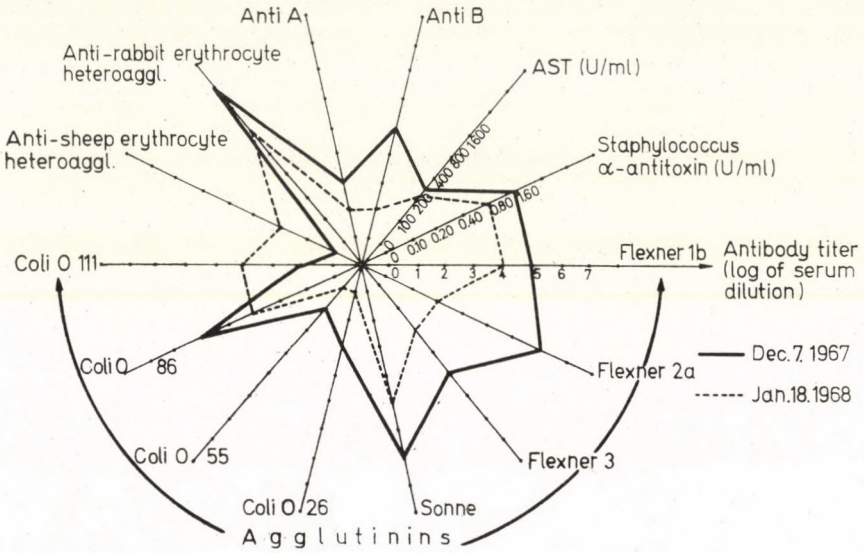

Fig. 3

Results and discussion

On the evidence of the figures presented in Table I, in untreated hyperthyroidism the increase in the titres seemed less distinct than in our erlier observations. However, in our former studies where 10 immunobiological parameters had been tested, distinct changes were generally confined to AST, CRP and the streptolysin skin test, the others remaining largely unchanged. On the other hand, in the present study where 14 antibodies were tested, the changes were disregarded unless involving at least 8 out of the 14 factors and being of the same direction, in other words, the titres of at least 8 out of 14 antibodies had either to be uniformly high or uniformly low. (An increase in titre of all the 14 antibodies is merely a theoretical possibility since exposure of the same individual to all of the antigens at the same time would hardly be probable.) It was this antibody pattern which served for the evaluation of the increasing or declining tendency of antibody synthesis. Of the 104 blood donors there were only four (3.8 per cent) in which this pattern was demonstrable, regardless of the season, whereas in the central hypermetabolism group (sympathicotonia) the ratio was as high as 35.8 per cent, in other

Table I

Immunogram

Diagnosis	Number of cases	No change	Increase		Reduction
			++	+	
1. Hyperthyroidism	47	37	3	2	5
2. Vegetative dystonia	39	24	9	5	1
3. Hypothyroidism	13	13	—	—	—
4. Earlier strumectomy	11	8	2	1	—
4. Controls (patients)	59	53	1	3	2
6. Controls (healthy donors)	104	100	1	3	—

Statistical analysis 2—5: $p < 0.02$
4—5: $p = 0.02$
2—6: $p < 0.01$

words, 14 out of 39 subjects exhibited this distribution of antibody titres The difference between the two groups was statistically significant, $p < 0.01$. The silent pattern was confined to the cases of idiopathic hypothyroidism where the basic values practically corresponded to those of the controls. Of the hyperthyroid subjects, five revealed an increase and another five a decline, of the titres. Their antibody production was, however, unstable and did not seem to vary in any definite direction. In the thyroidectomized subjects (with clinical hypothyroidism all throughout) the immunogram was similar as in vegetative dystonia of the central type.

The observed patterns admitted the following interpretation.

According to NILZÉN [9], thyroidectomy interferes with antibody synthesis, a finding consistent with the later results of FILIPP, SZENTIVÁNYI and MESS [4] referred to earlier on the evidence of which anaphylaxis induced in guinea-pigs and fatal in 87 per cent of the controls was inhibited by thyroidectomy and reduced to a minimum (5 per cent) by a hypothalamic lesion. The hypothalamic lesion had, however, no inhibitory effect on the development of shock if it had been combined with adrenalectomy or metirapone administration. Thyroxin treatment parallel with hypothalamic lesion had a similar effect. These results clearly illustrate the significance in antibody production of thyroid deficiency on the one hand and of thyroxin substitution on the other.

In the present study the metabolic aspects of antibody production have been approached from the angle of hypermetabolism. Its results support our earlier view that antibody production is enhanced in hypermetabolism in the framework of (and in relation to) the increase in protein synthesis resulting from this condition. The pattern is particularly convincing in the central type of hypermetabolism. A significant rise in the titres was found

in the subjects with sympathicotonia as also in those where elimination of the feed-back inhibiting pituitary activity resulted in a release of the hypothalamus from intrinsic feedback and thus in its hyperactivity, on the other hand, the antibody titres did not change in the other types of hypothyroidism (idiopathic) owing to the absence of central stimulation.

The so-called internal feedback results from a hyperactivity of the anterior lobe of the pituitary and controls the production of releasing factors in the medial eminence of the hypothalamus [6]. According to HAYNAL et al. [5], in this case the diencephalic impulses are acting directly through sympathetic pathways by enhancing at the periphery the oxidative (metabolic) activity of the cells. In congenital or idiopathic hypothyroidism, TSH and TRF functions are abolished, a fact which has been turned to therapeutic use by SZÁNTÓ et al. [11]. As the results of FILIPP and MESS [4] indicate, thyroid deficiency by itself would be reflected in the immunogram by a declining tendency of the titres, enhanced synthesis being confined to states of hypermetabolism.

Evaluation of the immunograms of patients subjected to various treatments (Table II) has confirmed our earlier findings in that therapeutic factors directed against central stimulation are more efficacious than those of peripher-

Table II

Effect of treatments on the immunogram

Type of treatment	Number of changed parameters	Titres increased	Reduced	Coefficient increase/ reduction
Central	53	41	12	3.4
Antithyroid	54	37	17	2.1
Symptomatic	19	10	9	1.1

al inhibitory action on thyroid hormone synthesis, since here, in the long run, the further course of hypermetabolism would depend on the outcome of the competition between the reduction of hormone production and the hypothalamic stimulation steadily increasing owing to the feedback effect on the pituitary. A possible stimulatory influence of the reduction of ACTH-adrenocortical function in consequence of the pituitary feedback may well constitute one of the factors accounting for the modified antibody production.

No relationship was found between the antibody titres and the serum gamma globulin levels which had been estimated in 38 cases. Nor did the severity of the clinical condition in hyper- or hypothyroidism display a relationship to the antibody titres. A similar divergence occurs in other immunobiological phenomena of the thyroid gland, too; in fact, the LATS level is likewise unrelated to the clinical severity of hyperthyroidism [3].

The immunogram of patients having had other types of treatment remained practically unchanged. This refers to all kinds of symptomatic therapy the necessity of which had arisen prior to specific treatment, e. g. adrenocorticoid substitution, rehydration, control of diarrhoea, etc.

*

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Dr. László Mosonyi
Dr. György Dóbiás
Dr. Judit Gönczi
Dr. Éva Perényi

Budapest XIII., Szabolcs u. 35, Hungary

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ANTIKÖRPERMANGELSYNDROM BEI PARAPROTEINÄMIE

Von

A. PATAKFALVI, R. BACKHAUSZ, JUDIT LAJOS, Á. GÓGL und M. BALÁZS

I. MEDIZINISCHE KLINIK (DIREKTOR: PROF. DR. T. JÁVOR) DER MEDIZINISCHEN UNIVERSITÄT PÉCS, IMPFSTOFFERZEUGUNGS- UND FORSCHUNGSINSTITUT HUMAN (DIREKTOR: DR. G. VERES) BUDAPEST

(Eingegangen am 18. April 1970)

Bei 40 paraproteinämischen Kranken wurden die klinischen Symptome des Antikörpermangelsyndroms fortlaufend untersucht. Zur Ermessung des humoralen Immundefekts wurden Papier-, Agar- und Immunelektrophorese vorgenommen und die zirkulierenden Antikörper und Immunglobuline bestimmt. Zur Bewertung des zellulären Immundefekts kamen mit verschiedenen Antigenen durchgeführte Kutanteste und Lymphozyten-Blasttransformationsuntersuchungen zur Anwendung. In 1/3 der Fälle meldete sich die Paraproteinämie in der Form eines Antikörpermangelsyndroms, welcher Umstand die Klärung der Grundkrankheit ermöglichte. Die bereits im Zeitpunkt der Diagnose schwer geschädigte Antikörper- und Immunglobulinsynthese verschlechterte sich parallel mit dem Fortschreiten der Krankheit. Die Schädigung der Antikörpersynthese war unabhängig von der Paraproteingruppe. Die Untersuchungen ergaben, daß sich die Produktion von allen drei Immunglobulinen, am ehesten aber die des IgA, vermindert hat.

Die zellulären Immunreaktionen zeigten keine wesentliche Abweichung. Bei Paraproteinämien IgA-, IgU- und IgM-Typs war die IgG-Abnahme weniger ausgeprägt, als bei IgG-Paraproteinämie.

Die Möglichkeit eines kausalen Zusammenhanges zwischen den die Leukämie begleitenden Infektionen und der verminderten Gammaglobulinproduktion hat ROTKY [1] bereits 1914 erwogen. LÖFFLER [2] vertrat die Ansicht, daß die bei einem seiner Patienten bestehende Prädisposition zu Infektionen der Insuffizienz der Antikörper-Produktion zuzuschreiben ist. Diese Beobachtungen gerieten indessen in Vergessenheit. Der 1954, also 3 Jahre nach Veröffentlichung der Mitteilung von LÖFFLER erschienenen Arbeit von ARENDS und Mitarb. [3] ist es zu verdanken, daß die Problematik der sekundären Immuninsuffizienz abermals in den Mittelpunkt des Interesses gelangte: der genannte Verfasser beobachtete bei einem an malignem Lymphom leidenden Patienten schwere bakterielle Infektionen und einen, der angeborenen Agammaglobulinämie ähnlichen totalen Gammaglobulinmangel.

Im Falle des wohlbekannten normoproteinämischen Antikörpermangelsyndroms können die zirkulierenden Antikörper, trotz der Anwesenheit der Globulinfraktionen vollkommen fehlen, und ihre Produktion setzt sich nicht einmal auf Einwirkung eines Antigenreizes in Gang. Ähnliche Schwierigkeiten vermag auch die Diagnostizierung des sekundären Antikörpermangelsyndroms zu verursachen, besonders im Falle einer Paraproteinämie, bei der die Anti-

körper bzw. die Immunglobuline, trotz der durch die pathologischen Eiweiße verursachten Globulinvermehrung, vollkommen fehlen können [4, 5, 6].

In unserem Krankengut befanden sich vier Fälle, deren Krankheitsverlauf unsere Aufmerksamkeit auf die Wichtigkeit der Kenntnis des sekundären Antikörpermangelsyndroms und der Untersuchung der humoralen und zellulären Immunität lenkte; es handelte sich hierbei um an Myelom leidende Patienten, bei denen das klinische Bild jahrelang durch die charakteristischen Symptome des Antikörpermangelsyndroms beherrscht wurde, ohne daß der eigentliche Antikörpermangel bzw. die latente Grundkrankheit diagnostiziert worden wäre. Laut der anamnestischen Daten spielten sich bei den ersten zwei Patienten im Laufe von 10 bzw. 8 Jahren 14 bzw. 28 Lungenentzündungen ab, während in der 9jährigen Anamnese des dritten Kranken 4 Lungenentzündungen und 6 Nasennebenhöhlenentzündungen vorkamen. Der vierte Patient wurde wegen verschiedener eiternder Hautprozesse (Injektionsabszeß, Hautgangrän usw.) im Verlauf von 5 Jahren mehrmals an chirurgischen und dermatologischen Abteilungen behandelt. Die Aufstellung der richtigen Diagnose wurde durch die Umstände erschwert, daß die angeführten Symptome die Aufmerksamkeit der Kliniker in Richtung der pulmonalen bzw. Hautkrankheiten lenkten und daß sich die Symptome der sich im Bild des Antikörpermangelsyndroms meldenden Plasmazytome in einem Teil der Fälle von der üblichen Erscheinungsform unterschieden.

Aufgrund der in diesen Fällen ermittelten Erfahrungen haben wir unsere Untersuchungen ausgebreitet, und zwar wurden seit 1964 in einem jeden paraproteinämischen Fall die auf Antikörpermangelsyndrom zurückführbaren Symptome fortlaufend untersucht; diesem Zweck dienten nebst papier-, agar- und immunelektrophoretischen Untersuchungen auch die auf die zirkulierenden Antikörper gerichteten Bestimmungen. Diese Untersuchungen wurden später mit quantitativer Immunglobulinbestimmung, die zelluläre Immunität analysierenden Kutantesten und der Untersuchung der Lymphozyten-Blasttransformation ergänzt, außerdem wurde auch die Einwirkung der Grundkrankheit auf die Antikörper- und Immunglobulinsynthese fortlaufend registriert. Die Kontrolluntersuchungen fanden in Intervallen von 3, 6, 12 Monaten statt. Bei 29 der 40 untersuchten Patienten bot sich nach der Diagnostizierung eine lange Zeit hindurch (in 22 Fällen bis zum Tod der Patienten; die durchschnittliche Überlebenszeit betrug 22,2 Monate) die Möglichkeit zur regelmäßigen Kontrolle der auf Antikörpermangelsyndrom zurückführbaren Symptome und der Gestaltung des Antikörperspiegels. Die Klärung der folgenden Fragen wurde erstrebt:

1. die Häufigkeit des Antikörpermangels bei Paraproteinämie;
2. Grad der Antikörper- und Immunglobulinsynthese-Schädigung;
3. die Korrelation zwischen dem mit immunologischen Methoden nach-

weisbaren Antikörpermangel und der Häufigkeit der klinischen Symptome des Antikörpermangelsyndroms;

4. die Eigenschaften des sich zur Paraproteinämie gesellenden Antikörpermangelsyndroms.

Material und Methodik

Die zu den verschiedenen Bestimmungen angewandten Verfahren waren wie folgt, Papierelektrophorese nach GRASSMANN und Mitarb. [7], Agarelektrophorese nach WIEME [8]: Immunelektrophorese und Leichtkettenanalyse nach BACKHAUSZ [9], Isoagglutinine und auf die Umgebungsantigene einwirkenden Antikörper nach BACHKAUSZ und Mitarb. [10]. Die bei den Patienten ermittelten Werte wurden mit den Durchschnittswerten von 114 gesunden Personen verglichen. Die quantitative Immunglobulinbestimmung erfolgte mit dem Radialimmundiffusionsverfahren von MANCINI und Mitarb. [11], während zur Bestimmung des in die mit dem IgG-Paraprotein identische Immunglobulingruppe gehörenden normalen IgG-s die anguläre Immundiffusionsmethode von BACKHAUSZ [12] zur Anwendung kam. Zu dieser Untersuchung wurden die durch das Impfstofferzeugungs- und Forschungsinstitut Human hergestellten Anti-IgG-25-B-, Anti IgA-30-K-, Anti-IgM-1-L-, Anti-IgM-110-L- und Anti-IgG-37-L-Immunseren verwendet. Zur Untersuchung der Reaktion vom Tuberkulin-Typ wandten wir die durch die Vakzinabteilung des genannten Instituts zur Verfügung gestellten, in Tabelle I angeführten Antigene an. Als das Kriterium der positiven Kutanreaktion galt eine 10×10 mm große, oder größere Hautröte bzw. -schwellung. Als 100% wurde die Gesamtpositivität der mit den 9 Antigenen durchgeführten Kutanteste betrachtet. Die bei den Patienten wahrnehmbare Positivität der Kutanreaktion haben wir mit den Ergebnissen von 29 gesunden Personen verglichen. Die Lymphozytenzüchtung erfolgte mit der Methode von MOORHEAD und Mitarb. [13]. Als nicht spezifischer Stimulator kam das Bacto-Phytohämagglutinin-P (Difco Laboratories Detroit, Mich. USA) zur Anwendung. Am 3. und 6. Tag wurde in der Kultur das prozentuale Verhältnis der Lymphoblastoidzellen bestimmt.

Die Antikörperuntersuchungen fanden bei 40 Patienten statt. Die der Paraproteingruppe entsprechende Verteilung war wie folgt: IgG: 24; IgA: 7; IgU: 5; IgM: 2; IgG+IgA: 1; IgG+IgM: 1. Die der Grundkrankheit entsprechende Verteilung zeigte folgendes: Plasmozytom: 37 Fälle; Makroglobulinämie: 2 Fälle; lymphoide Leukämie: 1 Fall. Quantitative Immunglobulinbestimmung wurde in 20 Fällen durchgeführt. Kutanteste wurden in 8 Fällen angewandt, während die Untersuchung der Lymphozytenblasttransformation bei 6 Patienten vorgenommen wurde.

Tabelle I

Antigene zu Kutantesten

A) Staphylococcus aureus haemolyticus
B) Staphylococcus albus
Micrococcus (Grampositiv)
C) Streptococcus haemolyticus
Lancefield A, C, G.
D) Streptococcus viridans
Streptococcus pneumoniae
Streptococcus haemolyticus L. D.
E) Neissera pharyngis
Klebsiella pneumoniae
Haemophilus influenzae
Micrococcus (Gramnegativ)
F) Bordatella pertussis
Bordatella parapertussis
Bordatella bronchioseptica
G) Escherichia coli
Pseudomonas aeruginosa
Bacillus proteus Providentia
H) Brucella abortus
I) Mantouxsche Probe 1 : 100 000

Tabelle II

Wichtigere Angaben der Antikörpermangelsyndrom-Fälle

Nr., Name,	Geburtsjahr	Typ der Paraproteingruppe	Knochen-Röntgen	A		Symptome des Antikörpermangelsyndroms													
				vor	nach	Lungenentzündung □		Bronchitis □		Sinusitis □		Pyoderma □		Zystopyelitis □		Enterokolitis □		Sepsis □		Meningitis □	
1. J. B.	1901	IgGK	o. R.	—	?	6		2	—	—	—	—	—	—	—	—	—	—	—	—	—
2. M. Cs.	1905	IgGL	O	124	52+	14	9	—	6	—	—	—	1	—	—	—	—	—	—	—	—
3. K. R.	1909	IgGK	o. R.	—	?	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4. Gy. G.	1893	IgGL	o. R.	—	2+	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—
5. F. K.	1898	IgGK	o. R.	—	?	—	2	—	1	—	—	—	—	—	—	—	—	—	—	—	—
6. Gy. K.	1923	IgGK	o. R.	—	4+	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—
7. J. G.	1904	IgG	neg.	97	13+	28	2	—	—	—	—	—	—	—	2	—	—	—	—	—	—
8. N. M.	1901	IgGK	o. R.	—	11+	—	1	—	—	—	—	—	1	—	—	—	—	—	—	—	—
9. Gy. M.	1898	IgGL	o. R.	20	72+	4	2	—	2	—	1	—	2	—	1	—	—	—	1	—	—
10. J. R.	1912	IgGK	o. R.	16	49K	2	1	x	2	—	—	—	—	—	—	—	—	—	—	—	—
11. J. S.	1891	IgGK	o. R.	—	2+	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
12. I. Sz.	1914	IgGK	o. R.	—	9+	1	1	—	1	—	—	—	—	—	—	1	—	—	—	—	—
13. J. W.	1913	IgGL	o. R.	—	8+	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
14. Gy. K.	1898	IgGL	o. R.	13	1+	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
15. A. K.	1908	IgGL	o. R.	42	1+	1	1	—	1	—	—	—	—	1	1	—	—	—	—	—	—
16. D. K.	1906	IgGL	neg.	100	34+	4	1	—	x	6	2	—	—	—	1	—	—	—	—	—	—
17. P. F.	1900	IgAL	O	5	15+	—	—	—	—	—	—	—	—	1	1	—	—	—	—	—	—
18. S. M.	1899	IgAK	O	55	23+	1	5	—	x	—	—	x	x	—	—	—	—	—	—	—	—
19. I. Cs.	1903	IgAK	o. R.	88	25	2	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—
20. S. I.	1899	IgA	o. R.	—	?	—	—	—	20	—	—	—	—	—	—	—	—	—	—	—	—
21. A. M.	1905	IgA—IgG	o. R.	72	7+	2	1	—	2	—	—	—	—	—	—	—	—	—	—	—	1
22. I. M.	1909	IgU	O	—	33K	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
23. J. F.	1910	IgM	o. R.	—	8+	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
24. A. F.	1912	IgM	neg.	—	24K	—	2	—	4	—	—	—	—	—	—	—	—	—	—	—	—

A. = Dauer des Antikörpermangelsyndroms (Monate) vor bzw. nach der Diagnosestellung der Paraproteinämie
o. R. = osteolytische Rarefraktion
O = Osteoporose
? = Zur Kontrolle nicht erschienen

\+ = gestorben
K = unter Kontrolle
□ = Zahl der Infektionen vor der Diagnose
x = häufiges Auftreten der Infektionen

Ergebnisse

1. *Häufigkeit der Infektionen.* In 24 der 40 Fälle traten auf Antikörpermangel zurückführbare klinische Symptome in Erscheinung; Verteilung und Häufigkeit dieser Symptome, Gruppe bzw. Typ des Paraproteins, Beginn des Antikörpermangelsyndroms, Zeitpunkt der Diagnose und Todeszeitpunkt sowie Verteilung der nachweisbaren Knochenveränderungen veranschaulicht Tabelle II. Was die Häufigkeit anbelangt, stehen die Atemweginfektionen und unter ihnen die Pneumonien an erster Stelle. Anläßlich der Zusammenstellung der Infektionshäufigkeit wurden nur die auch röntgenologisch verifizierten Pneumonien und Sinusitiden bzw. ausschließlich die mit hohem Fieber einhergehenden, eine stationäre Behandlung beanspruchenden Bronchitiden berücksichtigt, was gleichzeitig auch soviel bedeutet, daß die tatsächliche Infektionshäufigkeit höher lag. Hautinfektion kam seltener vor, bei einem Patienten meldete sie sich jedoch in einer schweren und bis zum Tode bestehenden Form. Harnweginfektionen ließen sich ziemlich häufig beobachten.

Unseres Erachtens ist der Tatsache, daß die Mehrzahl der Infektionen bereits vor der Diagnostizierung der Paraproteinämie auftrat, d.h., daß sich Myelom bzw. Makroglobulinämie in Form eines Antikörpermangelsyndroms meldeten — eine wesentliche praktische Bedeutung beizumessen. Obwohl sich die entzündliche Veränderung in diesen Fällen auf Einwirkung der antibiotischen Behandlung zurückentwickelte, blieb die Senkungsgeschwindigkeit der Blutkörperchen auch nach Abklingen der Entzündung wesentlich erhöht. Aufgrund dieser Symptome erhob sich der Verdacht einer, im Hintergrund des Antikörpermangelsyndroms stehenden Paraproteinämie. Nicht weniger beachtenswert ist auch der Umstand, daß in den klinisch schwersten Antikörpermangelsyndrom-Fällen (Tabelle II, Fälle 2, 7, 16 und 18) keine Rarefraktionen der Knochen nachzuweisen waren, auf den Rtgaufnahmen ließ sich zumeist Osteoporose erkennen, es kamen aber auch negative Knochenbefunde vor.

2. *Antikörper.* Die im Zeitpunkt der Diagnostizierung und anläßlich der letzten Untersuchung ermittelten Ergebnisse sind in Tabelle III dargestellt. Mit der Ausnahme von zwei Fällen waren die Titer bereits im Zeitpunkt der Diagnostizierung stark vermindert, die Mehrzahl der Werte lag unter dem Normaldurchschnitt. Ein bedeutender Teil der 13 untersuchten Antikörper konnte überhaupt nicht nachgewiesen werden. Die Schädigung der Antikörpersynthese erstreckte sich auf sämtliche, in alle drei Immunglobulingruppen gehörende Antikörper. Das Maß der Schädigung erwies sich als unabhängig von der Gruppe bzw. dem Typ des Paraproteins. In 20 Fällen zeigten die kurz nach dem Tod bestimmten Antikörpertiter im Vergleich zu den im Zeitpunkt der Diagnose ermittelten Resultaten eine weitere Verminderung.

Tabelle III

Antikörper-Durchschnittswerte bei Kontrollpersonen

	Titerreziprokwerte der auf die				
	Anti		Sh. sonnei	S. typhi	
	A	B		0	Vi
Kontrollwerte n = 114	32	75	22	12	8
Bei der Diagnose der Paraproteinämie n = 40	15,2	13	3	2,2	1,5
Vor dem Tode n = 22	10	9	2,4	2	1,4
Antikörpermangelsyndrom n = 24	18	14	3	2,2	1,7
Fälle ohne klinischen Symptome des Antikörpermangels n = 16	12	10	3,2	2	1,5

In der terminalen Phase ließ sich in manchen Fällen keiner der untersuchten Antikörper nachweisen.

