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IN MEMORIAM

DR. JÁNOS PÓRSZÁSZ
1923—1974

Dr. János PÓRSZÁSZ was born in Budapest in 1923 where he graduated M. D. in 1949. As a medical student he joined the Institute of Pharmacology lead by Professor B. Issekutz sen. and was working there till 1955. His first scientific work dealt with the mode of action of analgesic drugs. Later, in collaboration with Dr. K. Nádor, he investigated the relationship between chemical structure and pharmacological activity of aminoketones. These studies lead to the discovery of the analeptic action of several aminoketones with nicotine-like activity. One of these compounds was 1-piperidinomethyl-cyclohexen -2-on (Karion®). He demonstrated that several aminoketones with antinicotinic activity depressed the polysynaptic reflexes and inhibited the facilitatory function of the reticular formation; an example of these is the widely-used preparation methyl-p-tolylpiperidine propanone (Mydocalm®).

In 1955 he joined the Institute of Physiology, University Medical School, Szeged, first as assistant lecturer and later as senior lecturer. In this new scope of activity he created a firm physiological basis for his pharmacological research work. In the course of his studies of the organization of the vasomotor centre he demonstrated the decisive role of the dilator centre in reflex vasodepression, as the excitation of this centre inhibits vasoconstrictory tone. This work was the subject of his D. Sc. thesis in 1968.

In 1967 he was invited to the Institute of Experimental Surgery in Szeged. Here, he continued his own research work and in collaboration with Professor G. Petri participated in investigations into drug treatment of intestinal paralysis. They discovered that an increased sympathetic tone played a decisive role in the development of paralytic ileus, and that the abolition of the increased sympathetic tone had a beneficial effect.

In 1970 he was appointed professor of Pharmacology at the University Medical School, Pécs. There he continued his research on the physiology of

bulbar vasomotor and sympathetic centres. His early death, however, prevented him to accomplish these studies.

He was an experimenter to the core. He developed several new methods in drug research. He did not accept unfounded prejudices and had a flair for distinguishing important facts. His papers, remarks and contributions gave special colour to the Meetings of the Hungarian Physiological Society. He spent much of his time on guiding and educating young scientists. His academic lectures were popular and highly esteemed by the students.

He accepted his disease with a clear and quiet mind and endured his sufferings with patience and self-control. He is survived by four children and his wife, also a doctor, who shared not only his life, but as a constant collaborator, also his work.

F. VARGA

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Physiologia

PULSATILE CEREBRAL IMPEDANCE: A METHOD FOR MONITORING CHANGES IN CEREBRAL CIRCULATION

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A method of measuring pulsatile cerebral impedance is described. Effects of hypercapnia and hypoxia on the amplitude of pulsatile cerebral impedance are demonstrated.

Since the introduction of the nitrous oxide method by KETY and SCHMIDT (1948), a number of methods have been developed for measuring cerebral blood flow (CBF). An ideal method should

1) give values for total CBF or CBF in ml · $100g^{-1}$ · min^{-1};
2) be suited for measuring CBF in unanaesthetized, unrestrained animals;
3) permit continuous measurement of CBF;
4) be applicable to circumscribed areas of the brain.

No method available so far fulfils all the criteria listed; at most, three of the requirements could be complied with by any of the methods.

Measurement of electrical impedance pulsations in the brain, the so-called pulsatile cerebral impedance (PCI), has proved sensitive to changes in cerebral circulation. The method yielded new information on brain haemodynamics, such as selective vasodilation in the hypothalamus during arousal in the cat (BIRZIS et al. 1968).

A modification of the method of BIRZIS and TACHIBANA (1962) is described below.

Methods

The schematic diagram of the impedance circuit is shown by Fig. 1. The audio-oscillator was set at 20 kc and the output was adjusted to give 100 mV across the electrodes. The initially balanced impedance bridge was subsequently imbalanced by decreasing or increasing the variable resistance, while capacitance was always set at a value required for the bridge to be balanced for capacitance.

The electrodes were made of platinum wire 0.3 mm in diameter insulated with Araldite except at the sharp 0.5 mm long tip. In some cases along the electrode a polyethylene cannula was attached, into which a fine copper–constantan thermocouple was inserted up to the level of the electrode tip just before the experiment (Fig. 2).