3. *Quantitative Immunglobulinbestimmung.* In drei Fällen waren oberhalb der Normalgrenze liegende IgG- (Fall Nr. 14) und IgA-Werte (Fall Nr. 9 und 20) vorzufinden. Bei IgG-Paraproteinämien zeigten alle drei Immunglobuline eine starke Verminderung. Bei IgG-, IgA- und IgU-Paraproteinämien waren sowohl die IgA- als auch die IgM-Werte in bedeutendem Maße vermindert, bei den IgG-Paraproteinämien trat aber eine ausgeprägtere Verminderung der IgG-Werte in Erscheinung, als bei den übrigen Paraproteinämien (Abb. 1, Tab. IV).

Werden die Antikörpertiter und die mit Immundiffusion bestimmten Immunglobulinwerte verglichen, ist es augenfällig, daß die Antikörperverminderung im allgemeinen ausgeprägter ist, als es anhand des Immunglobulinspiegels anzunehmen wäre. In mehreren Fällen konnten — trotz des vollkommenen Antikörpermangels — verschiedene Immunglobulinmengen nachgewiesen werden.

Was die Häufigkeit der Infektionen und das Maß der Antikörperspiegelverminderung anbelangt, ließ sich feststellen, daß in den mit häufigen Infektionen komplizierten Fällen die Antikörperspiegelverminderung stets ausgeprägt war. Demgegenüber verursachte ein hochgradiger und sogar ein sich auf die untersuchten Antigene beziehender totaler Antikörpermangel nicht in einem jeden Fall klinische Symptome. In 16 der 40 Fälle meldeten sich im Laufe der Beobachtungszeit keine klinischen Symptome des Antikörpermangels. Zwischen den Antikörpertitern dieser 16 Fälle und den entsprechenden Werten der 24, mit verschiedenen Infektionen einhergehenden Fällen ließ sich kein Unterschied erkennen (Tab. III).

4. *Allergie vom Spättyp und Lymphozyten-Blasttransformation.* Die mit 9 Antigenen vorgenommenen Kutanreaktionen zeigten im Vergleich zu den

und bei Paraproteinämie

Antigene einwirkenden Antikörper							
Sh. flexneri		E. coli		Sh. flexneri 3	E. coli		Staphylokokkus-α antitoxin (IE)
2a	1b	055	026		086	0111	
11	41	10	14	56	14	12	0,75
3,4	3,7	3,5	3,5	5,4	2,1	1,9	0,58
3	4	1,6	2,2	3,6	1,5	1,5	0,31
3,2	4,5	3,1	3,5	5,5	2,7	2,5	0,42
3,3	3	3,6	3,3	4.2	2	1,8	0,33

Tabelle IV

Immunglobulinwerte der Kontrollpatienten und bei Paraproteinämie

Gruppe	Immunglobuline		
	IgG mg/100 ml	IgA mg/100 ml	IgM mg/100 ml
Normalwerte n=60	1570±52,3	220±12	193±44
Paraproteinämie n = 20	555±82	71,5±22	36,3±5
IgG n = 11	348±50	51,2±17	24,7±6
IgA n = 4	783±334	—	66,7±37
IgU n = 3	798±140	20,8±8	38,3±9
IgM = 2	880	259	—

Tabelle V

Verteilung der Kutantest-Positivität

Gruppe	Anzahl der Fälle	Ablesezeitpunkt der Reaktion		
		15 Min %	24 St %	48 St %
Normalwerte	29	29	30,2	30,2
Paraproteinämie	8	—	23,6	18

Kontrollwerten eine Verminderung (Tab. V). In 6 Fällen kam es in den Lymphozytenkulturen mittels eines aspezifischen Antigens (Phytohämagglutinin) zur Analyse der zellulären Immunantwort. In einem Fall, in dem die Untersuchung während der zytostatischen Behandlung stattfand, war keine Blasttransformation vorzufinden, in den übrigen Fällen ließen sich die Blasten am 3. und 6. Tag der Züchtung in der in der Tabelle VI angeführten Proportion beobachten. Die Ergebnisse können als Zeichen der entsprechenden zellulären Immunantwort bewertet werden.

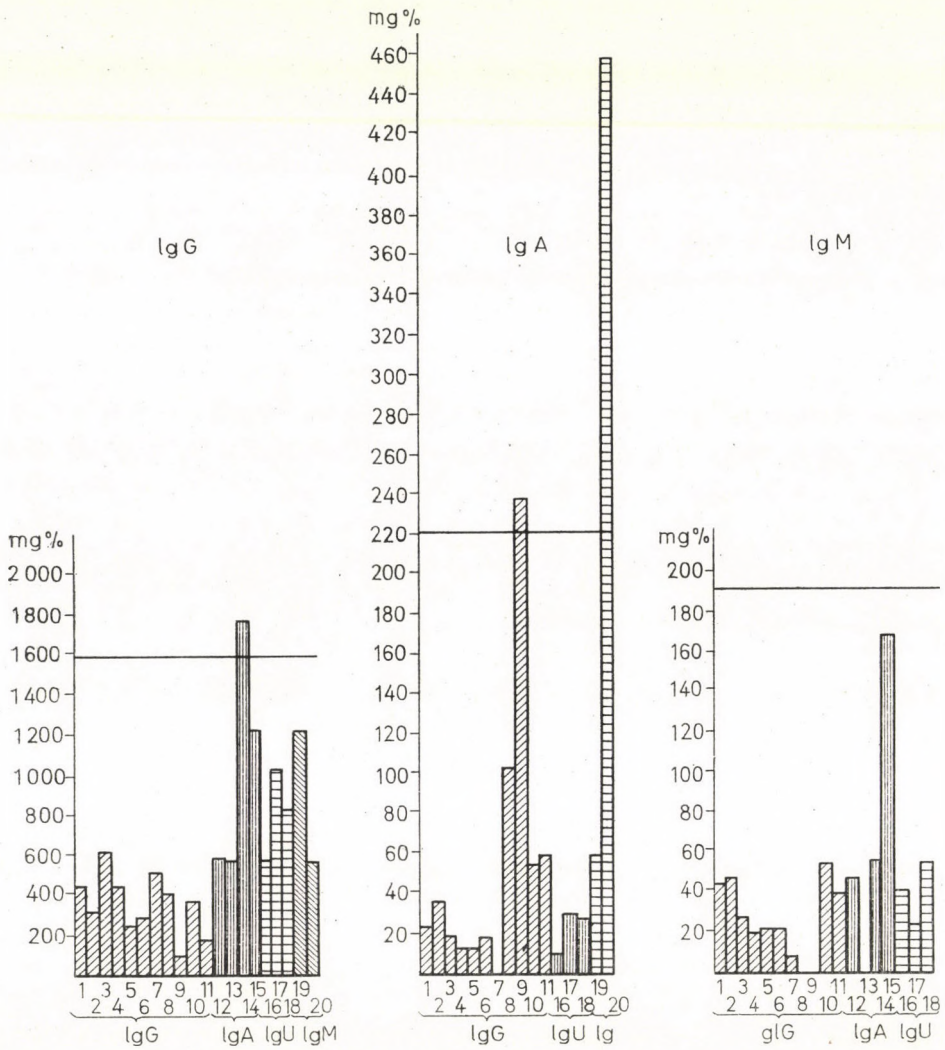

Abb. 1. Immunoglobulinwerte von 20 Patienten in einer der Gruppe bzw. dem Typ des Paraproteins entsprechenden Aufschlüsselung

Tabelle VI

Ergebnisse der Lymphozyten-Blasttransformationsuntersuchungen

	3. Tag		6. Tag	
	PHA	Kontr.	PHA	Kontr.
1. J. Cs.	72	0	24	0
2. A. K.	0	0	0	0
3. É. Sz. K.	36	0	25	0
4. A. S.	38	0,2	44	0
5. J. A.	68	0	29	0
6. F. A.	59	0	32	0

PHA = Phytohämagglutinin

Besprechung

Nach altbekannter klinischer Beobachtung besteht bei Myelom und bei der malignen Wucherung des lymphoretikulären Systems häufig eine Infektionsneigung [14, 15]. Eine weniger oder kaum bekannte Erscheinung ist dagegen — wie darauf auch unsere Fälle hinweisen — daß sich die Paraproteinämien Jahre oder sogar ein Jahrzehnt hindurch im Bild des Antikörpermangelsyndroms melden können. Besonders beachtenswert ist die Beobachtung, daß in den klinisch schwersten Fällen von Antikörpermangel keine osteolytischen Knochenrarefraktionen nachzuweisen waren, der Röntgenbefund negativ ausfiel oder auf Osteoporose verschiedenen Grades hinwies (Tab. II). Außer den im Vordergrund des klinischen Bildes stehenden Symptomen des Antikörpermangelsyndroms, spielte aller Wahrscheinlichkeit nach der letzterwähnte Umstand darin eine Rolle, daß sich die Aufmerksamkeit der Kliniker auf die lokalen Lungen- und Hautkrankheiten richtete und die im Hintergrund verborgene Grundkrankheit lange Zeit hindurch nicht erkannt wurde. Im diesem Zusammenhang muß aber erwähnt werden, daß im Falle eines negativen Knochenbefundes bzw. Osteoporose nicht in einem jeden Fall rezidivierende Infektionen auftraten.

Das sich zur Paraproteinämie gesellende Antikörpermangelsyndrom kann sich — und dies dürfte als das wesentlichste Charakteristikum des pathologischen Zustands betrachtet werden — scheinbar in selbständiger Form, als das erste Symptom der Krankheit melden. Die übrigen Formen des sekundären Antikörpermangelsyndroms treten im Falle einer bereits bekannten Diagnose (z. B. Lymphadenose) zumeist nach langjähriger Krankheitsdauer auf. Primäre Hypo- oder Agammaglobulinämie kommt bei bejahrten Personen praktisch nicht vor und ist auch bei jungen Erwachsenen eine seltene Erscheinung [19, 20, 21, 22].

Unsere Erfahrungen sprechen somit dafür, daß falls bei einem erwachsenen Patient ohne nachweisbare primäre Hypo- bzw. Agammaglobulinämie die Symptome des Antikörpermangelsyndroms sowie auf dem Papierelektrophoretogramm die Erhöhung der Gammaglobulinfraktion zu beobachten sind, besonders aber wenn die Senkungsgeschwindigkeit der Blutkörperchen auch in entzündungsfreien Perioden gesteigert bleibt, die Möglichkeit eines sich zu Paraproteinämie gesellenden Antikörpermangelsyndroms erwogen werden muß. Hierbei sei nachdrücklich betont, daß sich Bronchiektasie auch zufolge des Antikörpermangels entwickeln kann [23, 24, 25].

Was die Laboratoriumsdiagnostik des sich zu Paraproteinämie gesellenden Antikörpermangelsyndroms anbelangt, sind der Gesamteiweiß- und elektrophoretische Befund eher irreführend, genauer gesagt, sind diese Werte nur bei sog. normoproteinämischem und mikromolekulärem Plasmozytom pathognostisch, da sich nebst niedrigerem Gesamteiweißspiegel häufig Hypogammaglobulinämie beobachten läßt [26, 27, 28]. Diesen Umständen zufolge ist bei Paraproteinämie den Antikörperuntersuchungen eine besondere Bedeutung beizumessen. Früher wurden praktisch nur die Isoagglutinine bestimmt [29, 30], und diese Untersuchungen wurden später mit der Bestimmung von Streptolysin-O-Antitoxin sowie Staphylokokkus-α-Antitoxin ergänzt [31, 32]. Manche Verfasser begnügen sich auch heute noch mit der Bestimmung der Isohämagglutinine und des Diphtherie-, Tetanus-Antitoxin-, Antistreptolysin-Titers [33, 34, 35]. Anhand der Untersuchung von einigen Antikörpern ist aber die Bewertung des humoralen Immunzustands schwierig. HARTL und Mitarb. [32] haben z. B. normale Antistreptolysintiter vorgefunden. ZINNEMANN und HALL [36] und auch andere Verfasser [37, 38, 39] fanden nach Anwendung verschiedener Vakzinen (Diphtherie, Typhus, Paratyphus, Mumps) bzw. nach Verabfolgung eines Pneumokokkus-Polysaccharidenantigens stark verminderte Antikörperreaktionen.

Die quantitative Bestimmung der Immunglobuline ermöglichte die Herstellung der monospezifischen Immunseren; diese Bestimmungen wurden vorerst mit dem direkt-linearen Verfahren von OUDIN [40] vorgenommen, neuestens findet dagegen eher die Radial-Diffusionsmethode von MANCINI und Mitarb. [11] eine Anwendung.

BACKHAUSZ [12], HOBBS [41], MÜLLER [42], RENTSCH [43] und SCHEURLEN [44], die bei einer großen Zahl von paraproteinämischen Kranken quantitative Immunglobulinbestimmungen durchführten, vermochten im allgemeinen die ausdrückliche Verminderung der Immunglobuline festzustellen.

Die verhältnismäßig große Zahl der Isoagglutinine und der auf die Umgebungsantigene einwirkenden Antikörper gaben eine entsprechende Aufklärung über den Zustand des humoralen Immunsystems, die Schädigung der in die einzelnen Immunglobulingruppen gehörenden Antikörper konnte aber nur anhand der Ergebnisse der gleichzeitig durchgeführten quantitativen

Immunglobulinbestimmungen bewertet werden. Beim Vergleich der Immunglobulin- und Antikörpertiter stellte es sich heraus, daß es stets die Antikörperwerte waren, die die hochgradigere Verminderung aufwiesen. Nebst totalem Antikörpermangel — welche Erscheinung häufig zu beobachten war — konnten die Immunglobuline in verschiedenen Mengen nachgewiesen werden. Anhand dieser Beobachtung dürfte eventuell auch mit der Anwesenheit von funktionsgeschädigten Immunglobulinen gerechnet werden. Ob die zellulären Immunreaktionen aufgrund der Kutanteste und der Blasttransformation bewertet werden können, ist noch eine offene Frage. Unsere Untersuchungen sprechen dafür, daß bei Paraproteinämie die zelluläre Immunität keine nennenswerte Schädigung erleidet.

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Dr. Albert PATAKFALVI; I. Belklinika, Pécs, Ungarn.

Dr. Richárd BACKHAUSZ, Dr. Judit LAJOS: Human Oltóanyagtermelő és Kutató Intézet, Budapest X., Szállás u. 5—7, Ungarn

Dr. Árpád GÓGL, Dr. Mihály BALÁZS: I. Belklinika, Pécs, Ungarn

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EFFECT OF ANGIOTENSIN ON CORTICOID PRODUCTION OF THE RAT ADRENAL IN VITRO

KATHLEEN SZ. SZALAY and KATHLEEN MIHÁLY

INSTITUTE OF EXPERIMENTAL MEDICINE, HUNGARIAN ACADEMY OF SCIENCES, BUDAPEST

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A 25 or 50 μg/ml dose of angiotensin added to the incubation medium at 15-minute intervals stimulated corticosterone production and left aldosterone production unaffected; a 100 μg/100 ml dose enhanced both. Between 20% and 40% of the angiotensin is destroyed during incubation. The high angiotensinase activity of the rat adrenal cortex cannot explain the insensibility to angiotensin.

The part played by angiotensin in controlling aldosterone secretion is no longer under discussion (cf. review by DAVIS [3]), except in respect of the rat. Several authors found that in the laboratory rat angiotensin administered failed to stimulate aldosterone secretion [5, 8], or stimulated it only when given in very large doses [1, 4]. KAPLAN and BARTTER [7] incubated rat adrenal tissue slices with 5 μg/100 mg and GLÁZ and SUGÁR [6] with 100 μg/100 mg of angiotensin and could induce no increase in aldosterone production; nor could SPÄT et al. [13], by the continuous infusion of 0.5 μg/min. of angiotensin. MÜLLER [10], on the other hand, found an increase in aldosterone output after 330 μg/ml of angiotensin.

The present work was based on the assumption that the adrenal of the rat contains more angiotensinase than that of other species, and that this is why only large doses of angiotensin increase aldosterone production in the rat. To establish the validity of this assumption we have studied the effect on corticosterone and aldosterone production of repeated angiotensin doses added to incubated rat adrenals and determined the proportion of destroyed o retained, i.e. actually effective, angiotensin.

Materials and methods

Female Wistar rats weighing 170—200 g were decapitated and the adrenals were removed and halved. The experiments were arranged in randomized blocks, each block consisting of 2, 3, or 4 units depending on the number of treatments. Within each block the halved adrenals were distributed evenly so as to have 12 halves in each flask. Incubation was carried out for 40 minutes in a Warburg apparatus, in 5 ml of Krebs–Ringer bicarbonate buffer containing 3.6 mEq of potassium (according to MÜLLER [10]) at 37 °C, with a mixture of 95% O_2 and 5% CO_2 continuously bubbled through the medium. At the end of this preincubation period the medium was discarded. Added to the control flasks were 4.25 ml of Krebs–Ringer bicarbonate buffer containing 5.9 mEq potassium, and to the angiotensin-treated flasks 4 ml of the buffer and a dose of angiotensin in 0.25 ml of buffer to a final angiotensin concentration of 25, 50, or 100 μg/ml. Incubation lasted 90 minutes, and every 15 minutes

(in the first experimental series every 30 minutes) a fresh dose of angiotensin in 0.25 ml of buffer was added to the medium. At the same time, the same quantity of buffer solution was added to the control flasks. After incubation for 15, 45, and 75 minutes, 0.1 ml of medium was withdrawn from each flask before adding fresh angiotensin, to determine the concentration of angiotensin. The same quantity was withdrawn from each control flask. (In the first series of experiments angiotensin concentrations were not determined.) In a number of experiments, angiotensin was added to the flasks without adrenal tissue, to see whether it would be regained in the absence of angiotensinase.

The corticosteroids were extracted from the medium with chloroform and isolated by paper chromatography [14].

For angiotensin determination, 0.9 ml of physiological saline were added to the 0.1 ml of medium removed in the course of incubation, and the mixture was boiled in a water bath for 15 minutes to destroy the angiotensinase still present in the medium. The samples were stored at 0 °C until measuring. Angiotensin was determined in nephrectomized and ganglion-blocked (1 mg/kg of intravenous mecamylamine) male rats on the basis of its hypertensive action. The hypertensive action of the diluted incubation medium was compared with that of standard CIBA Hypertensin.

The results were evaluated by two-way analysis of variance, and the group averages compared by SCHEFFÉ's multiple comparison test [11].

Results

Doses of 25, 50, or 100 μg/ml of angiotensin added to the incubation medium at 30-minute-intervals had no effect on either corticosterone or aldosterone production (Fig. 1). A dose of 25 or 50 μg/ml added every 15 minutes

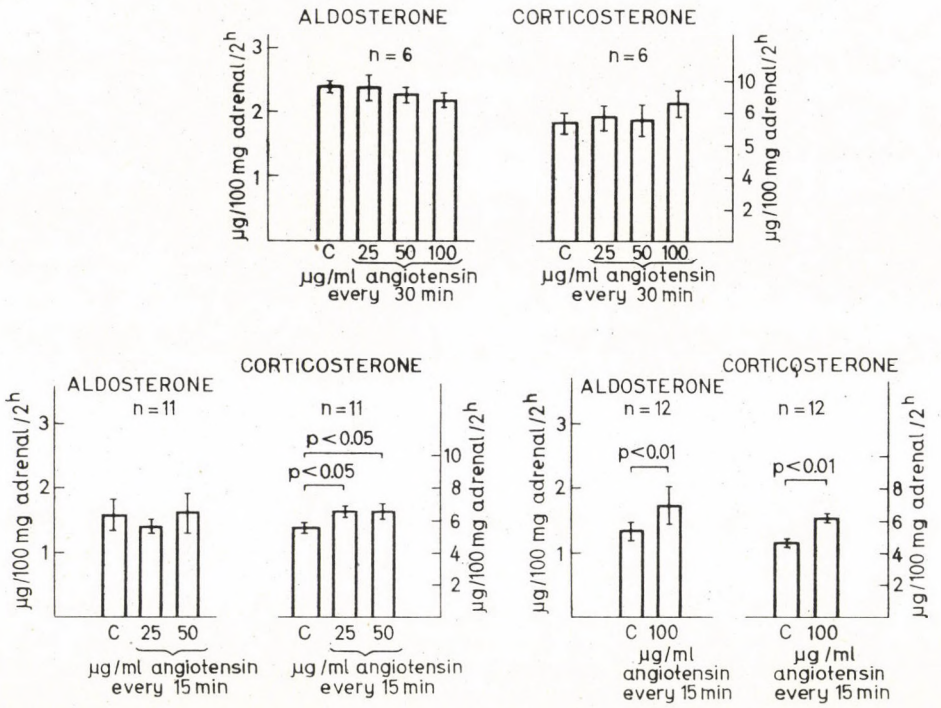

Fig. 1. Effect of angiotensin on aldosterone and corticosterone production of the rat adrenal. C: control

increased corticosterone production significantly ($p < 0.05$) and left aldosterone production unaffected. Fifteen-minute additions of 100 μg/ml angiotensin significantly stimulated the production of both aldosterone and corticosterone ($p < 0.01$).

Loss of angiotensin was insignificant, 10—15% in the first 15 minutes, but at 45 and 75 minutes it fluctuated between 20% and 40% (Table I). Of the angiotensin incubated without adrenal tissue 93.6% was recovered.

Table I

Angiotensin recovery

	Determined after incubation for (min)	Calculated μg/ml	Recovered μg/ml	Loss per cent
25 μg/ml every	15	23.8	21.5±1.3*	9.7
15 min	45	66.6	39.9±1.7	40.1
n = 12	75	104.0	70.9±3.7	31.8
50 μg/ml every	15	47.6	42.9±2.9	9.9
15 min	45	133.0	107.7±7.5	19.0
n = 12	75	208.0	143.0±10.2	31.2
100 μg/ml every	15	118.0	100.1±5.1	15.2
15 min	45	316.0	194.5±10.3	38.5
n = 13	75	475.0	338.8±16.4	28.7

* mean ± S. E.

Discussion

In agreement with other investigators we found that in the rat very large doses of angiotensin are necessary for stimulating aldosterone secretion. The recovery rate of angiotensin shows that at least 100—200 μg/ml of it are required to be present in the medium to enhance aldosterone production in vitro. Only MÜLLER [10] has as yet reported increased aldosterone production by rat adrenals incubated with 330 μg/ml of angiotensin. Taking into due account the loss of angiotensin resulting from the angiotensinase activity in the rat adrenal it is reasonable to suppose that in his experiments also about 100—200 μg/ml of angiotensin must have been present during most of the incubation period.

Smaller doses of angiotensin sufficed to augment corticosterone production. Allowing for angiotensin losses, between 40 and 70 μg/ml of angiotensin are necessary for corticosterone production to increase. Several authors observ-

ed a rise in corticosterone production after doses of angiotensin which had no effect on aldosterone secretion [1, 2, 9, 12].

Angiotensinase activity in the rat adrenal destroyed 20—40% of the angiotensin during incubation. This activity, however, is not high enough to explain in itself why several times larger doses are needed to increase aldosterone production by the adrenal of the rat than of other species.

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Dr. Kathleen Sz. Szalay
Kathleen Mihály
Budapest VIII., Szigony u. 43, Hungary

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PRODUCTION OF UNCONJUGATED 20-DHF (11β, 17α, 20β, 21-TETRAHYDROXY-PREGN-4-EN-3-ONE) IN ACTIVE RHEUMATOID ARTHRITIS

By

V. KEMÉNY, K. FARKAS and B. GÖMÖR

NATIONAL INSTITUTE OF RHEUMATOLOGY AND BALNEOLOGY, RESEARCH LABORATORY

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In patients with active rheumatoid arthritis and normal subjects treated with 5 to 10 μC of 1—2 ^{3}H-hydrocortisone, the fractions THF (3α, 11β, 17α, 21-tetrahydroxy-5-pregnan-20-one), 20 DHF (11β, 17α, 20β, 21-tetrahydroxy-pregn-4-3-one) and 6β OHF (6β, 11β, 17α, 21-tetrahydroxy-pregn-4-en-3,20-dione) were isolated from urine, estimated, scanned for radioactivity, and their specific activities were calculated. In all of the cases studied, 20-DHF displayed a lower activity than did THF or 6β OHF. In the normal subjects where urinary 20 DHF was low, this fraction showed no activity. The daily hydrocortisone secretion rate calculated from the specific activities of THF and 6β OHF was consistent with the literary data concerning normal individuals. On the other hand, the specific activity of 20-DHF was one order of magnitude lower than that of THF. Thus, 20-DHF cannot be regarded as a direct regular metabolite of hydrocortisone and constitutes no suitable parameter for the estimation of its secretion rate. The possible factors of 20 DHF-production are discussed.

The factors underlying the production of the hydrocortisone derivative reduced at C-20, 20-DHF (11β, 17β, 20β, 21-tetrahydroxy-pregn-4-en-3-one) are poorly understood. On the evidence of studies in vitro, certain authors [1, 2, 3] regard 20-DHF as a direct metabolite of hydrocortisone. BRADLOW et al. [4] suggest that the compound might be formed as an intermediary product in the course of hydrocortisone metabolism prior to reduction of the ring A. BAILEY et al. [5] found 20-DHF in the urine of healthy subjects and a highly significant increase in its urinary excretion in rheumatoid arthritis.

20-DHF is excreted with urine in an unconjugated, free form of high polarity, with no reducing side chains, therefore its identification and estimation in urine is not easy. By a procedure developed by us we have found a marked increase in urinary 20-DHF in active rheumatoid arthritis, in opposition to active ankylopoietic spondylarthritis and to normal conditions where the urinary excretion of the substance is normal. No deficit in steroid supply is demonstrable when this fraction accumulates in the urine [6].

The present study was devoted to the specific activity of 20 DHF excreted by subjects with active rheumatoid arthritis after the intravenous administration of 1—2 ^{3}H-hydrocortisone, and to ascertain whether it corresponded to the specific activity of THF, 3α, 11β, 17α, 21-tetrahydroxy-5β, pregnan-20-one, one of the compounds generally regarded as one of the major regular

urinary metabolites of cortisone, and to that of 6 β OHF (6β, 11β, 17α, 21-tetrahydroxy-pregn-4-en-3,20-dione), excreted with urine in its unconjugated form similarly to 20 DHF. It was furthermore studied whether this metabolite represented a suitable reference substance for the calculation of the daily hydrocortisone secretion rate. In each of the cases studied we measured the specific activity of THF, 20 DHF and, in the majority of cases, that of 6 β OHF likewise excreted in a free, unconjugated form.

Method

The 1—2 ^{3}H-hydrocortisone used in the present study was a product of the Radioisotope Institute of the Hungarian Academy of Sciences, with a specific activity of 1 mc/mg and a radiochemical purity of 97%. It was submitted to weekly repeated chromatographic purification procedures prior to use. 1 ml of the alcoholic solution of 5 to 10 μC 1—2 ^{3}H-hydrocortisone in 20 ml sterile physiological saline was administered intravenously in five minutes, to each subject.

Urine was collected for 24 hrs after injection. Unconjugated 6 β OHF and 20 DHF were recovered from 100 ml urine samples by the method of FRANTZ et al. [7].

Chromatographic separation was made in the following systems. I. benzene : methanol: water (2 : 1 : 1); II, benzene : methanol : water : ethyl acetate (0.5 : 1 : 1 : 0.5); III. benzene: methanol : water : ethyl acetate (1 : 1 : 1 : 0.1).

For the identification of 6 β OHF and of 20 DHF in unconjugated form the samples were run together with reference steroids in the various chromatographic systems and the RF values were determined. After acetylation the fractions were re-run in a petrolether : benzene : methanol : water system (66 : 33 : 50 : 50) and the RF values were compared with those of the acetylated reference standards. After periodic acid side chain oxidation by the method of ROMANOFF [8], identification of the fractions in the form of Zimmermann-positive ketosteroids was made with reference standards in the chromatographic systems, I, benzene : formamide (100 : 20) after impregnation with 20% methanol; II, n-heptane : methanol : benzene (30 : 11 : 59) after impregnation with propylene glycol : methanol (3 : 7). After final chromatography, the fractions 6 β OHF and 20 DHF isolated from urine were eluted from the paper and evaporated in vacuum to dryness. The dry residue was diluted in 1 ml absolute ethanol. Aliquots of this were used for the estimation of 6 β OHF by the Chen-reaction [9]. Estimation of 20 DHF was based on the micro-Zimmermann-reaction [10] of the 17-ketosteroid resulting from periodic acid oxidation.

THF was determined in 100 ml urine samples in the presence of 0.3 *M* acetate buffer pH 4.8 after enzymatic breakdown for 36 hrs at 37 °C and the addition of 2000 U/ml of β-glucuronidase (Reanal). Extraction, purification and separation were performed by the method of COPE [11]. After final chromatography, THF was evaporated in vacuum to dryness and dissolved in 1 ml absolute alcohol. Aliquots were used for quantitative estimation by the Chen-reaction, and 0.5 ml for radioactivity measurement.

The reference 20-DHF was prepared from hydrocortisone as follows. 1 mg hydrocortisone was dissolved in 1 ml methanol, subsequently 0.5 sodium borohydride was dissolved in 1 ml pyridine. After reduction at 15 °C for 15 minutes, 1 or 2 drops of glacial acetic acid were added to the solution. After evaporation in vacuum to dryness, 20 DHF was purified and separated in the chromatographic systems, I. benzene : methanol : water (1 : 1 : 1); II. benzene : methanol : water (2 : 1 : 1); III. benzene : methanol : water : ethyl acetate (0.5 : 1 : 1 : 0.5); IV. benzene : methanol : water : ethyl acetate (1 : 1 : 1 : 0.1). After acetylation and oxidation of the side chain, identification was made in the unconjugated form with reference to the standard sample of 20 DHF of the Steroid Reference Collection. The benzene : methanol : water : ethyl acetate (0.5 : 1 : 1 : 0.5) system was found to ensure a reliable separation of the reduced product prepared for reference purposes in our laboratory as well as of the 20 α fractions of the urinary extracts. The 20 α metabolites were located on the chromatogram by UV absorption, the negative tetrazolium blue reaction, furthermore by the presence of 11 hydroxy-androstenedione (11 β, hydroxyandrost-4-ene-3,17-dione) resulting from oxidization.

Table I

Daily hydrocortisone secretion rate and specific activity (0 to 24 hours) of THF, 6-OHF, 20-DHF in patients with active rheumatoid arthritis and in normal subjects, after intravenous 1—2 ^{3}H-hydrocortisone administration

Serial number	Specific activity d. p. m./μg			Injected radioactivity d. p. m. × 10^{-6}	Hydrocortisone mg/24 hrs on THF	Excretion the basis of 6 β OHF
	THF	6 β OHF	20 DHF			
1	380	145	48	5.02	13.2	34.6
2	261	304	22.4	5.02	19.2	16.5
3	395	366	13	5.02	12.7	14.1
4	190	182	1.6	5.02	26.4	27.6
5	235	173	6.8	5.02	21.3	29.2
6	730	614	18.8	10.04	13.7	17
7	880	720	39	10.04	11.4	14.5
8	380	241	24	5.02	13.2	20.6
9	326	—	—	5.02	15.4	—
10	291	341	58	5.02	17.2	14.7
11	310	308	—	5.02	16.1	16.3
12	410	242	15	5.02	12.3	20.6
				mean value	16 ± 1.2	17.3 ± 2.5
Control			*			
I	1020	750	—	10.04	9.8	13.4
II	310	395	—	5.02	16.1	12.7
III	620	710	—	10.04	16.2	14.1
IV	600	690	—	10.04	16.7	14.5
V	720	820	—	10.04	13.9	12.3
				mean	14.5 ± 1.27	13.4 ± 0.4

Normal range on the basis of THF, 4 to 31 mg/24 hrs (11)

* = no measurable activity

Radioactivity was measured in a Packard Tricarb liquid scintillation spectrometer (Model 3375) with a net efficiency of 25%, in a scintillation liquid of the following composition: 4 g PPO and 0.35 g POPOP in 1 L toluene. Specific activity was expressed in terms of disintegrations d. p. m/μg.

ACTH as well as corticosteroids were withheld from the patients at least for 3 months before the studies.

Results

Table I sums up the specific activities of the fractions THF, 6βOHF and 20 DHF isolated from 100 ml urine samples derived from 2 urine collections for 24 hrs after 1—2 ^{3}-H-hydrocortisone administration in 12 subjects with

active rheumatoid arthritis. There were no significant differences in the specific activities of THF and 6βOHF between the two groups. On the other hand, the activity of 20 DHF was one or several orders of magnitude smaller than those of the two other metabolites. Fraction DHF isolated from patients 9 and 11, and from a normal subject, displayed no measurable acitivity. The two last columns of Table I show the daily hydrocortisone secretion calculated

Table II

Data of patients with active rheumatoid arthritis

Serial number	Initials	Sex	Age, years	Duration of disease	ARA crit.	Stage	BSR	Latex	Urinary output ml/24 hrs
1	P. L.	F	17	6 months	5	II.	63	neg.	1400
2	B. K.	M	58	20 years	7	IV.	22	neg.	1020
3	M. I.	F	70	3 years	7	III	135	++++	900
4	J. E.	M	55	7 years	6	III.	29	++++	450
5	B. J.	M	67	21 years	9	IV.	51	++++	700
6	R. J.	M	58	3 years	8	III.	35	++++	1200
7	K. J.	M	59	14 years	7	III.	12	+++	600
8	P. J.	M	55	22 years	7	III.	68	++++	510
9	K. M.	M	60	11 years	9	III.	60	++++	300
10	H. M.	M	64	1.5 years	6	II.	57	neg.	750
11	K. K.	M	72	14 years	8	IV.	25	++++	1075
12	D. I.	M	44	1 years	8	III.	46	++++	590

from the specific activity of THF and of 6βOHF. The figures 16 ± 1.27 and $17.3 + 2.5$ and the subsequent control figures 14.5 ± 1.27 and 13.4 ± 0.4 mg/24 hrs correspond to the average range of 4 to 31 mg/24 hrs referred to in the literature. No difference in daily hydrocortisone output between normal subjects and those suffering from active rheumatoid arthritis was demonstrable, whether calculated from the specific activity of the regular metabolite THF or from that of 6βOHF excreted in an unconjugated state.

Age, sex of patients, urinary output and relevant clinical data are shown in Table II.

Table III shows the 24 hr excretion of THF, 6βOHF and 20 DHF on the day of 1—2 ^{3}H-hydrocortisone administration in the 12 patients with active rheumatoid arthritis. THF and 6-OHF excretion found in these patients was consistent with data in the literature. The 24 hr values of 20-DHF ranged from 52 to 216 μg in normal subjects and from 240 to 816 μg in those with rheumatoid arthritis. BAILEY et al. [5] found 204 μg/24 hrs in normal individuals

and 960 μg/24 hrs in active rheumatoid arthritis. Apart from some major individual variations, the values yielded by our method agreed well with these figures.

Table III

Urinary excretion in μg/24 hrs of conjugated THF, unconjugated 6β OHF, and unconjugated 20β DHF, in patients with active rheumatoid arthritis (1—12) and in normal subjects (I.—V)

Serial No.	THF, μg	6 β OHF, μg	20 DHF, μg
1	1624	499	560
2	2001	341	816
3	963	190	288
4	620	720	720
5	1190	1260	280
6	1620	972	390
7	1350	540	696
8	1147	204	590
9	1260	—	296
10	662	180	240
11	663	256	279
12	690	299	382
I	832	650	60
II	748	250	149
III	920	820	216
IV	1092	730	78
V	672	410	52

Normal range, THF: 220 to 1200 (COPE 1958) 6β OHF: 180 to 760 (FRANTZ 1961)

Discussion

According to the present findings in accordance with those of BAILEY et al. [5] and with our earlier results, urinary 20 DHF excretion is greatly increased in the active stage of rheumatoid arthritis. Since this was the first and, for the time being, the only parameter of steroid metabolism which revealed distinctive differences between normal individuals and those being in the active stage of rheumatoid arthritis, it seemed interesting to study its production more closely. In our earlier studies fractions of greater polarity than THF were separated from urine collected after 1—2 ^{3}H-hydrocortisone

administration. After the last step of separation the activity was estimated on 1 cm strips of the chromatogram. In this way activity peaks at the site of 20-DHF were found in several instances [6]. The aim of the present study was to reproduce these results in a larger patient material and to gain further information by a close identification of these polar fractions and by reliable measurements of specific activity.

20-DHF has been isolated from the urine of 12 patients with active rheumatoid arthritis and of 5 normal subjects, after the intravenous administration of 1—2 ^{3}H-hydrocortisone; the compound was then identified in its unconjugated, acetylated and oxidized forms in various chromotographic systems using as reference standards 20 DHF of our production and that of the Steroid Reference Collection.

As Table III shows, the estimated amounts of THF, 6 β OHF and 20 DHF, including their individual deviations, agreed with the values reported in the literature. On closer analysis of the data it seemed unlikely that 20 DHF should be a direct regular metabolite of hydrocortisone. Daily hydrocortisone secretion calculated from the specific activity of THF, a regular hydrocortisone metabolite corresponded in all instances to the values reported by other authors. Calculation of hydrocortisone secretion on the basis of the specific activity of 6 β OHF also yielded figures for hydrocortisone secretion which, though not quite identical with those quoted above, were still acceptable as being within the limits of reality. Owing to methodological difficulties we had to refrain from the estimation of complete activity passing into the urine from the administered ^{3}H-hydrocortisone. Incidentally, the corrected hydrocortisone values derived from such calculations would not have significantly affected the evaluation. On the other hand, the specific activity of 20-DHF was one or two orders of magnitude less. Daily hydrocortisone secretion as calculated on this basis would amount to ten- or hundredfold of those calculated from the regular metabolites. It makes, therefore, a decisive difference from which urinary metabolite the daily hydrocortisone secretion rate is calculated, as it has been pointed out by numerous authors [12, 13].

These considerations give support to our view that the normal metabolic process of hydrocortisone accounts only for a small fraction of the excessive amounts of 20-DHF excreted in active rheumatoid arthritis. Accumulation of the metabolite is probably due to a shift in time of the individual steps of breakdown, or to abnormal tissue transformation. Further studies are needed for clarifying, for which reason 20-DHF is excreted in an unconjugated form in rheumatoid arthritis and for throwing light on the factors inhibiting the further steps of its reduction in that disease.

*

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Mrs Dr. Veronika Kemény
Prof. Dr. Károly Farkas
Dr. Béla Gömör

Országos Reuma és Fürdőügyi Intézet
Kutató Laboratóriuma
Budapest II., Frankel Leo u. 17/19, Hungary

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PAPIERCHROMATOGRAPHISCHER NACHWEIS DER CORTICOSTEROIDFRAKTIONEN IN DER MENSCHLICHEN LEBER BEI VERSCHIEDENEN TODESURSACHEN

Von

I. GY. FAZEKAS und A. T. FAZEKAS

INSTITUT FÜR GERICHTLICHE MEDIZIN (DIREKTOR: PROF. DR. I. GY. FAZEKAS)
DER MEDIZINISCHEN UNIVERSITÄT SZEGED, UNGARN

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Aus dem Lebergewebe von an verschiedenen Ursachen gestorbenen Personen (18 Männer und 10 Frauen) wurde 1½—10 Stunden nach Eintritt des Todes ein Extrakt bereitet und im Bush 5-System papierchromatographisch auf Corticosteroide untersucht. Von der Todesursache abhängend wurden in der Leber 2—8 Corticosteroidfraktionen in verschiedenen Mengen nachgewiesen. Auffallend viele Corticosteroide wurden in der Leber bei Carcinosis peritonei festgestellt. Nach der Größenordnung folgten danach die an Pneumonie und dann die in alkoholfreiem Zustand wegen Herzlähmung plötzlich Verstorbenen. Sehr niedrige Corticosteroidwerte wurden bei unter Alkoholwirkung eingetretenen Todesursachen gefunden im Gegensatz zu den gleichen oder ähnlichen Todesursachen im alkoholfreien Zustand.

In früheren Mitteilungen wurde festgestellt, daß mit dem Leberextrakt von Kaninchen mit Nebennierenhypertrophie adrenalektomierte Mäuse am Leben erhalten werden können [5, 6] und ferner nach Einspritzung eines aus der Leber normaler (intakter) Kaninchen gewonnenen Extraktes der Leber- und Muskelglykogengehalt sowie der Blutzuckergehalt adrenalektomierter Ratten zunimmt [7, 8], d. h. dieser Extrakt auf den Kohlenhydratstoffwechsel eine der der Nebennierenrindeglukocorticoiden analoge Wirkung entfaltet. In anderen Untersuchungen wiesen wir nach, daß der Leberextrakt normaler, intakter Kaninchen den Na-Gehalt des Blutserums gesunder Kaninchen erhöhte und dessen K-Gehalt verringerte, d. h. den Na- und K-Stoffwechsel auf die gleiche Weise beeinflußte, wie die Mineralocorticosteroiden der Nebennierenrinde [9]. Papierchromatographisch wurden in dem Leberextrakt von Ratten [10, 11], Meerschweinchen [12, 13] und Kaninchen [14, 15] verschiedene Corticosteroidfraktionen gefunden. Es schien wünschenswert zu klären, ob die menschliche Leber endogene Corticosteroide enthält und falls ja, welche Fraktionen und in welcher Menge. Die vorliegende Mitteilung berichtet über Untersuchungen, in denen wir feststellen konnten, daß die Leber von an verschiedenen Ursachen gestorbenen Menschen tatsächlich endogene Corticosteroide enthält.

Untersuchungsmaterial und Methodik

Die Corticosteroidfraktionen des aus dem Leberextrakt von 28 (18 männlichen und 10 weiblichen) im Alter von 21—93 Jahren an verschiedenen Ursachen gestorbenen Personen wurden papierchromatographisch bestimmt. Die Aufarbeitung der Leber erfolgte 1½ bis

10 Stunden nach Eintritt des Todes, indem in den einzelnen Fällen 81—254 g Lebergewebe sofort nach der Herausnahme für 2 Stunden in den Kühlschrank bei +2 °C gehalten, dann herausgenommen, homogenisiert, extrahiert und die erhaltenen Extrakte chromatographiert wurden.

Das Untersuchungsmaterial wurde aufgrund der Todesursachen in sechs Gruppen geteilt, wie in Tabelle I ersichtlich. Bei den über 40 Jahre alten Personen waren anläßlich der Sektion neben Herzmuskelhypertrophie und Herzmuskelvernarbung, Koronarsklerose sowie allgemeiner Arteriosklerose, stauungsbedingte Milz-, Leber- und Nierenveränderungen feststellbar.

Aus dem menschlichen Lebergewebe wurden mittels Kombination des von ENDRŐCZI [4] für Nebennierengewebe ausgearbeiteten Aceton-Wasser, und des von BUSH und FERGUSON [1, 2] für Blut ausgearbeiteten Äther-Äthylacetat-Verfahrens in der von uns vorgenommenen Modifikation Extrakte hergestellt.

Die mit Benzin zweimal gereinigten Extrakte wurden im Bush 5-System chromatographiert. (Hinsichtlich Extraktion, Chromatographie sowie Auswertung s. [10—15].)

Parallel mit den Leberextrakten wurden die folgenden Standard-Corticosteroide chromatographiert: 0,5—1,0—1,5—2,0—2,5—3,0—4,0—5,0 bzw. 10.0 μg/l) Tetrahydrocortisol, 2) Tetrahydrocortison, 3) Cortisol (= Hydrocortison), 4) Cortison, 5) Corticosteron und 6) 11-Dehydrocorticosteron.

Die Chromatogramme wurden zunächst nativ im UV-Licht bei Verwendung eines Ug 5-Filters untersucht und dann nach BUSH und MAHESH [3] in einem Gemisch von 2 n NaOH und 0,1%igem Tetrazoliumblau 9 : 1 entwickelt. Die erscheinenden lilafarbenen Formazan-Flecke wurden auf ihre R_f-Werte geprüft und nach Vergleich ihrer Größe und Intensität mit denen der entsprechenden Standardproben sowie der Größe und Intensität der gleichen Standard-Flecke mit den aus den Extrakten erhaltenen, gelbe NaOH-Fluoreszenz gebenden Flecken deren μg-Werte bestimmt. Als Resultat wurden die Mittelwerte der beiden Daten betrachtet und — auf 100 g feuchtes Gewebe berechnet — in μg% ausgedrückt. Diejenigen Steroide, deren entsprechende Standard-Verbindungen nicht gestartet werden konnten, sind als Verbindungen X_1, X_2, X_3 und X_4 bezeichnet und durch ihren R_f-Wert, sowie ihre Tetrazoliumblau-Reaktion und NaOH-Fluoreszenz charakterisiert.

Ergebnisse

Die Qualität und Quantität der im menschlichen Lebergewebe nachgewiesenen Corticosteroidfraktionen sowie ihre Gesamtsteroidwerte gehen in der nach Todesursachen aufgestellten Gruppierung aus Tabelle I hervor.

In den Leberextrakten konnten — in Abhängigkeit von der Todesursache — gewöhnliche 2—8 Corticosteroidfraktionen nachgewiesen werden.

In den Lebern der 4 an Pneumonie gestorbenen Personen wurden 4—7 Corticosteroidfraktionen gefunden (R_f: 0,03; 0,13; 0,15; 0,18; 0,30; 0,47; 0,81; 0,89), und zwar in Mengen von 3,4—16,4 μg%, was einem Gesamtsteroid-Mittelwert von 7,2 μg% entspricht. In allen vier Fällen waren in der Leber Tetrahydrocortisol, Tetrahydrocortison und Cortisol nachweisbar, in zwei Fällen auch Verbindung X_1 und Cortison und in einem Falle 11-Dehydrocorticosteron. Verbindungen X_3 und X_4 kamen in der Leber der an Pneumonie Verstorbenen nicht vor.

In den Lebern von 6 an Herzlähmung Gestorbenen waren 1—6 Corticosteroidfraktionen (R_f: 0,03; 0,13; 0,15; 0,18; 0,30, 0,81) in Mengen von 0,4—6,4 μg%, d. h. ein Gesamtsteroidgehalt von 2,2 μg% nachweisbar. Verbindung X_1 kam in 2, Tetrahydrocortisol in 1, Verbindung X_2 in 3, Tetrahydrocortison in einem, Cortisol in 5 und Corticosteron in 4 Fällen vor. Verbindung

X_3 und X_4 sowie 11-Dehydrocorticosteron waren in den alkoholfreien Herzlähmungs-Fällen nicht nachweisbar.

Die Lebergewebe der vier unter Alkoholeinwirkung an Herztod Gestorbenen enthielt 1—3 Corticosteroidfraktionen (R_f: 0,03; 0,13; 0,30; 0,47; 0,89) in Mengen von 0,6—1,6 μg%, also durchschnittlich 1,2 μg% Gesamtsteroid, was nur die Hälfte der in den alkoholfreien Herzlähmungs-Fällen erhaltenen Werte erreicht. In 2 von den 4 Fällen enthielt die Leber Verbindung X_1, in einem Tetrahydrocortisol, in zweien Cortisol, in zweien Cortison und in einem nur 11-Dehydrocorticosteron, während Verbindung X_2, X_3 und X_4 sowie Tetrahydrocortison und Corticosteron im Lebergewebe dieser vier Fälle fehlten.

In den Lebern der beiden im Alkoholrausch einem Unfall zum Opfer gefallenen Männer war nur je eine Corticosteroidfraktion nachweisbar (R_f: 0,30 bzw. 0,81), der Gesamtsteroidwert betrug 0,6 bzw. 0,5 μg% (Mittelwert: 0,55 μg%), was rund ¼ dessen ist, was wir im Falle der in alkoholisiertem Zustand an Herzlähmung Verstorbenen fanden.

Die Leber eines Erhängten wies 4 Corticosteroidfraktionen (R_f: 0,30; 0,47; 0,81 und 0,89), also ein Gesamtsteroidwert von 1,1 μg% auf. In der Leber einer vom Blitz erschlagenen Frau waren zwei Corticosteroidfraktionen (R_f: 0,30; 0,81) im Gesamtwert von 1,3 μg% nachweisbar und in einer im alkoholisiertem Zustand wegen Apoplexia cerebri gestorbenen Frau nur eine Fraktion (R_f: 0,30), insgesamt 0,9 μg%. Die Leber einer an Carcinosis peritonei gestorbenen alten Frau enthielt 6 Corticosteroidfraktionen (R_f: 0,03; 0,13; 0,18; 0,30; 0,47; 0,65) mit einem Gesamtsteroidwert von 25,2 μg%. Unter allen untersuchten Fällen war dies der höchste Corticosteroidgehalt in der Leber.