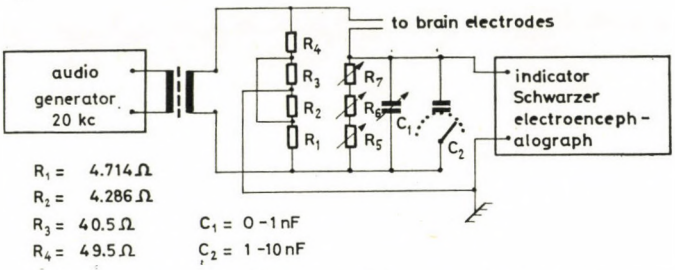

Fig. 1. Schematic diagram of the impedance circuit

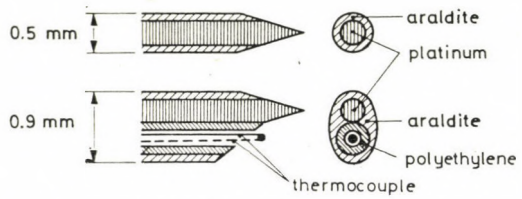

Fig. 2. Schematic diagram of the platinum electrodes

Albino rats of both sexes from the Institute's colony weighing 200—300 g were used. Under hexobarbital anaesthesia in the skull two holes were drilled 2 to 4 mm apart on one side of the midline. After the dura was incised at both sites, the two platinum electrodes were introduced into the brain to equal depths, 3, 3.5, 4.5, 5, 6, 7, 8 or 8.5 mm from the surface of the skull. Dental cement and acrylate were used to fix the electrodes to the skull. One or two screws served to secure the electrode assembly in place.

On the day of the experiment the rats were superficially anaesthetized with a small intravenous dose of hexobarbital. In some cases 30—40 mg urethane per 100 g body weight was given intraperitoneally to reduce activity. Fine copper-wire electrodes were inserted subcutaneously into one foreleg and one hindleg to record the ECG. In experiments in which no ECG was recorded, no anaesthetics were used.

After the effect of hexobarbital had worn off, the rat was placed into the metabolic chamber, which, in turn, was placed into a thermostatically controlled water bath.

The responsiveness of pulsatile impedance to changes in CBF was tested by introducing into the metabolic chamber gas mixtures containing 3, 6 and 9% CO_2 in air, or 8 and 12% O_2 in N_2, at an ambient temperature of 30°C.

PCI and ECG were recorded by a Schwarzer electroencephalograph, either continuously or for periods of 15—20 sec every minute. The amplitude of PCI was calculated by averaging 10 consecutive waves at the end of every minute. Values for PCI amplitudes were expressed in millimeter, since the amplification of output from the impedance bridge varied in different experiments. Experiments, in which PCI amplitude could not be amplified higher than 2—3 mm on the original record under control conditions, were discarded.

Each animal was used at least on two occasions, the interval between two experiments being at least 4 days.

Results

Interelectrode impedance was composed of 7 to 19 k Ω resistance and 270—460 pF capacitance, respectively, depending on the distance and the position of the electrodes. In every experiment the change in interelectrode resistance was in the range of 0 to 200 ohm in either direction during inhalation of hypercapnic or hypoxic gas mixtures.

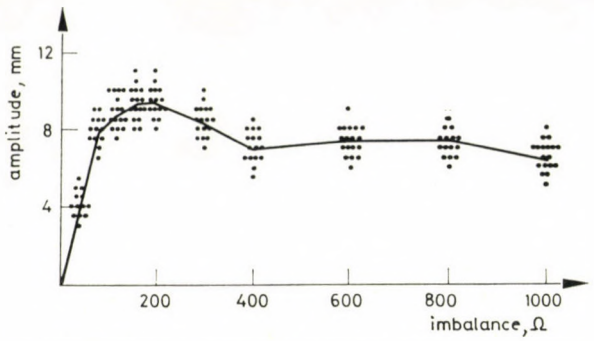

Fig. 3. Effect of the degree of imbalance of the impedance bridge on the amplitude of PCI. Unanaesthetized rat. Interelectrode distance 2 mm, depth 6 mm

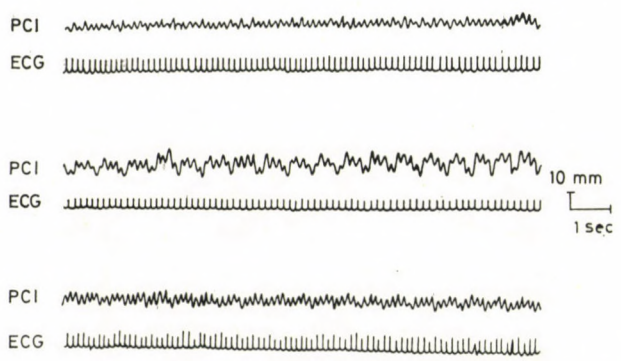

Fig. 4. Records of ECG and PCI in three different experiments

The effect of the degree of imbalance with respect to resistance in the impedance bridge is shown by Fig. 3. When both resistance and capacitance were balanced, no pulsations were obtained in the impedance record. On increasing the degree of imbalance of the resistive component, a sudden rise in amplitude could be seen reaching a peak value at about 200 ohm imbalance. Between 400 and 800 ohm imbalance, practically no further change in amplitude could be observed. This means that within a range of about 400 ohm, PCI was independent of the degree of imbalance. Thus the standard procedure has been to apply an imbalance of 500—600 ohm from the actual value of interelectrode resistance. Considering that the maximum change did not exceed 200 ohm in the course of an experiment, we worked within a range of imbalance in which a slow shift in interelectrode resistance per se did not influence the pulsatile component of impedance.

Fig. 4 shows the PCI and ECG records from three different experiments. The most prominent component of impedance pulsation was synchronous with the cardiac cycle. An additional but slow oscillation in impedance was related to respiration and varied from animal to animal.

It has long been known that CO_2 inhalation causes in mammals, including the rat, an increase in CBF. A continuous record of PCI can be seen in Fig. 5. Pure CO_2 gas was introduced for 10 seconds into the persplex box, which accommodated an unanaesthetized rat. This strong but rather short stimulus caused an almost immediate and marked rise in PCI. Simultaneously, impedance changes associated with respiratory movements appeared. At the height of the response, PCI stabilized at a considerably higher level for some 30 sec and than diminished gradually towards the initial level.

The effect of inhalation of 3, 6 and 9% CO_2 in air can be seen in Fig. 6. 3% CO_2 had practically no effect on PCI, while 6% as well as 9% CO_2 caused a considerable rise in PCI, the effect of the latter gas mixture being more prominent. This effect of CO_2 on PCI could generally be reproduced in the same experiment and on some occasions in the same animal in experiments carried out several days apart (Fig. 7).

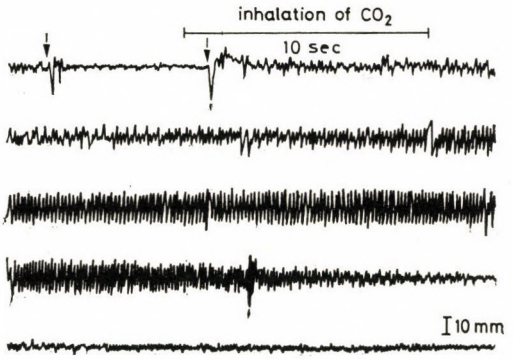

Fig. 5. Continuous record of PCI 6 days after operation. 40 mg urethane per 100 g body weight intraperitoneally. Interelectrode distance, 2 mm; depth, 6 mm. Arrows indicate bursts of gross movements of the rat