Im Falle einer wegen Isonicid-Vergiftung zustandegekommenen Bronchopneumonie konnten in der Leber 6 Corticosteroidfraktionen (R_f: 0,03; 0,13; 0,15; 0,30; 0,47) mit einem Gesamtsteroidwert von 4,5 μg% nachgewiesen werden. Die Lebergewebe zweier wegen Wofatox-Vergiftung an Bronchopneumonie Gestorbenen enthielt je 6 Corticosteroidfraktionen (R_f: 0,03; 0,30; 0,47; 0,65; 0,81; 0,89) mit einem Gesamtsteroidwert von 6,1 bzw. 4,0 μg%. In diesen drei vergiftungsbedingten Bronchopneumonie-Fällen waren im Lebergewebe annähernd gleiche Gesamtsteroide zugegen wie in den Fällen mit bakterieller Pneumonie. Bei der im alkoholisierten Zustand eingetretenen Cyan-Vergiftung enthielt die Leber 2 Fraktionen (R_f; 0,30 und 0,81) im Werte von 1,8 μg% Gesamtsteroid. Zwei Fraktionen (R_f: 0,30 und 0,47) wurden auch bei im Alkoholrausch erfolgten Barbiturat-Vergiftung gefunden, der Gesamtsteroidwert betrug 0,5 μg%. Bei CO-Vergiftung wies die Leber 3 Fraktionen (R_f: 0,03; 0,47 und 0,81) mit 0,2 μg% Gesamtsteroid auf. Die Leber eines an Alkoholvergiftung + Erfrieren gestorbenen Mannes enthielt vier Corticosteroidfraktionen (R_f: 0,30; 0,47; 0,81 und 0,89) mit einem Gesamtcorticosteroidwert von 0,7 μg%.

Tabelle I

Corticosteroid-Fraktionen der Leber

Nativ blaue Fluoreszenz Tetrazolium-blaue Reaktion NaOH-Fluoreszenz Rf-Werte								
Gruppe	Lebensalter	Geschlecht	Todesursache	Zeit zwischen Tod und Untersuchung Stunden	Körpergewicht kg	Gewicht der beiden Nebennieren g	Gewicht des untersuchten Organs g	Blutalkohol ‰
I/a	63	♂	Influenza mit Bronchopneumonie	4½	52	10	156	∅
I/b	55	♀	Schenkelbeinbruch Bronchopneumonie	6	37	11,5	156	∅
I/c	72	♀	Schenkelbeinbruch Bronchopneumonie	8	78	15,5	161	∅
I/d	78	♀	Lobare Pneumonie	6	47	13,0	81	∅
II/a	21	♂	Herzlähmung, Aortaklappeninsuffizienz	2½	61	6,5	131	∅
II/b	60	♂	Herzlähmung	3	64	10,9	145	∅
II/c	68	♀	Herzlähmung	5½	65	9,1	121	∅
II/d	51	♂	Herzlähmung	1½	78	12,0	254	∅
II/e	63	♂	Herzlähmung	4½	52	10,0	92	∅
II/f	41	♂	Herzlähmung	4	73	9,7	120	∅
III/a	52	♂	Herzlähmung	7	69	8,8	100	2,0
III/b	40	♂	Herzlähmung	9	77	11,5	137	1,18
III/c	57	♂	Herzlähmung	10	53	9,5	130	0,75
III/d	67	♂	Herzlähmung	4	56	12,2	96	0,60
IV/a	52	♂	Verkehrsunfall, Aortaruptur	2	63	9,2	110	0,85
IV/b	69	♂	Verkehrsunfall, Med. oblong.-ruptur	2	56	8,2	193	0,62
V/a	45	♂	Selbsterhängung, Ersticken	5	73	12,5	158	∅
V/b	40	♂♀	Blitzschlag	3	81	9,3		∅
V/c	59	♀	Apoplexia cerebri	5	57	8,0	121	1,0
V/d	83	♀	Magencarcinom, Carcinosis peritonei	10	28	9,8	130	∅
VI/a	40	♂	Isoniacid-Vergiftung Bronchopneumonie	1½	48	10,6	123	∅
VI/b	45	♀	Cyan-Vergiftung	3	62	16,5	110	0,28
VI/c	41	♂	Barbiturat-Vergiftung	5	64	10,5	141	0,36 0,48
VI/d	92	♀	Co-Vergiftung	5	34	8,0	108	0,49
VI/e	41	♂	Wofatox-Vergiftung Bronchopneumonie	6	44	10,5	126	∅
VI/f	44	♂	Wofatox-Vergiftung Bronchopneumonie	3	60	8,1	103	∅
VI/g	26	♂	Alkohol-Vergiftung, Erfrierung	5	73	7,3	226	2,28 3,72
VI/h	48	♀	Co-Vergiftung	5	71	8,2	100	∅

bei verschiedenen Todesursachen

+	—	—	—	—	—	—	—	—	—	
+	+	—	+	+	+	—	—	+	+	
+	—	+	—	+	+	+	+	+	+	
0.03	0.13	0.15	0.18	0.30	0.47	0.52	0.65	0.81	0.89	

Name u. Menge μg% der Steroid-Fraktionen

X_1	Tetrahydrocortisol	X_2	Tetrahydrocortison	Cortisol	Cortison	X_3	X_4	Corticosteron	11-dehydrocorticosteron	Gesamtsteroide μg%
—	0,2	1,0	0,2	1,0	1,2	—	—	—	—	3,6
0,6	0,4	—	0,6	0,8	Ny	—	—	1,0	—	3,4
2,0	0,6	—	0,7	0,5	0,5	—	—	1,0	0,2	5,5
7,5	2,5	—	2,5	3,9	—	—	—	—	—	16,4
								Mittelwert		7,2
—	—	1,0	—	—	—	—	—	—	—	1,0
—	—	—	—	0,4	—	—	—	—	—	0,4
—	—	—	—	0,8	—	—	—	0,3	—	1,1
0,7	—	0,5	—	1,0	—	—	—	Ny	—	2,2
0,5	0,6	2,1	0,5	2,0	—	—	—	0,6	—	6,3
—	—	—	—	2,0	—	—	—	0,5	—	2,5
								Mittelwert		2,2
0,5	—	—	—	—	0,5	—	—	—	—	1,0
—	—	—	—	—	—	—	—	—	0,6	0,6
—	—	—	—	0,4	1,2	—	—	—	—	1,6
Ny	0,8	—	—	0,7	—	—	—	—	—	1,5
								Mittelwert		1,2
—	—	—	—	0,6	—	—	—	—	—	0,6
—	—	—	—	—	—	—	—	0,5	—	0,5
								Mittelwert		0,5
—	—	—	—	0,6	Ny	—	—	0,2	0,3	1,1
—	—	—	—	1,0	Ny	—	—	0,3	—	1,3
—	—	—	—	0,9	—	—	—	—	—	0,9
2,9	8,0	—	8,0	3,1	0,7	—	2,5	—	—	25,2
0,9	0,4	0,4	—	1,0	1,8	—	—	—	—	4,5
—	—	—	—	0,8	—	—	—	1,0	—	1,8
—	—	—	—	0,3	0,2	—	—	—	—	0,5
Ny	—	—	—	—	1,0	—	—	1,0	—	2,0
1,8	—	—	—	2,5	0,8	—	—	0,4	0,6	6,1
0,5	—	—	—	1,0	2,0	—	0,5	0,5	—	4,0
—	—	—	—	0,2	0,3	—	—	0,2	Ny	0,7
—	—	—	—	2,0	0,8	—	—	—	—	2,8

Besprechung

Nach unseren Untersuchungen wurden die höchsten Corticosteroidwerte in der Leber (und in den Organen) einer an Carcinosis peritonei gestorbenen Frau gefunden. Danach folgte nach Größenordnung der Corticosteroidgehalt der Leber der an Pneumonie Verstorbenen und dann der der in alkoholfreiem Zustand an Herzlähmung gestorbenen Personen und schließlich der unter Alkoholeinwirkung an Herzlähmung Gestorbenen. Bei unter Alkoholeinwirkung entstandenen verschiedenen Vergiftungen war der Gesamtcorticosteroidgehalt der Leber (und der Organe) niedriger, als bei derselben Vergiftung ohne alkoholischen Zustand. Dies beweist, daß der alkoholische Zustand den Corticosteroidgehalt der Leber verringert. Dies steht im Einklang mit unseren tierexperimentellen Befunden und auch mit den bei anderen menschlichen Organen gemachten Feststellungen. Es sei erwähnt, daß bei verschiedenen Todesursachen im Vergleich mit den anderen Organen immer die Leber die wenigsten Corticosteroiden enthielt, wie es auch aus Tabelle II hervorgeht. Dies ist verständlich, da nach den Untersuchungen anderer Forscher die Leber die wichtigste Abbaustelle der Corticosteroiden darstellt und so dort eine kleingradige Speicherung zu erwarten ist. Im Gegensatz dazu wurde bei Carcinosis peritonei in der Leber und den anderen Organen eine Erhöhung der Zahl und Menge der Corticosteroidfraktionen hervorgerufen. Es scheint also, daß der Alkohol den Corticosteroidabbau in der Leber (und in anderen Organen) steigert, bei Carcinosis peritonei dagegen der Steroidabbau in der Leber und in den anderen Organen verringert wird. So dürfte bei Carcinom eine gesteigerte Nebennierenfunktion entstehen und dies den höheren Corticosteroidgehalt der Leber und anderer Organe erklären. Die Klärung dieser Frage bleibt weiteren Untersuchungen vorbehalten.

Tabelle II

Gesamtcorticosteroidgehalt der Organe

Todesursache	Milz	Lungen	Muskel	Nieren	Herz	Leber
Pneumonie	17,2	16,4	14,0	12,7	11,25	7.,2
Herzlähmung	8,1	5,6	5,7	6,6	7,50	2,2
Herzlähmung + Alkohol	1,9	2,2	1,2	4,3	4,00	1,2

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Dr. I. Gyula FAZEKAS, Szegedi Orvostudományi Egyetem, Igazságügyi Orvostani Intézet, Ungarn
Dr. T. Attila FAZEKAS, Szeged, Kossuth Lajos sgt. 40, Ungarn

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IMMUNOSUPPRESSIVE THERAPY OF MYASTHENIA GRAVIS

By

A. SZOBOR and GY. PETRÁNYI

SECTION OF NEUROLOGY, RÓBERT KÁROLY HOSPITAL, BUDAPEST
AND FIRST DEPARTMENT OF MEDICINE, UNIVERSITY MEDICAL SCHOOL, DEBRECEN

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After a brief survey of the autoimmune aspects of myasthenia gravis, the results of immunosuppressive (cytostatic) therapy obtained in 25 cases are reviewed. The drug used was the cytostatic agent mesoerythrite which exerts its effect primarily on the lymphoid system. The results are tabulated with reference to age of the patients, duration and clinical type of the disease, and to the character, degree and duration of response. In 20 cases immunoserological studies were performed the results of which were correlated to the clinical pattern and responsiveness of the individual cases. The theoretical basis and practical aspects of immunosuppressive therapy of myasthenia are discussed. It is first of all in the cases of major severity, particularly those which have failed to respond to other therapeutic measures, including drugs, where immunosuppressive treatment enters into consideration. As regards the type of drug best suited for this purpose, it is felt that non-toxic agents with a potent selective inhibitory action on the lymphoid system and at the same time of protective properties, but of no or little anti-inflammatory effect, deserve priority.

It was the theory put forward by SIMPSON [39] which had initiated investigations into the autoimmune aspects of myasthenia gravis [3, 18, 21, 41, 47]. On the evidence of these studies, 30 to 50% of the affected subjects displayed serological reactions consistent with an autoimmune mechanism. These experimental results have confirmed earlier clinocopathological observations which were clearly pointing to autoimmune factors being involved in the genesis of myasthenia. There is first of all the close, though in certain respects still problematic, pathogenetic part played by one of the essential organs of immunocompetence, the thymus. There is, furthermore, the striking prevalence of associations between myasthenia and various diseases of confirmed or probable immune origin (hyperthyroidism, chronic progressive polyarthritis, polymyositis, pernicious anaemia, Sjörgen's disease, haemolytic anaemia, systemic lupus erythematosus, pemphigus erythematosus, etc.). It is generally known that the thymus hardly ever contains germinal centres [14, 32], whereas in myasthenia these structures first described by SLOAN [40] have been demonstrated by various authors [6, 16, 33, 49]. The thymus contains germinal centres also in other diseases of confirmed or probable autoimmune origin [11], on the other hand, other lymphoid tissues contain no germinal centres in myasthenia gravis. Moreover, certain clinical features of the disease have long been regarded as indirect evidence of an autoimmune process [15, 47].

Table I

Immunosuppressive therapy.

Patient, number, name, sex	Age	Duration	Myasthenia type	Crisis
1. F. J. ♂	26	2 years	General	
2. C. J. ♀	29	3 years	General	++(my.)
3. S. L. ♀	37	4 years	General	
4. M. S. ♀	51	7 years	General	
5. P. E. ♀	16	4 years	General	++(my.)
6. L. R. ♀	58	1 year	General	
7. B. J. ♂	49	9 years	General	++(my.+chol.)
8. B. I. ♂	32	3 years	Ocular	
9. S. J. ♀	16	4 years	General	+++(my)
10. S. L. ♀	29	15 years	General	+(my.)
11. N. I. ♀	42	2 years	General	+++(my)
12. K. J. ♀	25	1 year	General	++(my)
13. S. T. ♀	25	8 years	General	+++(my)
14. L. L. ♂	26	1 year	Oculoskeletal	
15. M. J. ♂	46	3 years	Ocular	
16. I. L. ♀	76	1 year	General	
17. N. A. ♀	34	12 years	Oculoskeletal	
18. B. I. ♀	61	1 year	General	++(my)
19. M. F. ♀	71	6 years	Oculoskeletal	
20. M. M. ♀	47	1 year	General	
21. N. M. ♀	46	27 years	General	++(my)
22. K. J. ♀	61	1 year	Oculobulbar	
23. B. A. ♂	70	1 year	Oculobulbar	
24. N. I. ♀	54	52 years	General	
25. S. A. ♀	23	4 years	Skeletal	+(my)

One of the strongest arguments against a possible autoimmune nature of myasthenia gravis [42] is its unresponsiveness to steroids [9, 23, 24, 42, 45, 46, 47]. Failure of steroid therapy in this disease has led to attempts with drugs of other mechanisms, particularly with cytostatic, i.e. indirectly immunosuppressive, agents. Considering the value of immunosuppressors in other muscle diseases, particularly in autoimmune myositis [30], we found it reasonable to test this treatment in patients with myasthenia gravis. The first reports on similar studies [4, 8, 15, 19] coincided with our trials. The promising results obtained in our first 13 cases which had formed the subject of an earlier report [31] justified to continue this therapy. The present report concerns 25 patients with myasthenia gravis having been subjected to immunosuppressive therapy in the course of two years, to which treatment we have referred in an earlier comprehensive study on the disease [45].*

* Meanwhile further 7 cases have been treated with this method of immunosuppressive therapy, so all in all 32 myasthenic patients have been treated and observed.

Patients and data

Earlier therapy	Prognosis	Associated diseases	Immunosuppressive therapy	
			Prognosis	History
Drugs	C		A	2 years
Drugs, X-ray	B	Cured syphilis	A	2 years
Drugs, X-ray	C	Myositis	B	2 years
Drugs, X-ray	D		B	2 years
Drugs, X-ray	Perm. crisis		C	3 months
Drugs	D	Pulmonary carcinoma Myopathy	C	2 months
Drugs, Surgery, X-ray	C ⇄ B	Carcinoma of thymus	B ⇄ C	1 year
Drugs, X-ray	C		B	1 year
Drugs, X-ray	C ⇄ D	Nephritis	B	1 year
Drugs, X-ray	D	Muscle atrophy	C ⇄ B	1 year
Drugs, X-ray	D	Muscle atrophy	C ⇄ D	4 months
Drugs	C ⇄ D		B ⇄ C	3 months
Drugs, Surgery, X-ray	D	Muscle atrophy	D	Stationary
Drugs	B ⇄ C	Myositis	B	8 months
Drugs, X-ray	B		B	8 months
Drugs, X-ray	D	Myopathy	C	6 months
Drugs, X-ray	C ⇄ B		B	8 months
Drugs	C ⇄ D	Cerebral arteriosclerosis	C	3 months
Drugs, X-ray	C	Cerebral arteriosclerosis	B	7 months
Drugs	C		B	5 months
Drugs, Surgery, X-ray	C ⇄ D		B	5 months
Drugs	D	Cerebral obesity	C	5 months
Drugs, X-ray	C		B ⇄ C	2 months
Drugs	C		B	3 months
Drugs, X-ray	C		B	3 months

(Symbols, my. = myasthenic crisis; chol. = cholinergic crisis; + = severity of crisis

Material and methods

Twenty-five patients with myasthenia gravis were subjected to immunosuppressive (cytostatic) therapy. Details concerning the patients are shown in Table I. Age of the patients, duration of disease and the clinical pattern are shown in Tables II, III and IV, together with the assessment of response to immunosuppressive treatment. Table V shows the types of (and responses to) earlier therapy in the cases being under immunosuppressive therapy at the time of the present study. The data presented reflect the condition of the patients before immunosuppressive therapy, and also show that the cases selected for this study were all of major severity having exhibited little response to earlier therapy. In the whole series of 25 patients there were only three in remission *B*, those of stages *C* and *D* numbering 22. The patients were assigned to the respective stages in accordance with the generally accepted standards [45]. Twelve patients had had one or more respiratory crises before, and 3 of them were in a state of permanent crisis at the time of this study. This alone would sufficiently illustrate our lines of selection referred to above.

Sera from 20 patients were subjected to immunoserological studies, from 9 repeatedly, before and after treatment (Table VI). Anti-organ antibodies were studied by passive haemagglutination. The antigen attached to tannin-coated erythrocytes was obtained from fresh human surgical or necropsy material and mainly contained surface-membranes. The rheumatoid factor (RF) was studied by the Rose-test. For the study of the antinuclear factor (ANF), too, the passive haemagglutination method was used, the antigen being derived

from calf thymus cell nuclei. The figures presented in the Table indicate at how many times higher dilutions than those of a pool of mixed healthy donor serum the patient's serum still gave a positive reaction: 0 and 2 was considered definitely negative, 4 still negative, 8 a borderline value (±), 16 or more definitely positive. The serial numbers of the patients are identical in Tables I and II.

The results of treatment are shown in Table VII, on the grounds of *a)* the patients' statements, *b)* clinical observations, *c)* the ergometric test devised by one of the authors [43]. Table VIII also concerns the results in respect to the required doses of cholinergic (anti-cholinesterase) agents, subsequent to immunosuppressive treatment.

The immunosuppressive agent used in all cases was mesoerythrite, developed by VARGHA and HORVÁTH [51, 52], and pharmacologically tested by CSÁNYI et al. [7]. The compound is a sugar alcohol, with an alkyl sulphonyl oxyethylamino group as the functional moiety. Its chemical structure is 1,4-di-2'-mesyloxy-ethylamino-1,4-didesoxy-m-erythrite-dimesylate). In animal experiments [7, 52] it displayed a selective inhibitory activity on lymphopoiesis while being free from any secondary or toxic effect in therapeutic doses. Intravenous LD_{50} was 45 mg/kg in mice. Its clinical benefits primarily concern lymphoid leukaemia and various types of myositis [29, 30]. Its use in myasthenia was justified on the basis of two considerations, i.e. the lymphoid character of the thymus, and its presumable involvement in the pathogenesis of the disease.

The drug was given in a dose of 30 mg intravenously, every day. With the exception of Cases 3 and 4 where 10 injections were given (total dose, 300 mg), all other patients had 20 injections with a total dose of 600 mg, apart from Case 9, a juvenile patient of low body

Table II

Response to immunosuppressive therapy according to age groups

Age group	A	B	C	D	Death
10—16 years		1	1		
20—30 years	2	3	2	1	
30—50 years		5	2		
50—70 years		2	3		
Over 70 years		2	1		
Total 25 patients	2	13	9	1	

Table III

Response to immunosuppressive therapy according to history

History	A	B	C	D	Death
2 years		3	6		
2— 5 „	2	5	1		
5—10 „		2	1	1	
10—20 „		1	1		
20 „		2			
Total 25 patients	2	13	9	1	

Table IV

Response to immunosuppressive therapy according to clinical pattern

Type of myasthenia	A	B	C	D	Death
General	2	6	8	1	
Ocular		2			
Skeletal		1			
Oculo-skeletal		2	1		
Oculo-bulbar		2			
Total 25 patients	2	13	9	1	

Table V

Results of earlier (non-immunosuppressive) therapy

Earlier treatment	A	B	C	D	Total
Drugs			7	1	8
Thymectomy				1	1
Irradiation of thymus		2	7	4	13
Thymectomy + irradiation		1	1	1	3
Total		3	15	7	25

weight and with chronic nephritis who had 25 mg daily (total dose 500 mg). Quantitative blood counts were made every 2nd to 3rd day, and differential counts, weekly. The counts were then checked one, two, three and six months after completion of treatment. Body weight, urinary output, liver function and appetite were also recorded. One of the patients (Case 13) had two courses.

Results

In view of the immunosuppressive nature of the therapy, the immunoserological findings and their therapeutic relevance will have to be considered first. The findings showed a considerable scatter, in accordance with our earlier observations [47]. Where the reaction with skeletal muscle antigen (Musc.) was positive (more than 8 in Cases 2, 5, 8, 23 and 25, Table VI), it was invariably positive with other homologous (allogenic) antigens, and often even at higher titres than with muscle antigen; for instance in Cases 2, 5, 8, 23 and 25 with thyroid (Thyr.), in Case 8 with kidney (Kidney), liver (Liver), parietal cells of the stomach (Gastr.) and calf thymus cell nuclei (ANF). In cases 23 and 25, circulating antibodies to kidney tissue were demonstrable and in Case 23 the rheumatoid factor (RF) was also markedly positive.

It is a point of some interest that some diseases are often associated with myasthenia gravis. Coincidence of myasthenia with hyperthyroidism in 5 to 8% of the cases is well-known from the literature [13, 23, 35, 37, 48]. Of our patients with demonstrable antithyroid antibodies (Cases 2, 4, 5, 8, 11, 13, 14, 23, 24, 25) two (Cases 2 and 5) displayed clinical signs of mild hyperthyroidism, two (Cases 14 and 25) were hypothyroid and one (Case 13) had an inactive goitre. Among the patients with circulating antibodies against kidney-antigen (Cases 8, 9, 11, 13, 14, 23, 24 and 25) there were only two (Cases 9 and 24) with clinically demonstrable renal lesion. The first, a 16-year old female has been suffering from chronic nephritis with proteinuria since the age of 8 years, while the second had an incipient nephrosclerosis. In Case 8, showing the highest titre, there was no evidence of renal abnormality. A patient with values bordering on negativity (Case 10) had recurrent renal stones. Of the cases with a seropositive reaction to liver-antigen (Cases 8, 9, 13, 14, 15) two patients (Cases 8 and 14) had been drinking regularly but were no actual addicts. The others had no evidence of liver disease. Antibodies against parietal cells of gastric mucosa or to duodenum were demonstrable in Cases 6, 7, 8, 9, 10, 11, 13, 14, 15. The findings in Cases 8 and 14 might have been compatible with alcoholic gastritis, in the other cases there was no evidence of any abnormality of the digestive system. The rheumatoid factor was strongly positive in Cases 9, 11 and 23. None of these patients had progressive chronic polyarthritis but Case 23 (a 70-year old male) displayed osteochondrosis and rheumatism affecting the small joints. In Case 3 with histologically verified myositis the RF was negative. The LE-cell phenomenon was negative in all of the cases. In the present series there were no patients with local or systemic LE, or with pernicious anaemia, vitamin-B_{12} malabsorption, Sjörgen's disease.

Assessment of the therapeutic effect revealed that the individual responsiveness to immunosuppressive therapy shows no, or at least no definite, relationship to age (Table II), duration (Table III) or the clinical type of the disease (Table IV). These results are consistent with our earlier observations [31, 47] as well as with other published evidence [1, 2, 15, 17].

Evaluation of the results in accordance with the generally adopted standards [46] showed that two patients attained the stage of remission *A*, and 13, that of remission *B*, in other words there was a distinct unmistakable improvement in the condition of 15 patients. Although evaluation was made difficult by the spontaneous fluctuations and the tendency to spontaneous remissions inherent in the clinical character of myasthenia gravis, it was none the less possible on a comparative basis, with due consideration of the patient's state at the start of treatment (Table V). Actually, none of the patients had been in remission *A* at the start (which would have made treatment, obviously, unnecessary), and there had been only three in remission *B*. The fact that, as

Table VI

Results of immunoserological studies

Patient, number, name, sex	Immunoserological test								
	Musc.	Thyr.	Kidney	Liver	Gastr.	Duod.	RF	ANF	LE
2. C. J. ♀	5120	80					2		neg.
	128	16					2		,,
3. S. L. ♀	4	2					4		,,
4. M. S. ♀	2	80					2		,,
5. P. É. ♀	320	40					2		,,
	320	20					2		,,
6. L. R. ♀	2	2	4	2	4	4	4	4	,,
	8	2	8	2	16	2	4	8	,,
7. B. J. ♂	4	8	4	2	4	4	2	2	,,
	4	4	8	2	8	16	4	8	,,
8. B. I. ♂	32	16	128	16	16	8	8	16	,,
	16	16	8	4	8	16	8	16	,,
9. S. I. ♀	8	8	16	16	8	16	1024	8	,,
10. S. L. ♀	2	4	8	4	4	16	512	2	,,
11. N. I. ♀	4	16	16	8	8	32	1024	16	,,
12. K. J. ♀	4	8	4	4	2	8	8	4	,,
13. S. T. ♀	2	2	2	2	2	8	4	2	,,
	2	16	16	32	64	64	4	8	,,
14. L. L. ♂	2	32	16	64	16	128		8	,,
15. M. J. ♂	2	4	8	16	8	16		8	,,
16. I. L. ♀	8	8	8	2	4	8	4	8	,,
17. N. A. ♀	2	4	4	8	4	4		2	,,
19. M. F. ♀	2	4	8	2	2	4		2	,,
23. B. A. ♂	16	16	16	4	4	2	1024	8	,,
	4	8	8	2	2	2	4	8	,,
24. N. I. ♀	8	16	16	8	4	4	8	2	,,
	8	16	16	4	4	4	4	16	,,
25. S. A. ♀	16	16	16	4	4	2	4	4	,,
	4	8	8	4	4	2	4	8	,,

pointed out before, all cases were of major severity and more or less resistant to earlier therapy added to the reliability of the evaluation.