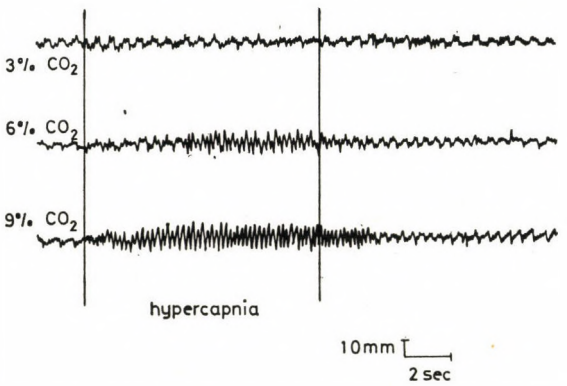

Fig. 6. Effect on PCI of breathing CO_2, 20 days after operation. 40 mg urethane per 100 g body weight. Interelectrode distance, 4 mm; depth, 4 mm. Periods of 2 sec at the end of every minute

Hypoxic gas mixtures containing 12% O_2 in N_2 did not cause any change in PCI, but 8% O_2 increased PCI very markedly (Fig. 8).

In most experiments the sensitivity of PCI to indicate changes in CBF was, therefore, tested by supplying to the rat 6% CO_2 and 8% O_2. As shown in Fig. 9, both 6% CO_2 and 8% O_2 elicited a marked increase in amplitude of approximately similar magnitude.

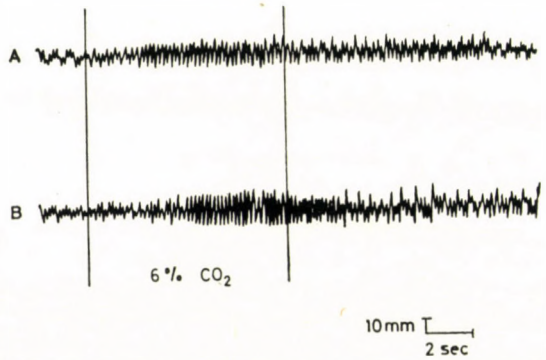

Fig. 7. Effect on PCI of breathing 6% CO_2; A: 5 days after operation; B: 15 days after operation. 50 mg urethane per 100 g body weight in both experiments. Interelectrode distance, 3 mm; depth, 8 mm. Periods of 2 sec at the end of every minute

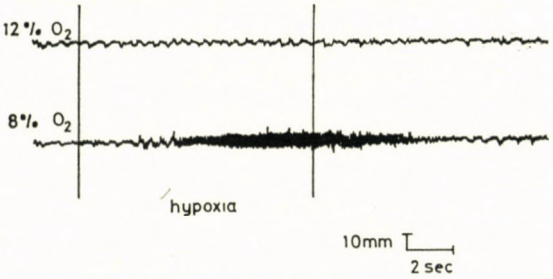

Fig. 8. Effect on PCI of breathing 12% and 8% O_2 recorded at the same experimental session as Fig. 6. Periods of 2 sec at the end of every minute

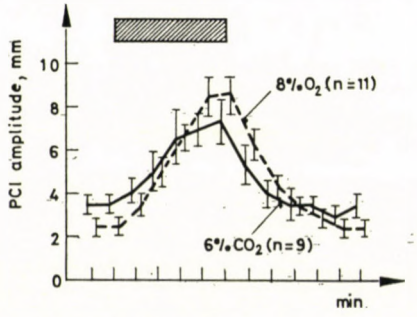

Fig. 9. Effect on PCI of breathing 6% CO_2 and 8% O_2. Horizontal hatched bar indicates exposure to hypercapnia and to hypoxia. Vertical bars: ± S.E.

Discussion

The methods in use for assessing CBF were dealt with in detail in a review by BETZ (1972), but only few words were devoted to the measurement of PCI.

SUGANO and INANAGA (1961) were the first to apply intracerebral impedance measurements for observing changes in cerebral haemodynamics in the cat. Detailed studies utilizing similar techniques furnished indirect evidence that this method indicates the pulsatile component of CBF in the area between the measuring electrodes (BIRZIS and TACHIBANA 1962, 1964; BIRZIS et al. 1968). It was also shown that the amplitude of pulsatile impedance is a measure of flow velocity of salt solutions in vitro (BIRZIS 1966). Another group of investigators utilized this method in man for recording differences between normal brain tissue and glioma (LAITINEN et al. 1966; LAITINEN and JOHANSSON 1968).