Further information emerges from Tables VII and VIII, where the results of treatment are presented under other aspects. One of the patients (Case 1), a young engineer, continues now for the third year in his usual field of activity in apparent good health without the need for drug or any other

therapy. A female patient (Case 2) also remained symptom-free for two years on a drug-free regimen but had to be started subsequently on pyrodistigmine in doses of 120 to 180 mg daily (actually one third of the former dose) and is doing well. Both cases would obviously raise the possibility of spontaneous remission. This, however, can safely be dismissed, considering the long duration of the process and its lack of adequate responsiveness to other types of treatment, which makes a conspicuous contrast to the rapid and sustained change for the better soon after the start of immunosuppressive therapy. This female patient had had two respiratory crises, one of which required assisted breathing for 8 days [46].

In all the other cases anticholinesterase therapy had to be maintained even after completion of immunosuppressive treatment, the dose requirement being a good indicator of the degree of remission [15, 23, 46]. As seen in Table

Table VII

Results of immunosuppressive treatment in the light of the patients' observation and of ergometric evidence

Result	Female	Male	Total
I. Remission A (cure)	1	1	2
II. Remission B (marked improvement)	8	5	13
III. Remission C (moderate or slight improvement)	9		9
IV. Status D (unchanged)	1		1
V. Deterioration (death, crisis)			
Total	19	6	25

Table VIII

Evaluation of immunosuppressive therapy in the light of subsequent cholinergic drug consumption

Results	Females	Males	Total
I. No need for cholinergic medication	1	1	2
II. Reduction of the former dosage by 50 to 75%	3	2	5
III. Reduction by 25 to 50%	6	1	7
IV. Unchanged dosage	8	2	10
V. Dosage higher than before	1		1
Total	19	6	25

VIII, a highly significant reduction (by 50 to 75%) of the daily doses was possible in 5 cases and a substantial reduction (by 25 to 50%) in 7 cases. Though allowance has to be made to psychic factors, i.e. hope or confidence in every new treatment [22, 44], misinterpretations arising from the source are readily avoided by keeping the required doses on record.

Slight amelioration with unchanged anticholinesterase requirement was noted in 7 cases. Deterioration which might have been connected with immunosuppressive (cytostatic) therapy was not observed in any of the cases. In a female patient (Case 13) the anticholinesterase doses had to be raised in the course, and after completion, of treatment.

Three female patients (Cases 5, 11 and 12) deserve special mention. Two of them had been on continuous, one on nearly continuous, artificial breathing. All three were candidates for thymectomy and immunosuppressive therapy was responsible for the improvement during which the intervention could actually be performed. In one of them (Case 11) surgery revealed a tumour of the thymus, with microscopic evidence of malignant degeneration.

Assessment of the duration of the beneficial effect presents similar difficulties. It was obvious in two patients in remission *A* but far less simple in all the others, considering the continuously changing character of myasthenia gravis and the modification in its course which may result from a variety of other diseases or factors. At the time of the present study there have been remissions of 2 years, one year and 8 months, respectively (Table I). Remissions in the last three cases have not yet reached the limit of evaluation. In the three later thymectomized patients referred to earlier there have been remissions of three to four months, allowing preoperative measures, actual surgery and postoperative care. In an elderly patient with continuous threat of crisis (Case 18) a transient improvement of minor degree was noted. A female patient (Case 5) with advanced myopathic myasthenia associated with pulmonary carcinoma showed striking improvement of short duration extending also to her general condition which had greatly been affected by the tumour. Response was slighter but far longer in duration (6 months) in a 76-year-old female (Case 16), where severe myasthenia gravis, though safe from crises was associated with paraproteinic myopathy. In a patient (Case 3) now in full remission for two years, muscle pains due to myositis have also ceased.

The signs of remission were noted in a few cases in the last week of treatment and in the majority in the first 2 to 6 weeks after its completion. One of the patients (Case 5) had a remission 4 months after treatment.

The results of serological tests performed in 9 cases and checked repeatedly in the course of treatment allowed to confront the clinical and serological response. Improvement of the serological titre was noted in 4 cases (2, 8, 23, 25). In one of these, the clinical response was excellent and of long duration (Case 2), in another it was fairly good (Case 25) and in two (Cases 8 and 23),

it was slight. The serological titre was unaffected in 4 cases (5, 6, 7 and 24); in one of these (Case 6), the patient with pulmonary carcinoma referred to earlier, response was striking though of shor duration; in the second one (Case 5) it was likewise short but none the less compatible with thymectomy, and moderate but of long duration in the male patient with carcinoma of the thymus (Case 7). Improvement in another patient (Case 24), though marked, was still too short for evaluation. In one of major severity, a young female patient (Case 13), impairment of the serological titre was noted without any apparent clinical benefit and the maintenance dose had to be increased transiently.

Secondary effects: There was a general steady decline in the leukocyte count without, however, imposing the necessity for discontinuing treatment in any of the cases. In a patient (Case 5) who had radiotherapy six months before immunosuppressive treatment, the leukocyte count persisted at the level of 3000/ml for three months but returned subsequently to normal without interfering with the planned thymectomy. A further patient (Case 7) who had had repeated courses of massive-dose radiotherapy for thymus carcinoma in the course of the last nine years, developed transient leukopenia and thrombopenia on completion of immunosuppressive (cytostatic) treatment. Impairment in liver functions or abnormal urinary findings were not noted. With the exception of the patient with pulmonary carcinoma (Case 6), body weight remained unaffected. Two patients were complaining of anorexia, one of nausea at the time of the first injections. Paravenous injection resulted in local necrosis with consecutive scarring in one case and in a sluggishly resolving cubital induration in another.

Discussion

The aetiology of myasthenia gravis is uncertain but seems to involve the thymus closely, as indicated by the prevalence of its association with hyperplasia or tumours of this organ and of its responsiveness to radiotherapy of the thymus, or to thymectomy [20, 23, 24, 34, 38, 42, 45, 46].

SIMPSON's autoimmune theory [39] evolved on a speculative basis but none the less confirmed later in many respects took some time to give rise to immunosuppressive therapy of myasthenia gravis. The cause of the delay was the fact that the corticoids which have proved their value in other autoimmune diseases are not only ineffective but even present certain hazards in the condition at issue [9, 23, 24, 46]. The absence of inflammatory factors in myasthenia gravis accounts for its unresponsiveness to steroids [28]: in fact, neither the nervous system, nor the muscles or the thymus present inflammatory signs in this disease [10, 14, 43, 46]. However, the mechanism

of action of the cytostatic agents is not confined to anti-inflammatory factors, it being primarily of antimetabolic nature. Therefore, theoretically, all cytostatic agents affecting nucleic acid and purine metabolism are expected to act as potent immunosuppressors in myasthenia gravis, too, presumably by their inhibitory effect on the immunocompetent lymphocytes or on their precursors. According to SCHWARTZ and DAMESHEK [36] they primarily benefit processes marked by delayed type hypersensitivity, their effect being of cellular rather than of humoral nature.

The immunosuppressor drugs act in a highly diverse manner. Some of them have a definitely suppressive, while others a protective action [5]. The activity of 6-mercaptopurine includes distinct anti-inflammatory factors [25], in contrast to methotrexate with next to no effect of this kind [50]. Azathioprine possesses marked anti-inflammatory and nuclein-inhibitory effects [5]. The inhibitory effect of methotrexate on nucleic acid metabolism does not involve its incorporation into DNA to which fact it owes its potential advantage of a lesser toxity [5, 25].

DELWAIDE et al. [8] as well as NOUZA and ŠMAT [19] used azathioprine and cylophosphamide in 5 and 2 cases of myasthenia gravis, respectively. The report by MERTENS et al. [15] on the immunosuppressive treatment of myasthenia gravis appeared at the time of our earlier publication on this subject. Their series of study in which 6-MP, azathioprine, methotrexate and actinomycin-C were used, includes 38 patients, all of major severity. The immunosuppressors were also used in combination with radiotherapy, thymectomy and/or ACTH if these alone had proved inadequate. Significant improvement evidenced by an increase in muscle power and a reduction in the dependence on cholinergic drugs, was attained in 22 cases. The schedule of treatment comprised repeated courses interrupted by drug-free intervals. It is emphasized that even in the cases where improvement was less marked, unexpected exacerbations remained absent and instead of showing the usual fluctuations, the clinical course was smooth. The response seemed to be unrelated to the clinical type of the disease as also to the immunosuppressive agent employed. Combinations of various immunosuppressors had no advantage over single drugs.

In the present study, too, immunosuppressive (lymphocytostatic) therapy was promising in many respects. Considering the degree and duration of response, the treatment amounted to a semiradical cure, like irradiation of the thymus or thymectomy. At any rate, it has certainly added to the therapeutic possibilities and particularly in those cases in which for some reason radiotherapy or thymectomy were impracticable [45, 46] or where conventional therapeutic measures had failed. A further point in favour of immunosuppressive (cytostatic) therapy has been referred to in our former paper [31]: in view of an eventual ectopy of the thymus the chances of delivering the total destructive radiation dose to the actual site of the thymus amount to 70 to

80%, whereas the success of immunosuppressive therapy is obviously little affected by the topography of the organ. This also applies to residual thymic tissue which has been spared by the operation (duplicate or multiple thymus, aberrant islets of thymus), compensatory hypertrophy of which may be a source of postoperative deterioration or recurrence [45, 46].

It will take a long time to establish the proper place of immunosuppressive therapy in the management of myasthenia gravis. Considering the low incidence of this disease and its incalculable course, assessment of the value of any therapeutic procedure is fraught with difficulties and requires a sufficient lapse of time. There are, in addition, some special issues which add to the difficulties. 1. There is no immunoserological test which would identify individual cases of myasthenia gravis involving an autoimmune mechanism thus providing us with a basis for the selection of cases for immunosuppressive (cytostatic) therapy. As already pointed out [31, 47], the response to immunosuppressive treatment seems to be unrelated to the results of immunoserological tests. But then, myasthenia gravis might well behave in this respect similarly to other autoimmune diseases where it is quite common to find no antibodies to the affected organ or tissue, while there are antibodies to antigens derived from clinically healthy organs or tissues, an observation which constitutes one of the chief arguments against the pathogenicity of circulating antibodies [26, 27]. 2. In the case of myasthenia gravis the plan of treatment is inseparable from the time factor. It is a general observation that the previous duration of the disease belongs to the main factors which decides the success of thymectomy: the shorter the history, the more successful the operation [20, 34, 38, 45]. With immunosuppressive therapy there is always the possibility of an undue loss of time: for instance, thymectomy or radiotherapy would have to be postponed for a couple of months in the case of leukopenia. In the light of these considerations it appears unwise to undertake immunosuppressive therapy before having exhausted all other therapeutic possibilities, so as not to jeopardize the chances of surgery or to miss its optimum point of time. In fact, thymectomy performed in proper time and on the grounds of correct indications is still superior to any other therapeutic procedure in myasthenia gravis. 3. Further studies will only show which of the immunosuppressor drugs ensures the fullest and longest remissions. The agent selected for the present study has an inhibitory effect on the lymphatic system, thus presumably also on the immunocompetent small lymphocytes (or upon their precursor cells). However, this selection by no means implicates that other immunosuppressors of different mechanisms might not be envisaged for further studies. 4. The relationship of immunosuppressive (cytostatic) therapy and corticosteroids is also open to controversy. Our personal views on the possible causes of the inefficiency of steroids and on their practical hazards (crisis!) have been expressed earlier. MERTENS et al. [15] have been led by their experience

with immunosuppressive agents in myasthenia gravis to reconsider their attitude towards the administration of ACTH and of steroids in this disease. Similar considerations have recently prompted us to resort to steroids in a case of a female patient with myasthenia gravis of utmost gravity. On the 3rd day of this treatment she developed a respiratory crisis and had to be kept under IPPB for two days. There was no other demonstrable factor which might have precipitated the crisis. This accident has only confirmed us in our negative attitude to steroid therapy, shared by numerous earlier experiences. 5. There still remain the questions *a)* whether it is safe to repeat the immunosuppressive courses and if so, how many times; and *b)* whether an interruption of immunosuppressive treatment involves any hazard. The results on intermittent treatment are known [15]. Our personal experience is more favourable inasmuch as the remissions have been attained by a sole immunosuppressive course. One patient had two courses without, however, responding to either of them.

Despite all these controversial issues and unclarified points we may outline the value and present status of immunosuppressive (cytostatic) therapy in the management of myasthenia gravis as follows.

A) The possibility of autoimmunity being involved in the pathomechanism of myasthenia gravis in a number of cases justifies the use of immunosuppressive agents.

B) The results achieved in myasthenia gravis with immunosuppressive agents are by no means of symptomatic character but amount to those of a semiradical therapy taking effect through an inhibition of the immune mechanisms. The duration of this effect has yet to be ascertained. The character of response makes immunosuppressive therapy closely similar to thymectomy and thymus irradiation, the benefits of which are well-known. The therapy may have a particular value in cases where thymectomy or radiotherapy are impracticable for some reason and, considering its humoral character, in the case of an ectopic or aberrant thymus inaccessible to surgery, or islets of thymus spared accidentally by the operation.

C) The response to immunosuppressive agents bears little relationship to the clinical features of the individual case. This is also valid for the positivity or negativity of the serological reactions. It is therefore not the evidence of the serological tests which decides the necessity for immunosuppressive therapy.

D) The indication of immunosuppressive treatment depends solely on the clinical aspects of the case. It may be vital to avoid any delay for other successful therapy on account of immunosuppressive therapy takes, of necessity, a considerable length of time. Consequently, it is chiefly in cases of major severity, unresponsive to other kinds of therapy, particularly in drug-resistant cases, where immunosuppressive therapy has its place.

E) It would be desirable to investigate the entire range of immunosuppressor drugs of diverse mechanisms, in the framework of clinical observations. On theoretical grounds, the priority belongs to those cytostatic agents of relatively slight toxity whose inhibitory activity is primarily directed at the lymphopoietic system and which exert not only suppressive but also protective effects. Antiphlogistic effects of the agent are irrelevant to the success of therapy, since inflammatory factors play little part in the pathomechanism of myasthenia gravis.

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Dr. Albert SZOBOR, Budapest XIII., Róbert Károly u. 84, Hungary
Dr. Gyula PETRÁNYI, Debrecen, I. Belklinika, Hungary

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AN EXTENDED EXCRETION STUDY ON THE INGESTION OF A MONOFLUOROPHOSPHATE TOOTHPASTE BY CHILDREN*

By

J. A. HARGREAVES

DEPT. OF RESTORATIVE DENTISTRY, THE UNIVERSITY, EDINBURGH

G. S. INGRAM and B. J. WAGG

GIBBS DENTAL RESEARCH, UNILEVER RESEARCH LABORATORY, ISLEWORTH. MIDDLESEX, ENGLAND

A study has been made of the toothpaste ingestion habits of young children over a total period of eight weeks. The amounts of toothpaste swallowed varied widely from child to child and from day to day. When allowance had been made for dietary fluoride it was found that 98% of the children swallowed a mean of 0.0—0.25 g of paste per day. One child, drawn from the original 105, swallowed an average of 0.44 g of toothpaste per day.

Introduction

In an earlier article (HARGREAVES et al.) a short-term toothpaste ingestion study employing pre-school children in the low fluoride region of Edinburgh was reported. In that study 90% of children who used a fluorine-containing toothpaste for two weeks swallowed an average of less than 0.5 g of toothpaste per day.

The period of experiment was short and it was of interest to find whether those children who had, on occasions, swallowed quantities of toothpaste would repeat this behaviour with continued use or whether this was a random behaviour pattern.

In addition a longer experimental period would enable more accurate mean ingestion figures to be calculated for individual children.

Accordingly some of the original panellists from the short trial continued to use the toothpastes for a longer period and urinary fluoride determinations were used to follow toothpaste ingestion.

Experimental

i) *Panel members.* In the original study 105 pre-school Edinburgh children participated in the trial. A number of these were found to have ingested a measurable amount of toothpaste on some occasions. Most of these 'high ingesters' were included in a panel of 54 children who were to use a fluorine-

* Read at the XVIIth Congress of the European Organization for Caries Research Debrecen, Hungary, July 16, 1970.

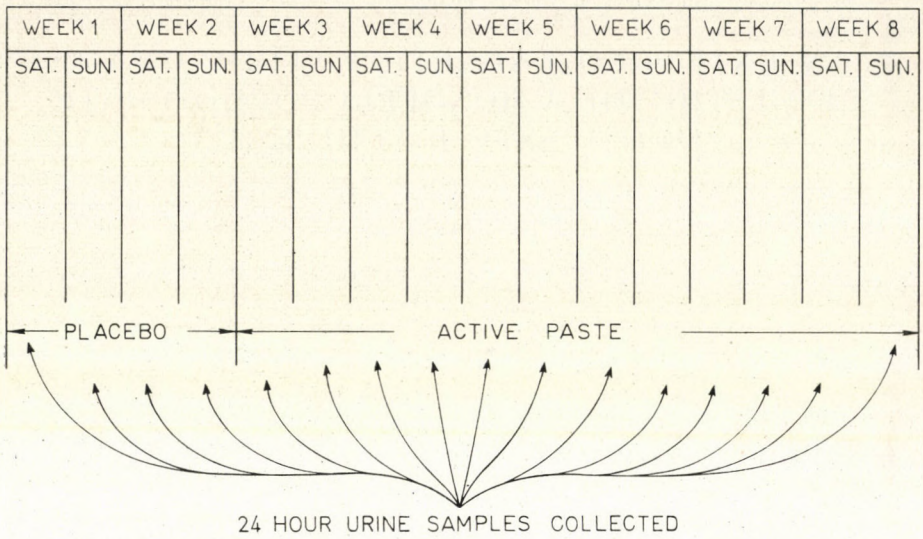

Fig. 1. Design of trial showing toothpastes used and urine sample collection

free toothpaste for two weeks and the fluorine-containing toothpaste for a further six weeks.

ii) *Toothpastes.* The same two toothpastes were used as in the earlier study; a fluorine-free placebo toothpaste and one containing sodium monofluorophosphate equivalent to 2.4 mg F/g. With such a paste the swallowing of any substantial amount should be reflected by an increased urinary fluoride level unconfused by variations in dietary fluoride. Both pastes were in stable formulations and were similarly and acceptably flavoured.

iii) *Trial procedure.* The 54 children were given the fluorine-free toothpaste to use for two weeks. On the Saturdays and Sundays at the ends of these weeks 24-hour urine samples were collected so giving four sample days corresponding to use of the fluorine-free toothpaste (Fig. 1). The urine volumes were measured and the fluoride contents determined (HARGREAVES et al.) so giving the daily fluoride clearance. The children were then given the toothpaste containing sodium monofluorophosphate to use for six weeks. As with the fluorine-free toothpaste, 24-hour urine samples were collected from the children on the Saturday and Sunday at the end of each week so giving 12 sample days from the period when the fluorine-containing paste was used (Fig. 1). As before, the fluoride contents and volumes of the urines were measured so giving the daily fluoride output by each child.

iv) *Collection of urine samples.* The method used in the earlier study was followed in that the urine samples were collected in wide-mouthed plastic jars containing a little toluene to inhibit bacterial growth.

The volume of each urine sample was measured and small portions were removed for determination of their fluoride contents.

v) *Determination of fluoride in urine.* In the previous study it was shown that monofluorophosphate was excreted in the fluoride form. This was determined in the urine using a method based on the Auto Analyzer (HARGREAVES et al.).

Results

The results of this longer term study were combined with the relevant data from the short term trial (HARGREAVES et al.) so that fluoride excretion figures were obtained for four weeks' use of a fluorine-free placebo toothpaste and eight weeks' use of the toothpaste containing sodium monofluorophosphate. The figures obtained in the study are shown as histograms in Fig. 2. The fluoride output of children using the monofluorophosphate toothpaste was compared with that when the control paste was used. The mean urinary fluoride output per child when using the placebo toothpaste was 0.09 mg/day compared with 0.11 mg/day when using the monofluorophosphate toothpaste.

In the previous publication we established that an average of one fifth of a deliberately administered dose of monofluorophosphate was excreted as fluoride in the urine by this group of children. The corresponding factor of five was applied to the daily fluoride outputs by the fifty-four children to give estimates of ingestion and the results were recalculated in terms of grams of toothpaste swallowed per day.

These values are shown in categories of individual children's average daily intakes over the trial period in Table 1 and Fig. 3. In Fig. 3, each vertical strip represents one sample day. The vertical axis is divided into 'limit zones' and the numbers are codes referring to individual children who have apparently swallowed fluorine equivalent to the indicated amount of toothpaste on that sample day. This demonstrates the frequency with which particular children exceeded certain levels of toothpaste intake.

The average daily fluoride excretion of those children who had apparently swallowed toothpaste on diverse occasions are compared in Table 2, with their average excretion when using the placebo. The differences are shown in terms of grams of toothpaste per day.

Table 1

Average daily toothpaste ingestion by panel members

(not adjusted for dietary fluoride)

61%	of children	ingested	0.00—0.25	g	paste/day
35%	of children	ingested	0.25—0.50	g	paste/day
4%	of children	ingested	0.50—0.70	g	paste/day

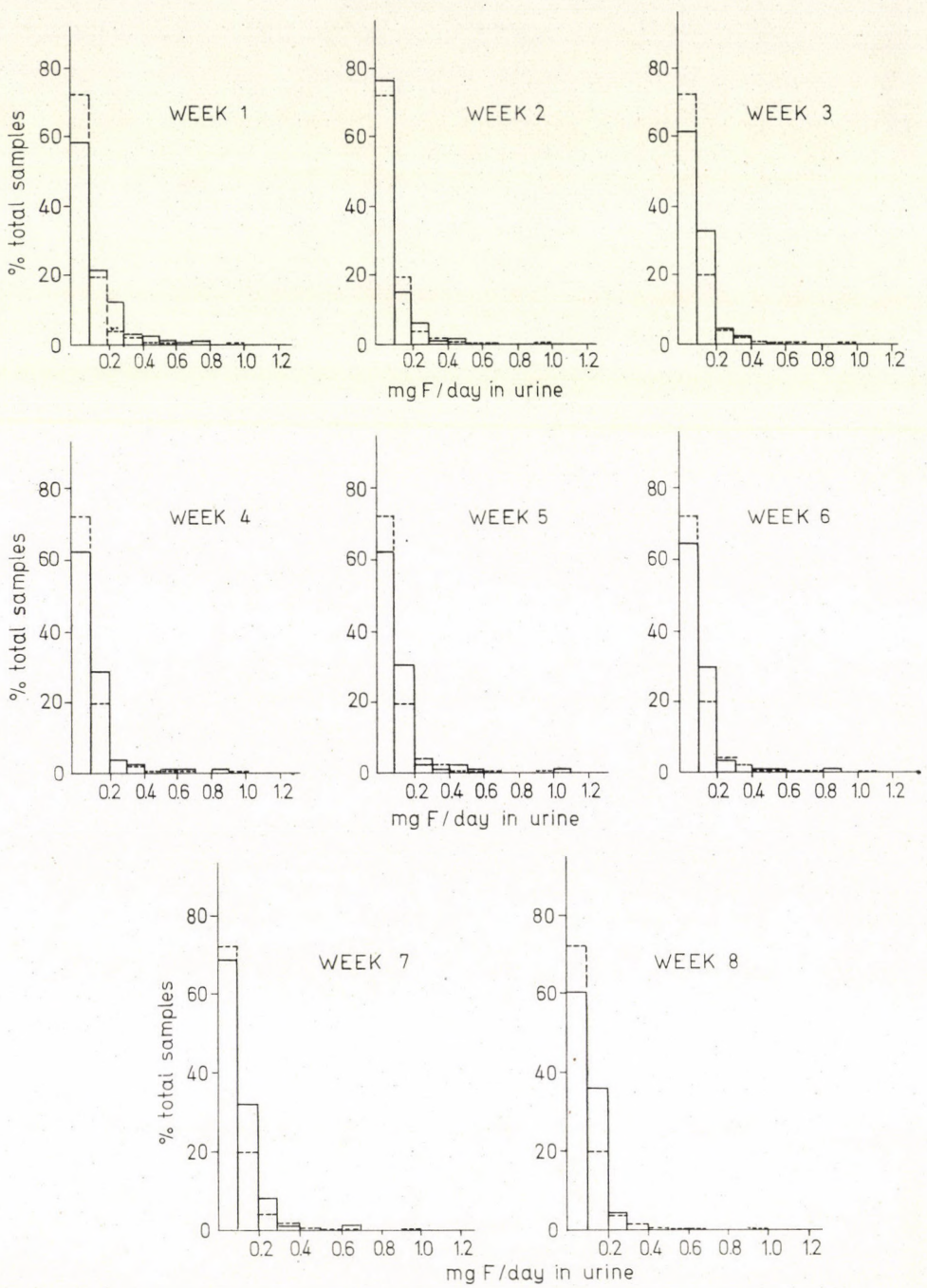

Fig. 2. **Histograms showing week by week changes in urinary fluoride outputs when using monofluorophosphate paste (solid lines) compared with mean output figures when placebo paste was used (dotted lines)**

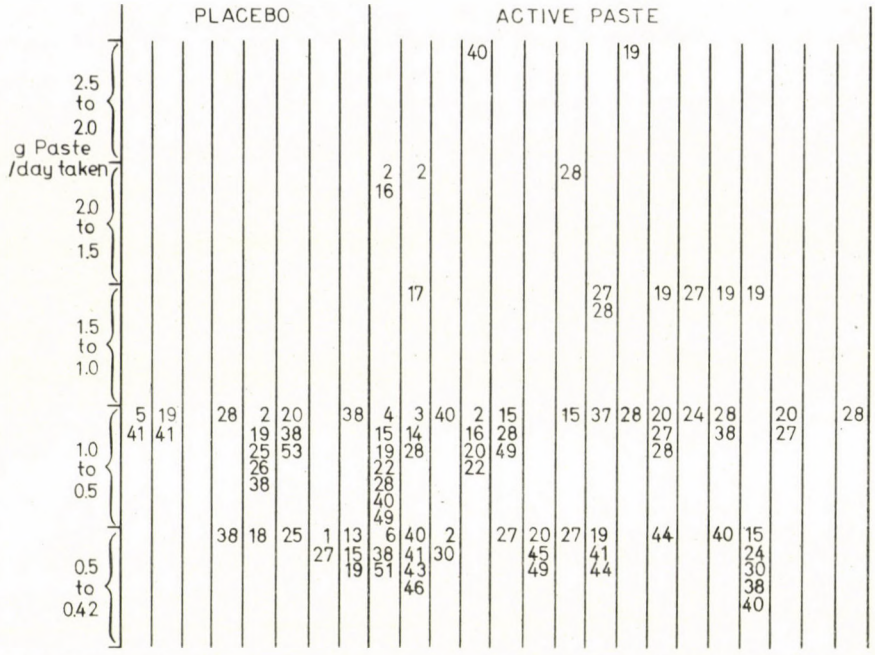

Fig. 3. Category chart showing individual children's total fluorine ingestions in terms of grams of toothpaste per day when using the placebo and the monofluorophosphate toothpastes. Each number refers to an individual child. No reference is made to those children with a calculated ingestion of less than 0.42 g paste in any one day

Table 2

Average daily toothpaste ingestion by those children swallowing appreciable amounts

Child No.	Urinary F (mg/day) using		Extra F ingested mg/day (i.e. (b—a)×5)	Estimated "active paste" ingestion (g/day)	
	(a) "Placebo"	(b)"Active paste"		Unadjusted	*Adjusted
2	0.13	0.21	0.40	0.44	0.17
15	0.12	0.18	0.30	0.38	0.13
16	0.06	0.16	0.50	0.33	0.21
19	0.19	0.28	0.45	0.58	0.19
20	0.13	0.15	0.10	0.31	0.04
22	0.11	0.16	0.25	0.33	0.10
27	0.14	0.24	0.50	0.50	0.21
28	0.13	0.34	1.05	0.71	0.44

* The "unadjusted" figure is calculated from total urinary fluoride. Allowance has been made in the "adjusted" figure for urinary F during use of placebo.

Table 3

Net average daily toothpaste ingestion by panel members
(adjusted for dietary fluoride)

98% of children ingested 0.00—0.25 g paste/day
2% of children ingested 0.25—0.50 g paste/day

Discussion

The purpose of the present work, itself an extension of an earlier study (HARGREAVES et al.) was to find whether the ingestion of toothpaste by individual children followed an established pattern or whether the behaviour was random. If the first premise were true then one could expect that those children who were observed to have ingested significant amounts of toothpaste during the short-term trial would continue to do so. Otherwise children might exhibit random behaviour varying between low and high toothpaste ingestion throughout the trial.

Such knowledge is essential to establish safe levels of active ingredients.

The experimental method was similar to that in our previous study; urinary fluoride outputs by the children were measured during use of placebo toothpaste and one containing an elevated level of sodium monofluorophosphate (2.4 mg F/g of paste). It was necessary to use this level of fluorine to differentiate genuine ingestions of toothpaste from dietary fluoride variations.

The histograms in Fig. 2 show how the gross urinary fluoride figures changed from week to week, compared with the mean figures observed with a fluorine-free toothpaste. It can be seen that there was a slight decrease in the proportion of samples containing the lowest levels of fluorine when using the sodium monofluorophosphate toothpaste and a corresponding increase in the higher levels.

The individual child's average daily toothpaste ingestion figures are shown in categories in Table 1. In this extended study, no child had an average daily intake in excess of 0.70 g and 96% of participants did not exceed 0.5 g. These figures can be compared with those of the earlier short-term study, when 90% of the children apparently swallowed up to 0.50 g per day and 4% of the panel had a mean daily intake of 0.75—1.10 g of toothpaste. This difference is probably a direct effect of lengthening the trial period so that the children became accustomed to the regime.

Neither histogram, overall mean figures nor intake categories, however, show the calculated toothpaste intakes by individual children on separate sample days. These data have been combined with those from the previous study (HARGREAVES et al. 1970) and presented in Fig. 3. It was found that the ingestion of toothpaste by young children varied widely from child to child and from day to day. From this random pattern it emerged that a few children had on occasions a propensity to swallow toothpaste. Thus it can be seen that

child No. 19 apparently ingested more than 1 g of toothpaste on four occasions out of sixteen and that three other children, Nos. 2, 27 and 28, exceeded this figure twice.

It should be stressed that these figures take no account of dietary fluoride intake in this low fluoride area and attribute all of the urinary fluoride to ingested toothpaste. McCLURE and ZIPKIN (1958) calculated that the diet of a group of children 4—6 years old contained an average of 0.2 mg of F per day, corresponding to approximately 0.1 g of the toothpaste per day but would vary from child to child. Accordingly the ingestion figures were re-examined in the light of each child's average fluoride output when using the fluorine-free toothpaste.

When the differences between 'placebo' and 'experimental' paste fluoride excretions were re-calculated in terms of milligrams of fluorine and grams of toothpaste swallowed per day it was seen (Table 2) that child No. 28 apparently acquired an additional 1.05 mg of fluorine per day from the experimental toothpaste. This corresponded to 0.44 g of toothpaste per day.

Table 3 shows the ingestion categories given in Table 1 when the individual corrections are applied.

It is, however, the figures obtained with the 'worst performer' drawn from our original experimental group which should be taken as a guide when considering the use of ingredients in toothpastes.

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Dr. J. A. HARGREAVES, Dept. of Restorative Dentistry, The University, Edinburgh, Scotland, UK

Dr. G. S. INGRAM, Dr. B. J. WAGG } Gibbs Dental Research, Unilever Research Laboratory. Isleworth, Middlesex, England, UK

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EFFECTS OF DEXTRANASE ON EXPERIMENTAL CARIES IN THE RAT*

By

A. BENAGIANO, G. GRIPPAUDO and M. CATTABRIGA

DEPARTMENT OF ODONTOLOGY (HEAD: PROF. DR. A. BENAGIANO) OF THE UNIVERSITY, ROME, ITALY

In rats made relatively gnotobiotic and infected thereafter with the Streptococcus strain PK1 and kept on a cariogenic diet, the addition of 200 U/g of dextranase to the food resulted in a significant reduction of dental caries incidence in an experiment of 60 days duration. Caries reduction was less in a group treated in the same way but receiving 400 U/g of dextranase in the food.

Studies on the formation, composition, and metabolism of the dental plaque are in the foreground of dental research owing to its importance in the pathogenesis of dental caries as well of periodontal diseases [7, 14, 17, 18, 20]. Factors influencing dental plaque formation have much been discussed in the past years and recently it has been recognized [3, 8, 9] that dextrans are of considerable importance in its formation. This polysaccharide is synthetized by some microorganisms in the presence of sucrose, and it tends to adhere firmly to the surface of the teeth, contributing thereby to the formation of the dental plaque matrix. — FITZGERALD and KEYES [6] showed that the microorganisms inducing dental caries are far more specific than thought previously. Several strains of cariogenic streptococci are producing a particular type of dextran [8, 12, 21]. — Studies *in vitro* and *in vivo* by FITZGERALD and KEYES [6], KÖNIG and GUGGENHEIM [13, 16], BOWEN [2], BLOCK et al. [1] on the enzyme dextranase — produced by *Penicillium funiculosum* and by *Penicillium lilacinum* under certain conditions — have demonstrated that it hydrolyzes the dextran produced by cariogenic microorganisms, and thus prevents the formation of a bacterial plaque adhering to the tooth surface, reducing hereby experimental caries in hamsters and rats.

In the present paper studies are reported on the effect of dextranase upon experimental caries in the rat.

* Read at the XVIIth Congress of the European Organization for Caries Research, Debrecen, Hungary, July 17, 1970.

Review of the literature

The first report upon the effect of dextranase was published by FITZGERALD and KEYES in 1968 [5]; they studied it in hamsters with experimental caries. It was shown that the dextranase produced by *P. funiculosum* markedly reduced the caries incidence when added to the drinking water at a concentration of 80 U/ml or to a sucrose rich cariogenic diet (diet 2000) in the amount of 80 U/g in the animals infected orally with Streptococcus strain E-49. In this experiment, however, a few animals were receiving only the dextranase. — In a second experiment on three hamsters, dextranase was administered in the drinking water (80 U per ml); the hamsters were infected with Streptococcus strain 6715, one more cariogenic than the former one: there was a notable caries reduction. It was noticed, however, that no caries was found in the uninfected controls. KÖNIG and GUGGENHEIM [16] checked the caries protective effect of a dextranase prepared from cultures of *P. lilacinum* in Osborn-Mendel rats fed the cariogenic diet No. 2000 of KEYES and JORDAN. After having been treated with antibiotics to eliminate the indigenous oral flora, the rats were infected with various strains of *Streptococcus mutans*, capable of producing dextran from sucrose. As has been shown previously, this type of dextran was hydrolyzed in vitro by dextranase. The dextranase was administered in the drinking water in a dosage of 170 U/ml in the first experiment, and 170 U/g and 170 U/ml, respectively with the diet and the water in the second one. In this latter instance, the Streptococcus strains OMZ 71 and OMZ 61, respectively, were applied. In the first experiment the dextranase had no favourable action, and in the animals whose oral flora had not been destroyed with antibiotics and no artificial infection had been induced, the prevalence of caries was higher than in the treated animals. In the second experiment, with a total dose of 340 U dextranase in diet + drinking water, and after inoculation with different Streptococcus strains, a slight but nonetheless clear reduction of caries was obtained. It was only the OMZ 61 strain of Streptococcus that was capable of inducing caries on the smooth surfaces of the teeth. In a more recent experiment the effect of 150 U/ml of *P. lilacinum* dextranase administered together with high doses of soluble sucrose added to the diet or to the drinking water was examined in Osborn-Mendel rats kept on a modified diet No. 2000. The animals were not infected artificially but were taken from a caries-susceptible colony, and harboured therefore a mixed oral flora. No reduction by dextranase of experimental caries was noted while total elimination of caries was observed in animals receiving sucrose in aqueous solution. — BLOCK et al. (1969) used gold hamsters kept on the cariogenic diet No. 2000. The animals were not infected artificially, and the dose of dextranase administered to the drinking water was 1000

U/ml. After 5 weeks a considerable reduction of caries incidence was noted in the treated animals.

All these reports showed that the effect of dextranase depended on its capacity of breaking down the polysaccharide synthetized by various strains of Streptococcus.

The present study had the aim of controlling *in vivo* the action of a recently produced dextranase on experimental caries in the rat.*

Materials and methods

Wistar rats of a colony not noted for its predisposition to caries were used. In order to make the animals gnotobiotic, they were given 0.1% erythromycin in the diet, then infected orally by the Streptococcus strain PK1, kindly supplied by DR. R. J. GIBBONS, a strain described in literature as being able to produce cariogenic dextran by polymerization of sucrose [10].

The cariogenic diet used was No. 200 of NAVIA et al. [19], which contains 67% sucrose. The dextranase was produced by a culture of *P. funiculosum* grown in a substrate of dextran produced by *Leuconostoc mesenteroides.*

Five groups of freshly weaned animals were used as follows:

1st group: 10 male animals kept on a normal diet;

2nd group: 10 male animals kept on diet No. 200;

3rd group: 15 male animals — kept for four days on diet No. 200 containing 0.1% erythromycin. The administration of the antibiotic was then stopped, and the same diet was continued for another 2 days. Thereafter, each rat was given orally four times every other day two drops of a culture of the erythromycin-resistant Streptococcus strain PK1, isolated from a human carious cavity;

4th group: 10 male animals, fed and treated as the previous group, but after four administrations of the culture of strain PK1, the diet was completed by 200 U per g of dextranase;

5th group: 10 male animals, treated as group 4, but with 400 U/g of dextranase in the diet.

Water of Nepi was given to the animals *ad libitum.* It was chosen as it had been used in previous experiments and its mineral content is exactly known [11].

After 60 days the animals were killed by ether inhalation and decapitated. The heads were fixed in 10% neutral formalin for 48 hours. Thereafter the mandible and the maxilla were dissected, all soft tissues having been carefully removed around these bones. The carious lesions were then assessed with KEYES' method [15] using a stain to show clearly the initial carious lesions [4].

Results

Carious lesions were observed in all animals in all groups. Lesions were seen predominatly in the sulci, a small percentage was found on the smooth lingual and/or vestibular surfaces. Some lesions were a cavity proper in the enamel and dentin, but a high number of lesions was demonstrated only with the use of a stain elective for less mineralized zones.

In Table I the mean caries scores are shown using KEYES' method of assessment.

* The enzyme used was produced by PIERREL Laboratory, Milano.

Table I

	Number of rats	Diet 200	Erythro-mycin	Strept. PK1	Dextra-nase 200 U/g	Dextra-nase 400 U/g	Mean caries score	
							in the sulci	on smooth surfaces
Group 1	8	—	—	—	—	—	7.8(±6.0)	0.2
Group 2	8	+	—	—	—	—	8.4(±3.2)	0.8
Group 3	13	+	+	+	—	—	12.1(±2.5)	0.3
Group 4	10	+	+	+	+	—	3.5(±3.0)	0.1
Group 5	10	+	+	+	—	+	8.5(±2.7)	0.4

In parenthesis: Standard error of the mean

Discussion

Between the treated groups, a certain difference has been noticed as to the mean caries score. A certain reduction of caries incidence was shown by animals treated with 200 U per g of dextranase. It may thus be concluded that at this dosage, dextranase influenced negatively the cariogenic activity.

In this way, our results are similar to those of KÖNIG and GUGGENHEIM [16], i.e. to findings reported in the 2nd experiment of their first paper, though the protection observed was far inferior to the outstanding results obtained by FITZGERALD and KEYES [5], and BLOCK et al. [1] in the hamster.

There was no great difference in caries incidence between groups 1 and 2. This may be explained by the fact that our animals had not originated from a caries susceptible colony. Moreover, the carious lesions observed in our experiments were not as frequent and deep as expected, in spite of the high sucrose concentration of the diet which, according to literature [19], is suitable for producing in a short time carious lesions both in the sulci and on the smooth surfaces. This might have been due to the fact that in order to have relatively gnotobiotic animals, prior to the experiment a wide spectrum antibiotic was administered which may have altered the caries susceptibility. The duration of the experiment too might have influenced the incidence of caries. It is well-known that in gnotobiotic animals the time necessary for the development of carious lesions depends on the strain of Streptococcus [22]; as to strain PK1, there are no data available in literature. The cariogenicity of the strain is, however, obvious from the comparison between groups 2 and 3, as in the latter the caries incidence was higher in spite of the previous antibiotic treatment.

Of certain interest is also the difference in caries incidence between group 4 whose diet contained 200 U/g of dextranase, and group 5, with 400 U/g of the enzyme. The different behaviour of the two groups may be connected

with a possible interference with the enzyme activity due to an increased concentration of its reaction products. The positive experience with 1000 U/g of dextranase of BLOCK et al. [1] apparently contradicts the above explanation. The contradictory finding in group 5 is interesting and should thus be studied further.

Conclusions

Although our results are partly encouraging as far as to the role of dextranase in reducing experimental dental caries in the rat, we must not forget that in animal experiments there have always been different conditions by which a comparison of the results is rendered difficult. The partly contradictory results reported by various research groups may be due to the fact of having used different animals with different biological background, with mixed oral flora or germ-free, or made relatively gnotobiotic using different antibiotics. The cariogenic diets applied have been of different qualitative and quantitative composition. The animals have been infected with cariogenic bacterial strains differing in quantitative and qualitative polysaccharide production. Finally, the dextranases used differed in the methods of their preparation, as to their specific activity and even as to dosage. The experimental methods were also different. — These five factors, i.e. differences in experimental animals, diet, infecting organism, dextranase, and experimental method, must have influenced in one way or other the results of individual experiments, reducing their objective value.

The scarcity of studies does not permit to draw definite conclusions as to the action of any type of dextranase in the control of experimental dental caries. It is therefore still premature to consider the clinical application of any enzyme preparation of this class. Nevertheless, as the amount of dextran produced and the action of dextranase are markedly influenced by the oral bacterial population and its metabolism, it would be rewarding to study how the dextran-dextranase system may influence the composition and metabolism of the bacterial population of the human oral cavity. It is therefore important to conduct studies on the effects of various types of dextranase in man under various experimental conditions. If positive results are obtained, and only in this case, will it be justified to regard dextranase as a new weapon in the control of dental caries.

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Prof. Dr. D. BENAGIANO
Dr. G. GRIPPAUDO
Dr. M. CATTABRIGA
Viale Regina Elena 287a, I.00161 Rome, Italy

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MORPHOLOGISCHE REAKTIONEN DER MILCHZAHNPULPA UNTER ZAHNKARIES*

Von

D. HATTYASY

I. KLINIK FÜR ZAHN- UND MUNDKRANKHEITEN DER MEDIZINISCHEN UNIVERSITÄT SZEGED

Eingegangen am 15. August 1970

Die Gewebsreaktionen der jungen sowie der »alternden« Pulpa von Milchmolaren mit Zahnkaries wurden an 44 Mustern untersucht. Die akut leukozytäre Reaktion in der Pulpa der 5jährigen wird in den älteren Zähnen durch Plasma- und Mast- sowie sonstige mononukleare Zellen ersetzt. Die Änderungen der normalen Komponente ließen drei verschiedene Typen erkennen. Die naheliegende Möglichkeit eines immunbiologischen Geschehens wird erörtert.

Über die Veränderungen der »alternden« normalen sowie kariösen Zahnpulpa ist in der Literatur, so z. B. in den neueren z. T. klinisch-monographischen Arbeiten von BRAUER u. Mitarb. [1] sowie von TAYLOR und BERMAN [22], auffallend wenig zu finden. HARNDT [9] macht stichhaltige, aber eher allgemein gehaltene Feststellungen; so hebt er u. a. die »ungewöhnliche Reaktionskraft der Milchzahnpulpa« und »ihre hohe biologische Leistungsfähigkeit« hervor. Aufgrund eigener früherer Befunde [10, 12] erschien eine genauere Untersuchung dieser Frage wünschenswert.

Material und Methoden

Als Kontrolle dienten kariesfreie Milchzähne von 7—10jährigen Kindern [10]**. Die kariösen Zähne — meist untere Milchmolaren — sind nach Alter, Kariestiefe und klinischem Befund in Tab. I geordnet; die nicht bezeichneten Zähne waren symptomfrei. Über das mikrotechnische Verfahren s. [5, 10, 19].

Ergebnisse

In sechs von zehn *kariesfreien Milchzähnen* war ein scheinbar normales Pulpagewebe zu finden, aber auch in diesen sechs Fällen waren viermal Plasmazellen (PL)-Infiltrationen zu sehen; in den weiteren vier war die PL-Komponente auffälliger. Als Nebenbefund waren zweimal perivaskuläre Infiltrationen, einmal kleinere Blutungen, viermal außer der PL diverse mononukleare (MO)

*Nach einem am XVII. Kongress der ORCA (Organisme Européen de Recherche sur la Carie) in Debrecen, Juli 1970 gehaltenen Vortrag.

** In Klammern: die Zahl der einschlägigen Fälle.

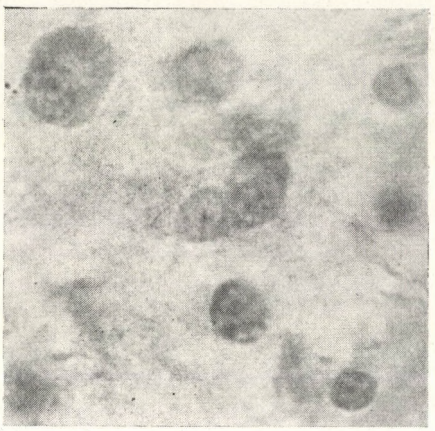

Abb. 1. Diverse mononukleare Zellen aus einer losen Infiltration in der Pulpakammer (Prot. Nr. 1218, 9. j. ♀, +e, keine Karies) Vergr. ca. 700×. H. E.

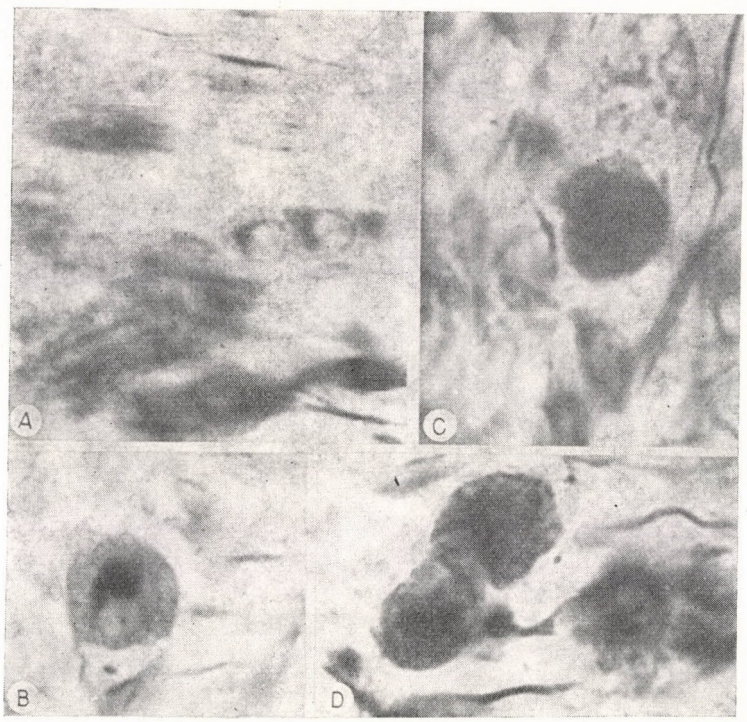

Abb. 2. Vier Etappen der Entwicklung von Mastzellen (MC): *a)* und *c)* links: junge, unentwickelte MC; *b)* junge, vollentwickelte MC mit hellem Kern, kleinem Nucleolus, intensiv imprägniertem Golgi-Netz und netzförmigem Plasma; *c)* ältere große MC mit »Balg« (welcher anscheinend entleert ist); *d)*: zusammenhängende oder sich abschnürende MC von einem ähnlichen Gebilde wie in c), jedoch dicht mit argentophiler Granula besetzt. (Prot. Nr. 1164, loj. ♂, e+, Karies prof.) Vergr. ca 1000×. Impr. n. Romanes

Zellanreicherungen zu verzeichnen, einmal auch kalkige Degeneration und dreimal eine auffallende Abnahme des Zellbestandes. Bei den zwei mehr als 10 J. alten Zähnen dominierte einmal die Blutung, ein andermal die Abnahme des Zell- und Faserbestandes. Auch in diesen Fällen waren die immunstoffetragenden Zellen reichlich vertreten.

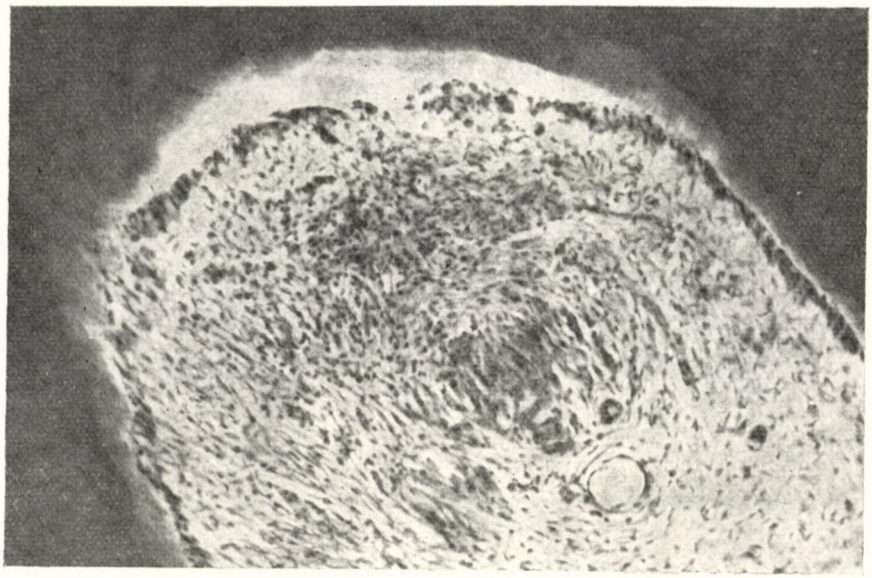

Abb. 3. Region des Pulpahornes bei Karies. Abhebung und Degeneration der Odontoblasten darunter lose mononukleare Infiltration und die Bildung eines »Knotens« (→). (Prot. Nr. 1152A, 11j. ♂, +e, sehr tiefe Karies ohne Schmerzen.) Vergr. ca. 120×. Van Gieson. Aufnahme Dr. D. TANKA (Landesinstitut für Rheuma und Physiotherapie, Budapest)

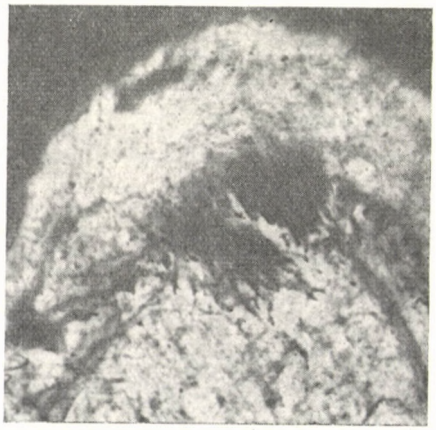

Abb. 4. Verbindung eines »knotenartigen« Gebildes rechts und links mit einem Nervenstrang. (Prot. Nr. 1166, 12j. ♂, +e, mesial und distal sehr tiefe approximale KL; keine Sensibilität.) Vergr. ca. 650×. Impr. n. Romanes

Kariöses Material. 44 kariöse, meist untere, Milchmolaren wurden in drei Altersgruppen (AG) geordnet: AG 1. Zähne von 5; AG 2. von 7—10; und AG 3. über 10jährigen Probanden.

Das *histologische Hauptmerkmal* wurde mit A, B, C, D, F bezeichnet, wobei A die Normalstruktur, B eine akut leukozytäre Reaktion, C PL und sonstige MO-Reaktion, D degenerative Veränderungen und F Nekrose bedeutet.

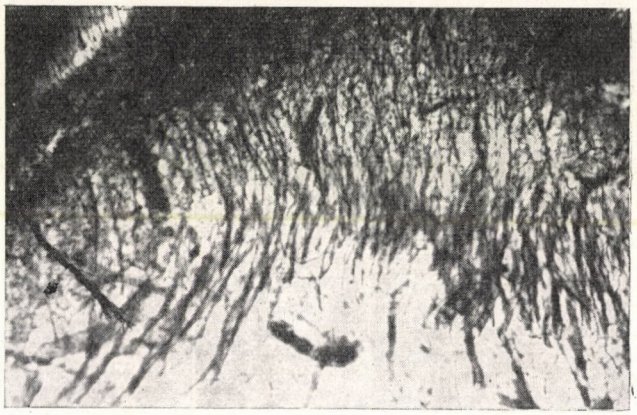

Abb. 5. Periphere Pulpapartie unter der Karies in einem Pulpahorn. Große Anzahl nahezu parallel gerichteter argentophiler Fasern. (Prot. Nr. 1165, 11j. ♂, —e, sehr tiefe Karies ohne Spontanschmerzen.) Vergr. ca. 150×. Impr. n. Romanes

Die Tiefe der kariösen Läsion (KL) wurde mit c, m, pr und *pr* bezeichnet, wobei c oberflächliche, m mittlere, pr tiefe und *pr* sehr tiefe KL bedeutet (Tab. I). Es sei hervorgehoben, daß in ¾ der Fälle eine tiefe KL vorlag.

Die akute *leukozytäre Reaktion* kam in AG 1 vorwiegend, in AG 2 in der Minderheit und in AG 3 nur in einem F-Fall als Nebenbefund vor. Ein umgekehrtes Verhältnis zeigt sich bei den C-Fällen: Reaktionen mit MO-Zellformen bilden in AG 2 und 3 die Majorität. Die D-Fälle sind in jeder AG in Minderheit; eine partielle Nekrose (F) war lediglich zweimal zu verzeichnen.

Tiefe der KL und Sensibilität. Eine systematische Sensibilitätsprüfung mussten wir unterlassen und haben nur die »spontanen« Sensationen verzeichnet, wobei geringe Empfindlichkeit bzw. Schmerzen und stärkere Schmerzen der Anamnese gemäß unterschieden und in Tab. I erstere mit +, letztere mit ++ unter Aufführung der Tiefe der KL angegeben wurden, wobei jeder Zahn einzeln aufgenommen wurde. Anamnestisch kamen Empfindlichkeit viermal, stärkere Schmerzen sechsmal — letztere bei pr und *pr* KL — vor. Demgegenüber zeigte die Mehrheit keine anamnestisch eruierbaren Sensationen (s. Tab. I). Das Minimum an Sensibilität zeigte Gruppe C (von 21 Fällen zweimal +, einmal ++).

Relation der Tiefe der KL und des pathologischen Hauptmerkmales. In AG 2 und 3 haben die C-Fälle fast den gleichen Anteil, so daß bei tiefer

KL die PL und MO Reaktionen eine dominierende Stelle einnehmen; an zweiter Stelle stehen Fälle der D-Gruppe.

Reaktion der Nerven und Tiefe der KL. Bei einer Reihe von Fällen war eine positiv-attraktive Wirkung seitens des kariösen Gewebes auf die Nervenfasern festzustellen. Die nervale Struktur entspricht entweder einem einfachen »spreading« der meist nicht markhaltigen Fasern in Richtung des kariösen Herdes (Abb. 5), oder es bildet sich ein aus Bindegewebs- und Nervenfasern bestehender »Knoten« nebst geringer zellulärer Beteiligung (Abb. 3, 4), welcher nicht in der äußersten Pulpaschichte, sondern in einer gewissen Tiefe vom kariösen Herd liegt. Als dritte Form kommen auch Nervenknäuel vor. Um Aufschluß über die Organisation des »spreading«-Typs zu gewinnen, wurde ein schon imprägniertes Präparat auch elektronenmikroskopisch untersucht. Obwohl die Qualität dieser Präparate nur eine Orientierung zuließ, hat es den Anschein, daß es sich tatsächlich um nervale Strukturen handelt (Abb. 6, 7). Das Vorkommen dieser divers erscheinenden Aktivierung der Nervenfasern, welche sich auf die Umgebung der KL beschränkt, gibt Tab. II wieder. Es zeigt sich eine deutliche Tendenz zur Häufung in AG 3 und eine Präferenz für tiefere KL.

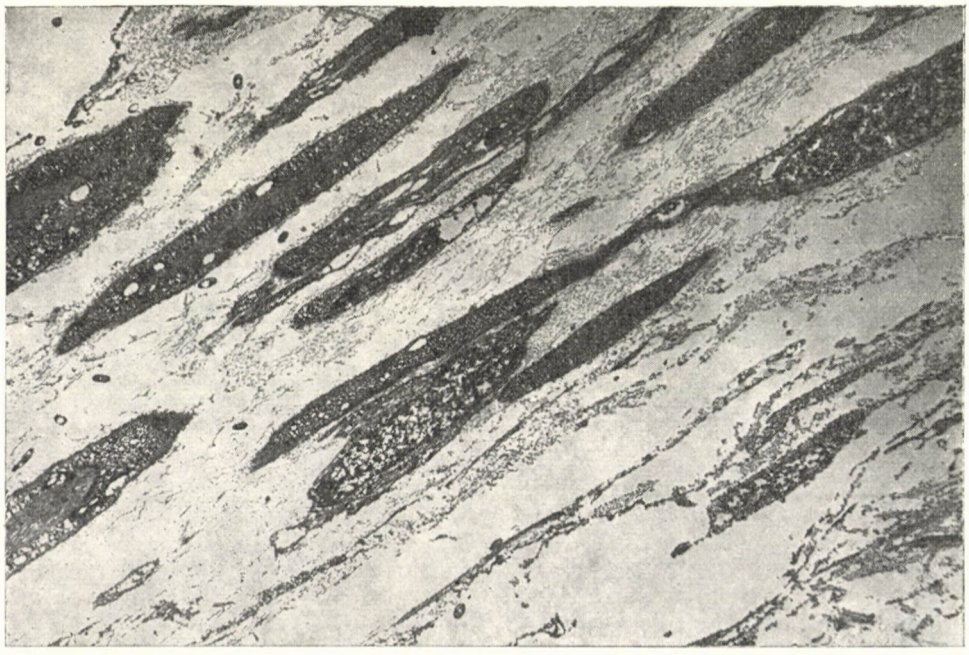

Abb. 6. Gleicher Fall wie Abb. 5. Der Raum zwischen faserartigen Gebilden ist durch Kollagen ausgefüllt. (Ein n. Romanes imprägniertes Präparat wurde nachträglich eingebettet.) Vergr. 2000×. Elmo-Aufnahme von Dr. G. ELEMÉR (Pathologisches Institut der Medizinischen Universität Szeged)

Tabelle I

Altersgruppe	Histol. Gruppe	Caries-Typ				Total		Bemerkung
		s	m	pr	*pr*			
1	A	1				1		
	B		2	1	1	4		*pr*++
(5 J.)	C			2	1	3		(2-mal 1e-Ein.)
	D		1			1	9	
	B		1	2	1	4		pr++
2	C		3	3	5	11		m+, pr+
(7—10 J.)	D	1	1	2	1	5		pr+
	F			1		1	21	pr++
	A			1		1		
3	C			2	5	7		*pr*++
(>10 J.)	D			3	1	4		pr+, *pr*++
	F		1		1	2	14	*pr*++ (1e-Ein.)
Total		2	9	17	16		44	

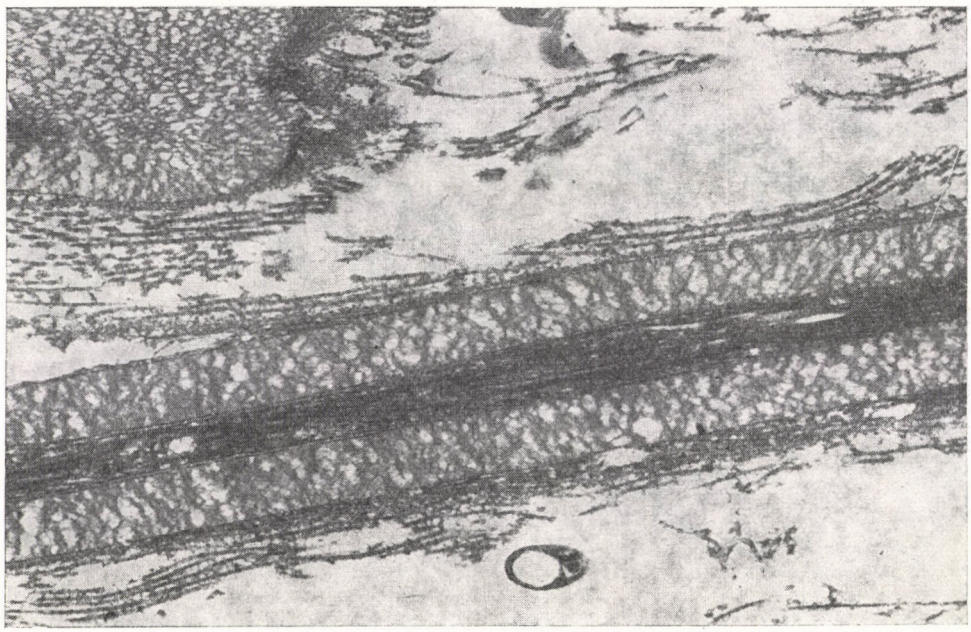

Abb. 7. Gleiches Material wie Abb. 6. Die Struktur erinnert an nervale Herkunft. Vergr. 15000×. Elmo-Aufnahme von Dr. G. ELEMÉR (Pathologisches Institut der Medizinischen Universität Szeged)

Auf die *Relation von Karies und Wurzelresorption* (Res.) wurden die Zähne der AG 2 und 3 geprüft (Fallzahlen der AG 3 sind in Tab. III kursiv gesetzt). Die Fälle wurden nach Res.-Grade in 1 geringe, 2 halbe, 3 vorgeschrittene und in eine 4 »gemischte« Kategorie geordnet. In letzterer sind in Tab. III die Res.-Werte in Klammern gesetzt. Es ergibt sich, daß in beiden AG bei den tiefer kariösen Zähnen die geringe Res., sowie die gemischte Form die

Tabelle II

Typ d. Wurzelres.	Aktivierte Nervenfasern in	
	AG 2	AG 3
1 (gering)	3 (1pr, 2*pr*)	7 (3pr, 4*pr*)
2 (halb)	1 (m)	1 *(pr)*
3 (vorgeschr.)		1 *(pr)*
4 (gemischt)	2 (2pr)	1 (pr)
Total	6 (aus 29)	10 (aus 16)

Tabelle III

Typ d. Wurzelres.	Caries-Fälle			Keine Caries	Total	
	m	pr	*pr*			
1 (gering)	2, *1*	4, *5*	4, *4*	4, *1*	14	*11*
2 (halb)			*1*			*1*
3 (vorgeschr.)	1		1, *1*	1, *1*	3	*2*
4 (gemischt)	2 (1—2)	1 (1—3) 1 (2—3) 1 (1—2—3) *1* (1—3)	1 (1—2) *1* (2—3)	1 (1—1—2) 1 (1—2—3) 1 (2—3)	9	*2*
?	1	1	1		3	
Total	6, *1*	8, *6*	7, *7*	8, *2*	29	*16*

Mehrheit bilden; die wenigen kariesfreien Zähne (10), meist in AG 2, zeigen geringe Res. In der gemischten Kategorie gewinnt man den Eindruck, daß an der Seite der KL die Wurzel weniger resorbiert wird.

Die Relation der *Wurzelresorption und der »aktivierten« Nervenfasern* scheint in AG 2 und 3 verschieden zu sein, indem in AG 3 in der Mehrzahl (10) nur geringe Res. vorhanden ist (Tab. II). Es fällt auch auf, daß die *pr*-Fälle in AG 3 (7) fast alle (6) und die pr-Fälle (6) zu 2/3 (4) aktiviert sind. Es ergibt sich, daß in AG 3 aus 14 Fällen bei 10, wogegen in AG 2 aus 15 Fällen nur bei 5 eine Aktivierung nachweisbar ist (vergl. Tab. II und III).

Besprechung

Um ein kompletteres Bild geben zu können, wären an jedem Muster eine Reihe von Methoden simultan anzuwenden; dies war für uns z. Zt. nicht möglich. Somit geben die dargestellten Ergebnisse nur beschränkte Aufschlüsse, besonders was die Zellidentifikation und Grundsubstanz sowie die Natur des Gewebsgeschehens betrifft.

Junge intakte Milchzähne konnten wir nicht untersuchen, nur solche von 8—10jährigen. Außer dem Normalbild erscheinen in der »alternden« Milchzahnpulpa jene Zellarten (Plasmazellen, Mastzellen, Histiozyten, verschiedene Speicherzellen usw.) in größerer Anzahl, welche auch hier ganz diverse biochemisch-physiologische Funktionen haben dürften. Die physiologisch verschiedene Einstellung der älter werdenden Milchzahnpulpa zeigt auch der sukzessive Schwund der markhaltigen Nervenfasern [10, 11]. Somit stellt die größere Anzahl argentophiler fixe und labile Zellen — etwa im Sinne von MARSHALL's metalophil cells [16] — e inenschon integrierenden Teil der älteren Milchzahnpulpa-Zellpopulation dar; diese Zellen sind aber in geringerer Anzahl auch im jüngeren Alter sowohl licht-, wie elektronenmikroskopisch nachgewiesen [21].

Den wichtigsten Unterschied zwischen den drei AG erblicke ich darin, daß auf die kariöse Einwirkung die junge Gruppe (AG 1) dominant mit akut leukozytärer Entzündung reagiert und diese in AG 2 nur mehr eine untergeordnete Rolle spielt.

Man sollte aber auch daran denken, daß in den Jahren zwischen AG 1 und 3 infolge der Karies viele Zähne entfernt worden sind; d. h. daß dieses Material das Ergebnis einer Auslese ist. Bei den verbliebenen Zähnen der AG 2 und 3 besteht mithin die theoretische Möglichkeit, daß entweder eine gewisse Resistenz der Zahnkaries gegenüber, oder aber eine Kariesattacke geringerer Intensität vorlag.

Die überwiegende Mehrheit in AG 2 und 3 (Tab. I) bilden jene Fälle, die als histologisches Hauptmerkmal eine größere Anzahl diverser, teils argen-

tophober (Plasmazellen, große Schaumzellen) teils argentophiler Zellen (RES-Zellen, Mastzellen, Histiozyten, Speicherzellen [14], nicht identifizierbare mononukleare Zellen) fixen und mobilen Charakters zeigen (Abb. 1). Die öfters vorkommenden kleinen argentophilen Zellen dürften wahrscheinlich in Entwicklung begriffene Mastzellen sein (Abb. 2) [4]. Als Negativum sei hervorgehoben, daß keine kleine Lymphozyten zu finden sind und auch eosinophile Leukozyten nur spärlich vorkommen.

Da diese Gewebsbilder öfters bei tiefer und sehr tiefer Karies vorkommen und es an der Stelle der maximalen Einwirkung keine stürmische Reaktion gibt (Abb. 3) und die genannten Zellen in Form mehr geschlossener oder diffuser Aggregate *mehr pulpainnen* erscheinen, gewinnt man den Eindruck einer gewissen Gewebstoleranz gegenüber — oder Symbiose mit — der kariösen Noxe.

Es wäre unangebracht, die Funktion dieser Zellen hierorts in extenso erörtern zu wollen. Ich werde mich daher auf wenige — gut begründete — Hinweise beschränken: Die Plasmazellen bilden ein wichtiges Glied des Immunsystems und sind immerhin eine Art von Produzenten von Immunglobulinen (Ig) bzw. Antikörpern gegen verschiedene Antigene [17]. Die Mastzellen (MC) als Produzenten von Heparin, Histamin, Hyaluronsäure, 5HT — und wohl noch anderer Stoffe — sind als pluripotente Zellen zu werten, welche besonders an Stellen eines lokalen Streß vorkommen und u. a. an der Granulombildung teilnehmen [20].

Solche und ähnliche Zellreaktionen kommen bei verschiedenartigen chronischen Erkrankungen vor, so u. a. auch bei dem primär chronischen Rheumatismus (PCP) [6]. Leider gibt das Vorkommen der erwähnten Zellsorten noch keinen Aufschluß über die Natur des Gewebsgeschehens, nämlich ob es sich um eine toxische Abwehr, eine lokale Antigen-Antikörper-Reaktion (AAR) oder ein anderes Immunphenomen handelt [15], z. B. um eine Autoimmunreaktion, ähnlich wie TOLLER [23] wegen dem erhöhten Ig-Spiegel in Radikulärzysten an diese dachte und welche auch bei dem als Beispiel angeführten PCP sehr wahrscheinlich sein dürfte [7].

Bei dem bakteriell-enzymatischen Abbau des Dentins entstehen Substanzen — prinzipiell ähnlich wie bei der Proteolyse — Proteosen, Peptone, Peptide und Aminosäuren [13], welche ungefähr den Stoffen bei dem Abbau von Zahnplaques entsprechen [3]. Solche und ähnliche Stoffe sind öfters Halbantigene (Haptene) und werden erst mit Gewebseiweiß gekuppelt zu Vollantigenen [2]. Diese Möglichkeit besteht eventuell auch hier, da die PL und MC usw. — Zellpopulation in Fällen der AG 2 und besonders AG 3 in tieferen Pulpaschichten erscheint.

Die relativ häufigen sonstigen histologischen Erscheinungen (Gefäßerweiterung, herdförmige und disseminierte Blutungen, Verdichtung der Grundsubstanz, Fibrolyse, nekrotische Säume) sprechen für die Möglichkeit einer

AAR und c. p. einer Analogie mit dem Zahngranulom, welches nach WERNER auch in die AAR-Kategorie gehören dürfte [24].

Die »andersgeartete« Gewebsreaktion auf die kariöse Einwirkung in der »alternden« Milchzahnpulpa mag z. T. auch dadurch bedingt sein, daß — wie schon erwähnt — durch die Änderung der nervalen Rezeption infolge der sukzessiven Eliminierung gewisser Nervenfasern (deren Schwund ich [10] an einem jüngeren menschlichen Material fand als HARGREAVES [8]), auch die Gewebsreaktion sich notwendigerweise ändert, da dieser Zustand m. E. einer partiellen Denervierung gleichzustellen ist. Dieser Vorgang scheint keineswegs im Widerspruch zu dem auf die Nervenelemente wirkenden im Raum der KL lokalisierten Effekt zu stehen.

Die in dieser Abhandlung als »spreading-Typ« bezeichnete Form wurde zuerst von PLAČKOVÁ [18] an Pulpen bleibender kariösen Zähne beschrieben. Dieser Effekt scheint nicht obligat zu sein und führt zu keiner erhöhten Sensibilität — trotz der Vermehrung der Nervenelemente — und beeinflußt auch nicht die zelluläre Komposition. Die hier beschriebene »Knotenform« (Abb. 3, 4) dürfte als eine *nerval-fibröse Überproduktion* aufzufassen sein, ausgelöst durch die aktivierende, gehemmt aber durch die übermäßig werdende kariogene Einwirkung.

Von den sich selber ergebenden Fragen wäre es meines Erachtens wichtig, Genaueres über die Natur der hier beschriebenen Pulpenreaktion zu wissen: vor allem ob es sich um einen immunoallergischen Prozeß (evtl. eine zellulargebundene Spätreaktion) handelt oder nicht. Das morphologische Bild und auch der klinisch-chronische Charakter halten diese Möglichkeit offen.

*

Für die Sammlung und Überlassung der Zähne sei auch hier Dr. med. dent. E. MOLNÁR und Dr. med. A. SOMKUTHY, für die technische Hilfe Dr. med. G. ELEMÉR und ganz besonders Frau I. CSETNEKI gedankt.

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Prof. em. Dr. med., Dr. sc. D. HATTYASY; Budapest XII. Maros u. 23, III. 4, Ungarn

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TIERVERSUCHE ZUR KARIESPROTEKTIVEN WIRKUNG VON GLYZEROPHOSPHAT*

Von

C. H. PLATHNER und M. WINIKER

POLIKLINIK FÜR KONSERVIERENDE STOMATOLOGIE (DIREKTOR: PROF. DR. MED. DENT. HABIL. C. H. PLATHNER) DES BEREICHS MEDIZIN (CHARITÉ) DER HUMBOLDT-UNIVERSITÄT ZU BERLIN

Mittels kariogener Diät vervierfacht sich am Goldhamster der Kariesgrad binnen acht Wochen um das Vierfache. Die kariogene Wirkung der Diät kann durch tägliche Gabe von 0,25 mg Fluorid fast vollständig verhindert werden. Die Schutzwirkung von einer Tagesdose von 0,025 g Dinatrium-beta-glyzerophosphat erwies sich als fast ebenso stark, während die Gabe von 0,04 mg Ammoniumvanadat praktisch keine Schutzwirkung gegen Karies zeigte.

Die unbestreitbar großen Erfolge, welche die Versuche zur Eindämmung der Zahnkaries durch Fluorverbindungen gezeitigt haben, könnten fast zur Annahme verleiten, die endgültige Überwindung der Karies wäre eigentlich nur noch ein »technisches Problem«, d. h. es käme lediglich darauf an, die in manchen Ländern gegen eine generelle Fluorprophylaxe erhobenen Bedenken zu zerstreuen und die Fluorprophylaxe selbst so zu vervollkommnen, daß die gesamte Bevölkerung erfaßt wird. Leider ist dem nicht so, denn wir sind nach wie vor mit folgenden Fakten konfrontiert:

1. Die Ätiologie und die Genese der Zahnkaries sind noch nicht restlos geklärt.

2. Der Wirkungsmechanismus der Fluorverbindungen ist ebenfalls noch nicht völlig erforscht.

3. Die Applikation von Fluorverbindungen wird allen bisherigen Erfahrungen zufolge bestenfalls nur zu einer ca. 60%igen Verminderung des Kariesbefalls führen.

Die Suche nach weiteren karieshemmenden Agentien ist somit aus theoretischen und praktischen Gründen von höchster Aktualität.

Was den praktischen Aspekt des Problems betrifft, so sollte unseres Erachtens im Hinblick auf die mit Fluorverbindungen erzielten Erfolge das Hauptinteresse auf solche Substanzen gerichtet sein, deren Einsatz eine Ergänzung der Wirkung der Fluorverbindungen erwarten läßt. Dies scheint nach Ansicht verschiedener Forscher, z. B. NIZEL und HARRIS [17], namentlich bei den Phosphaten der Fall zu sein.

Ausgangspunkt der weltweiten Propagierung einer Kariesprophylaxe durch Fluorverbindungen sind bekanntlich die Anfang der dreißiger Jahre

*Vortrag am XVII. Kongreß der Europäischen Arbeitsgemeinschaft für Kariesforschung in Debrecen, Ungarn, am 14. Juli 1970

von DEAN und Mitarbeitern [3, 4] begonnenen systematischen Untersuchungen des Zusammenhangs zwischen Kariesbefall und Fluorgehalt des Trinkwassers. Weniger geläufig dürfte sein, daß praktisch gleichzeitig (1929 bzw. 1931) LENNOX [13] den hohen Kariesbefall bei der weißen Bevölkerung von Südafrika auf einen Phosphatmangel der Nahrungsmittel zurückführte, während HEWAT [10] den auffallend guten Gebißzustand von Waisenkindern in Neuseeland als Folge des besonders hohen Phosphatgehalts der Kost deutete. In diesem Zusammenhang ist von Interesse, daß auch ÅSLANDER [1], der gewöhnliches Knochenmehl für die Kariesprophylaxe empfiehlt, ursprünglich von der Vorstellung ausging, die starke Ausbreitung der Karies bei der schwedischen Bevölkerung sei durch einen zu niedrigen Phosphatgehalt der einheimischen Ernteprodukte bedingt. An Hand eigener Tierversuche [18] konnten wir wahrscheinlich machen, daß der karieshemmende Effekt von Knochenmehl auf der Wirkung des darin enthaltenen Kalziumphosphats beruht.

KLEIN und MCCOLLUM [11] waren wohl die ersten, die an Ratten die kariostatische Wirksamkeit von Phosphaten demonstrierten. Seit diesen ersten Anregungen und Versuchen hat die mögliche Bedeutung von Phosphorverbindungen für das Kariesgeschehen in der ganzen Welt ein starkes Interesse gefunden. 1962 lagen bereits so viele Untersuchungen und Erfahrungen vor, daß mit dieser Thematik ein spezieller Kongreß in Cambridge (Massachusetts) einberufen werden konnte.

Leider ist es aus zeitlichen Gründen nicht möglich, im Detail auf die bisher publizierten Arbeiten einzugehen, doch seien uns einige kurze Hinweise gestattet, die für unsere eigenen Untersuchungen von Wichtigkeit sind.

Mit organischen Phosphaten wurden nur wenige Versuche gemacht. So berichten MCCLURE [15] sowie FEDOROV und SAPAGOVSKAYA [7] über Erfolge mit Phytat bei Ratten, was den Befunden von LIMBASUTA und Mitarbeitern [14] widerspricht. Weiterhin haben KÖNIG und Mitarbeiter [12] eine gewisse karieshemmende Wirkung des Dinatrium-β-glyzerophosphats bei Ratten beobachtet, die jedoch statistisch nicht gesichert war. Demgegenüber wird von FEDOROV [5, 6] über eine hervorragende kariostatische Wirksamkeit von Kalziumglyzerophosphat (ebenfalls bei Ratten) berichtet.

Verschiedene Forscher (BARNARD und JOHANSEN [2], MÜHLEMANN und Mitarbeiter [16], VAN REEN und Mitarbeiter [19]) stellten bei Ratten eine synergistische karieshemmende Wirkung von anorganischem Phosphat und Fluorid fest. In jüngster Zeit wurde von FEDOROV und SAPAGOVSKAYA [8] über Versuche an Albinoratten berichtet, deren Ergebnissen zufolge durch die gleichzeitige Verabfolgung von Natriumfluorid und Vitaminen sowie Kalziumglyzerophosphat eine ausgezeichnete Hemmung der experimentellen Karies erzielt werden konnte. Nicht zuletzt durch diese Arbeit angeregt, beschlossen wir, die Testung organischer Phosphorverbindungen in unser Kariesforschungsprogramm einzubeziehen.

Unsere ersten einschlägigen Versuche, über die im folgenden ein kurzer Bericht gegeben werden soll, hatten das Ziel, die karieshemmende Wirkung von Dinatrium-β-glyzerophosphat, Natriumfluorid und Ammoniumvanadat beim Goldhamster zu vergleichen. Wir bedienten uns der gleichen Versuchsmethodik wie bei unseren früheren tierexperimentellen Arbeiten[18]. Im einzelnen verfuhren wir wie folgt:

Insgesamt 75 syrische Goldhamster, die wir am Alter von 4—5 Wochen von einem ortsansässigen Züchter übernommen haben, wurden zunächst eine Woche lang bei normalem Futter (Gerste, Hafer, Sojakerne) an ihre neue Umgebung gewöhnt. Sodann wurden sie in 5 Gruppen von jeweils 15 Tieren eingeteilt. Von diesen 5 Gruppen wurde eine weiter mit normalem Futter und eine andere mit kariogener Kost versorgt, während die restlichen 3 Gruppen kariogene Kost mit einem Zusatz des zu prüfenden Agens erhielten, dessen Konzentration auf Literaturangaben (Glyzerophosphat) bzw. eigenen Erfahrungen (Fluorid, Vanadat) basierte.

Als kariogene Kost dienten selbstgebackene Kekse, deren Teig nach folgendem Rezept hergestellt worden war:

300 g Zucker,
300 g Weizenmehl,
380 g Maizena,
150 g Magermilchpulver,
10 g Backpulver,
375 ml destilliertes Wasser.

Alle Tiere bekamen zweimal wöchentlich etwas frisches Gemüse (Möhren, Salat). Als Trinkflüssigkeit diente gewöhnliches Leitungswasser.

Während der gesamten Dauer der Versuche (generell 8 Wochen) erfreuten sich die Goldhamster mit einigen Ausnahmen bester Gesundheit, wofür auch die eine stetige Zunahme aufzeigenden, hier nicht reproduzierten Gewichtskurven sprechen. Die im Verlauf der Versuche eingetretenen Verluste waren auf Darmerkrankungen zurückzuführen, die mit der Fütterung in keinem ursächlichen Zusammenhang standen. Am Ende der Versuche wurden die Tiere mit Leuchtgas getötet und die Schädel freipräpariert. Bei der Ermittlung der Karies wurden nur Defekte berücksichtigt, die mit Mikroskop und Sonde diagnostizierbar waren. Jeder kariöse Molar wurde, unabhängig von Anzahl, Größe und Tiefe seiner Läsionen, nur als eine Einheit gewertet. Der Kariesbefall wurde pro Gruppe in Prozenten ausgedrückt, d. h. es wurde die Summe der kariösen Molaren in den Ober- und Unterkiefern aller Tiere der jeweiligen Gruppe mit 100 multipliziert und durch die Summe aller Molaren geteilt. Die statistische Bewertung unserer Ergebnisse erfolgte mit Hilfe des χ^2-Tests.

Unsere in Tabelle I zusammengestellten Ergebnisse gestatten folgende Feststellungen:

Tabelle I

Eigene Versuchsergebnisse

Gruppe	Kostform	Anzahl der Tiere	Kariesbefall, Prozent
I	Normale Kost	14	8,9
II	Kariogene Kost	13	35,2
III	Kariogene Kost + 0,25 mg Fluor/Tag	14	13,7
IV	Kariogene Kost + 0,025 g Glyzerophosphat/Tag	11	19,7
V	Kariogene Kost + 0,04 mg Vanadin/Tag	13	28,1

1. Die kariogene Kost war voll wirksam [hoch signifikanter Unterschied (Irrtumswahrscheinlichkeit $\alpha = 0{,}1\%$) zwischen Gruppe I (normale Kost) und Gruppe II (kariogene Kost)].

2. Den stärksten karieshemmenden Effekt hatte das Natriumfluorid (Gruppe III), gefolgt von Dinatrium-β-glyzerophosphat (Gruppe IV) und Ammoniumvanadat (Gruppe V). Allerdings ist nur die Wirksamkeit von Natriumfluorid und Dinatrium-β-glyzerophosphat statistisch gesichert (Irrtumswahrscheinlichkeit $\alpha = 0{,}1\%$ bzw. 1%). Der Wirkungsunterschied zwischen diesen beiden Agentien selbst konnte statistisch nicht gesichert werden.

3. Keines der Agentien konnte die experimentelle Karies so stark eindämmen, daß der Kariesbefall in Gruppe I (normale Kost) erreicht wurde. Hierbei ist allerdings zu bemerken, daß der Unterschied im Kariesbefall zwischen Gruppe I (normale Kost) und Gruppe III (Natriumfluorid) statistisch nicht gesichert ist, also als zufällig angesehen werden kann.

Unter den von uns gewählten Versuchsbedingungen hat sich also Natriumfluorid als stärker karieshemmend erwiesen als Dinatrium-β-glyzerophosphat, wobei jedoch dieser Unterschied statistisch nicht gesichert ist. Zunächst scheint dieser Befund im Gegensatz zu den von FEDOROV [6] mitgeteilten Ergebnissen zu stehen, die für eine Überlegenheit des Glyzerophosphats sprechen (vgl. Tabelle II)

Tabelle II

Versuchsergebnisse von Fedorov

Gruppe	Kostform	Anzahl der Tiere	Kariesbefall, Prozent
A_1	Kariogene Kost + 0,025 g Ca-Glyzerophosphat/Tag	10	16,6
B_1	Kariogene Kost + Trinkwasser mit 1 mg/Liter Fluor	11	28,7
C_1	Kariogene Kost	12	47,2

Einen solchen »Widerspruch« kann man damit hinreichend erklären, daß FEDOROV mit einer anderen Tierart (Ratten) und mit einem anderen Salz der Glyzerophosphorsäure, nämlich dem Kalziumsalz, experimentierte als wir.

Zusammenfassend möchten wir das Ergebnis unserer Versuche wie folgt formulieren: Glyzerophosphat, dessen karieshemmende Wirkung von FEDOROV [5, 6] sowie von KÖNIG und Mitarbeitern [12] an Ratten demonstriert wurde, hat sich bei einer weiteren Tierart, dem Goldhamster, als kariostatisch wirksam erwiesen. Es kann zur Zeit noch nicht mit Sicherheit beurteilt werden, in welchem Verhältnis dieser Effekt zu dem von Natriumfluorid steht.

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Prof. Dr. C. H. PLATHNER
Dipl. Chem. M. WINIKER
104 Berlin, Invalidenstr. 87/89, DDR

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Ce Congrès suivre immédiatement le Congrès de la Société de Broncho-Oesophagologie et de Gastroscopie de Langue Française qui se tiendra également à LYON les 8 et 9 juillet 1971, sous la Présidence du Professeur P. GALY.

Toute demande de renseignements ou d'inscription pourra être adressée au Secrétaire Général du Congrès: Professeur Agrégé J. P. HAGUENAUER, Clinique O. R. L., Hôpital Edouard-Herriot, place d'Arsonval. 69 — LYON 3e.

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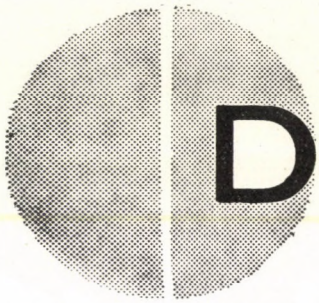

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РЕЗЮМЕ

ДЕЙСТВИЕ ЦИТОСТАТИЧЕСКОГО СРЕДСТВА И ФАКТОРА, СТАБИЛИЗИРУЮЩЕГО ФИБРИН, НА ХОЛЕСТЕРИНОВЫЙ АТЕРОСКЛЕРОЗ КРОЛИКОВ

Ш. БЕНКЁ, К. БЕНКЁ и Л. ТЁРЁК

Исходя из тромбогенной теории, авторы изучали действие тромбопении на развитие холестеринового атеросклероза и поведение ензима, стабилизирующего фибрин. Тромбоцитопения, вызванная дачей маннитмилерана, не влияла на степень холестеринового атеросклероза.

В другом эксперименте авторы установили, что в стенке сосудов, пораженных холестериновым атеросклерозом, накопляется значительное количество энзима, стабилизирующего фибрин.

ЛЕЧЕНИЕ АУТОИММУНОЛОГИЧЕСКИХ МИОЗИТОВ ПРЕПАРАТОМ R-74

Д. ПЕТРАНЬИ и Д. СЕГЕДИ

Авторы предлагают новое средство для лечения острых и подострых аутоиммуно логических миозитов.

Цитостатический препарат R-74 обладает энергичным иммунно-супрессивным и хорошим антилейкемическим действием, но среди аутоиммунологических заболеваний он оказывает необходимое избирательное действие только на миозиты. На основе пятилетних наблыдений и опыта, приобретенного в связи с лечением повторных рецидивов 23 больных, лечение препаратом R-74 само по себе или при комбинации со стероидотерапией быстро приводит к ремиссии. На вторичную дистрофию или атрофию мышц препарат не оказывает эффекта. Действие, оказанное на мышцы, довольно избирательное, влияние на кожное явление дерматомиозита менее выраженное, а на частичные явления прочей локализации препарат или мало влияет или совсем не вызывает эффекта. В части случаев уже на 3—7-й день лечения наблюдалось выраженное улучшение миозита. R-74 следует ввести внутривенно, при контроле картины крови, однако при даче рекомендуемой авторами эффективной дозы лечение безопасное.

Авторы обсуждают свои представления о механизме действия R-74.

ИССЛЕДОВАНИЕ ИММУННОГРАММОВ, СНЯТЫХ ПРИ РАЗЛИЧНЫХ ТИПАХ ГИПЕРМЕТАБОЛИЗМА

Л. МОШОНЬИ, Д. ДОБИАШ, Ю. ГЁНЦИ и Е. ПЕРЕНЬИ

1. Результаты сравнения титра противотел, действующих на 14 антигенов среды, подкрепляют прежний взгляд авторов, согласно которому при гиперметаболизме наблюдается тенденция к повышению синтеза противотел.

2. Гипертироксинемия повышает синтез противотел только до тех пор и только в такой мере, пока механизм обратной связи не оказывает противоположного влияния, уменьшающего синтез белков, посредством торможения гипоталамо-диэнцефалических образований.

СИНДРОМ НЕДОСТАТКА ПРОТИВОТЕЛ ПРИ ПАРАПРОТЕИНЕМИИ

А. ПАТАКФАЛЬВИ, Р. БАКХАУС, Ю. ЛАЙОШ, А. ГОГЛ и М. БАЛАЖ

Авторы следили с 1964 года за клиническими симптомами синдрома недостатка противотел у 40 больных парапротеинемией. Для установления гуморального иммунологического дефекта были проведены, кроме исследования бумажного, агарового и иммунологического электрофореза, также определения циркулирующих противотел и количества иммуноглобулинов, а для оценки клеточного иммунологического дефекта — кожные пробы с применением различных антигенов, а также исследование бластозного превращения лимфоцитов. В одной трети случаев парапротеинемия проявлялась в форме синдрома недостатка противотел, что способствовало распознаванию основной болезни. Синтез противотел и иммуноглобулинов был уже в момент постановки диагноза сильно нарушенным, и нарушение еще усиливалось по мере прогрессирования болезни. Нарушение синтеза противотел было незивисимым от типа парапротеинов. На основе количественных определений понизилось производство всех трех типов, однако наиболее выраженным было понижение синтеза IgA.

Клеточные иммунологические реакции не показали существенных отклонений. Понижение IgG было при парапротеинемия типа IgA, IgU и IgM менее выраженным, чем при парапротеинемии типа IgG.

ДЕЙСТВИЕ АНГИОТЕНЗИНА НА ПРОИЗВОДСТВО КОРТИКОИДОВ НАДПОЧЕЧНИКАМ КРЫС IN VITRO

К. САЛАИ и К. МИХАЙ

Добавление 25 или 50 μг ангиотензина на мл в минуту повышает производство кортикостерона надпочечниками крыс, in vitro при неизменном производстве альдостерона. Добавление 100 μг ангиотензина на мл каждые 15 минут повышает образование как альдостерона, так и кортикостерона.

Потеря ангиотензина составляет в течение инкубации 20—40%. Активность ангиотензиназы в надпочечниках не объясняет нечувствительность коры надпочечников крысы в отношении ангиотензина.

УСЛОВИЯ ОБРАЗОВАНИЯ НЕКОНЬЮГИРОВАННОГО 20 DHF/11 β, 17 α, 20β, 21-ТЕТРАГИДРОКСИ-ПРЕГН-4-ЕН3-ОН У БОЛЬНЫХ АКТИВНЫМ РЕВМАТОИДНЫМ АРТРИТОМ

А. КЕМЕНЬ, К. ФАРКАШ и Б. ГЁМЁР

У больных активным ревматоидным артритом и у здоровых лиц авторы после внутривенного введения 5—10 μC 1—2 H^3-гидрокортизона отделили из суточной мочи фракции THF (3α, 11β, 17α, 21-тетрагидрокси-5β-прегнан-20-он), 20 DHF (11β, 17α, 20β, 21-тетрагидрокси-прегн-4-3-он) и 6β OHF (6β, 11β, 17α, 21-татрагидроксипрегн-4-ен-3,20-дион). Они измеряли количество и радиоактивность отдельных фракций и исчисляли удельные активности.

Величины удельной активности 20 DHF во всех исследовавшихся случаях были более низкими, чем величины удельной активности THF и 6β OHF тех же больных. При подобных условиях меньшее количество 20 DHF здоровых лиц не показало измеряемой радиоактивности. Суточная величина секреции гидрокортизона, исчисленного на основе удельной активности THF и 6β OHF, совпадала с литературными данными, относящимися к здоровым лицам. В противоположность этому удельная активность 20 DHF, которая во всех случаях на один порядок величин оказалась более низкой удельной активности THF, непригодна для исчисления суточной секреции гидрокортизона, и поэтому 20 DHF нельзя рассматривать как непосредственный, регулярный метаболит гидрокортизона.

Обсуждаются условия возникновения 20 DHF.

МОРФОЛОГИЧЕСКИЕ РЕАКЦИИ ПУЛЬПЫ МОЛОЧНЫХ ЗУБОВ ПРИ КАРИОЗЕ ЗУБ

Д. ХАТТЯШИ

Поведение пульпы молочных зубов при кариозе было изучено на 44 молодых (5-летних) и «стареющих» (выше 7 лет) образцах молочных коренных зубов. В группе пятилетних моляров часто наблюдается острая лейкоцитарная реакция, все реже появляющаяся в стареющих зубах. На ее месте развивается тканевая реакция из плазменных и тучных клеток и из отдельных мононуклеарных клеток. Нервные элементы также показывают характерные, распределяемые на три типа, отчасти гиперплазические, изменения. Для объяснения указанных реакций выдвигается возможность местных иммунобиологических явлений.

ПРОТИВОКАРИОЗНОЕ ДЕЙСТВИЕ ГЛИЦЕРОФОСФАТА В ОПЫТЕ НА ЖИВОТНЫХ

К. Х. ПЛАТНЕР и М. ВИНИКЕР

Кариогенной диетой у хомяка за восемь недель степень кариоза увеличивается в четыре раза. Кариогенное действие диеты может быть почти полностью предотвращено дачей по 0,25 мг фторида. Едва слабее оказалось защитное действие дачи по 0,025 г дианатрий-бетаглицерофосфата в день, в то время как суточное введение 0,04 мг ванадата аммония практически не оказывало защитного действия от кариоза.

ВЫЯВЛЕНИЕ МЕТОДОМ ХРОМАТОГРАФИИ НА БУМАГЕ ФРАКЦИЙ КОРТИКОСТЕРОИДОВ В ПЕЧЕНИ ЛЮДЕЙ, УМЕРШИХ ПО РАЗЛИЧНЫМ ПРИЧИНАМ

Д. И. ФАЗЕКАШ и А. Т. ФАЗЕКАШ

Авторы изготовляли из печеночной ткани 28 лиц (18 мужчин и 10 женщин), умерших по различным причинам, полтора до 10 часов после смерти вытяжки и подвергли их в системе Буш 5 бумажной хроматографии. В зависимости от причины смерти в печени отдельных лиц выявили различные количества 2—8 кортикостероидных фракций. Поразительно большое количество кортикостероидов было найдено в печени умерших вследствие карциноза брюшины, происходившего из рака желудка и матки. В порядке величин следовало содержание кортикостероидов в печени умерших от пневмонии, а затем в печени умерших внезапно от паралича сердца в свободном от действия состоянии. Очень низкие величины кортикостероидов были выявлены в случаях смерти любой причины в алкогольном состоянии по сравнению с одинаковыми или подобными причинами смерти в свободном от алкоголя состоянии.

ИММУНОСУПРЕССИВНОЕ ЛЕЧЕНИЕ ТЯЖЕЛОЙ МИАСТЕНИИ

А. СОБОР и Д. ПЕТРАНЬИ

После краткого изложения автоиммунологического патомеханизма тяжелой миастении сообщаются результаты иммуносупрессивного (цитостатического) лечения 25 больных тяжелой миастенией. Авторы применяли для лечения препарат мезилэритрит (R-74), действиующий прежде всего на лимфатический аппарат. Результаты подытожены в таблицах, в разбивке по возрасту больных, давности заболевания, круга форм заболевания, а также по характеру достигнутого результата, степени и длительности ремиссии. У 20 больных были проведены также иммуносерологические исследования. Анализуются возможные связи терапевтических результатов с серологическими реакциями и с характерными клиническими данными заболевания. Обсуждаются теоретические основы и практическое значение иммуносупрессивного лечения миастении. Авторы считают целесообразным применение иммуносупресивной терапии прежде всего у тяжело больных, мало или совсем не реагирующих на другие методы лечения, резистентных к лекарствам. Они обсуждают также правильный выбор препарата с иммуносупрессивным эффектом и рекомендуют вещества с лимфатическим спектром действия, которые нетоксичные, оказывают интенсивное супрессивное и незначительное противовоспалительное действие.

ОПРЕДЕЛЕНИЕ КОЛИЧЕСТВА ЗУБНОЙ ПАСТЫ, СОДЕРЖАЩЕЙ МОНОФТОРФОСФАТ, ПРОГЛОЧЕННОЙ НА ПРОТЯЖЕНИИ ДОЛГОГО ВРЕМЕНИ ВО ВРЕМЯ ЕЕ ПОТРЕБЛЕНИЯ, НА ОСНОВАНИИ КОЛИЧЕСТВА ВЫДЕЛЕННОГО ФТОРА

Й. А. ХАГРЕВС, Г. С. ИНГРАМ и Б. Й. ВАГГ

Авторы исследовали на малых детях в течении 8 недель, сколько они проглатывают в день во время мытья зубов зубной пасты. Количество проглоченной зубной пасты в разные дни и у разных детей различное.

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ДЕЙСТВИЕ ДЕКСТРАНАЗЫ НА ЭКСПЕРИМЕНТАЛЬНЫЙ КАРИОЗ У КРЫС

А. БЕНАДЖИАНО, Г. ГРИППАУДО и М. КАТТАБРИГА

Авторы вызвали у крыс относительный гнотобиоз, а затем инфицировали животных стрептококками штамма РК1 и содержали их на кариогенном режиме питания при добавлении к пище 200 ед./г декстраназы. В течение 60-дневного экспериментального периода наблюдалось достоверное уменьшение возникновения кариоза зубов. В группе животных, получавших при одинаковых экспериментальных условиях 400 ед. декстраназы на г пищи, наблюдалось меньшее понижение возникновения кариоза.

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