Our platinum electrodes may have been relatively large for the small size of the rat's brain, but the interelectrode distance sufficed to leave enough brain tissue with intact circulation between the electrodes. As a safeguard only those observations were evaluated in which the amplitude of PCI waves excedeed 3 mm.

As for the reliability of PCI to monitor CBF, BIRZIS et al. (1968) found parallel changes in CBF measured with the electromagnetic method and by PCI. The same authors demonstrated that PCI was not sensitive to arterial pressure per se, since an increase in PCI followed the stimuli irrespective of the direction of the blood pressure change.

In the present investigation, measurement of PCI was also found useful for obtaining information about CBF. Breathing of hypercapnic and hypoxic gas mixtures elicited increases in PCI similar to the rise in CBF measured by other methods.

No attempt has been made to express PCI measurements in terms of CBF, since the method is not suitable for measuring CBF in absolute terms (BIRZIS et al. 1968). For the same reason, results obtained with electrodes at different depths have not been compared directly. The method permits, however, to study cerebral haemodynamics in practically awake, unrestrained animals; continuous records of PCI allow observation of transient and sudden changes in CBF. The relative ease of construction of the impedance bridge and of making the electrodes is a further advantage. In addition, the experiment can be repeated several times in the same animal over a prolonged period, extending in many instances to 2 to 3 months.

A further advantage of PCI is that it is not influenced by unequal changes in heat production, and/or in heat loss at the sites of the two electrodes.

Acknowledgements

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THE INFLUENCE OF BODY MASS ON THERMOREGULATORY RESPONSES OF NEWLY BORN AND ADULT GUINEA PIGS TO CHANGES IN AMBIENT TEMPERATURE, TO HYPOXIA, AND TO HYPERCAPNIA

By

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The relationship between body weight (BW) and the changes in colonic temperature (ΔT_c) was analyzed in guinea pigs aged less than 2 days, 3 to 6 days, 7 to 9 days, and 10 to 20 days, as well as in adult animals, in response to transfer from a thermoneutral environment to a moderately cold one (newly born: 20°C; adults: 20° and 10°C). Subsequently, the animals were exposed at these ambient temperatures (T_a) to 8% O_2 and to 6% CO_2. No correlation could be demonstrated between BW and ΔT_c in response to transfer to the colder environment in any age-group with the exception of the group aged 10 to 20 days. In none of the groups was significant correlation observed between BW and the increase in heat production (ΔO_2) in response to transfer to the cold. In the newly born, no relationship was found between BW and ΔT_c in hypoxia, whereas in adult animals exposed to hypoxia at T_a 20°C, the coefficient of correlation proved to be significant statistically, but indicated that the reduction in T_c tended to increase with increasing BW. In hypercapnia, a statistically significant correlation was observed between BW and T_c in the group aged 7 to 9 days, and coefficients of similar magnitude were obtained with adult animals both at T_a 20°C and at T_a 10°C, showing that the fall in T_c tended to be greater in the smaller animal. It was concluded that under conditions not leading to progressive hypothermia, BW, i.e. the mass–surface ratio is, at most, a minor determinant of the changes in T_c.

The newly born of many mammalian species fail to maintain core temperature within the range considered normal for the adult animal when exposed to a moderately cold environment. These observations are usually explained by the unfavourable body mass–body surface ratio. In a short communication based on observations made in the period 1963 to 1967, it has been shown that body weight (BW) was not an important determinant of the fall in core temperature in response to transfer from a thermoneutral environment to a moderately cool one, neither in the new-born rabbit, nor in the new-born guinea pig (FARKAS et al. 1972). Since these observations were confined to the response elicited by changes in ambient temperature (T_a), the relationship between body mass and core temperature was investigated under the effect of hypoxia and hypercapnia, i.e. under conditions not involving changes in the thermal environment.

Methods

Newly born and adult guinea pigs of both sexes, bred and reared in the Institute's animal house and fed a standard diet, were used. The observations on new-born guinea pigs were made throughout 1971 and 1972, those on adult animals in 1973. For the sake of comparison, observations on new-born guinea pigs from the years 1963 to 1967 have been included. Between 1967 and 1970 the guinea pig colony of the Institute had been reduced in numbers, and moving of the animals into the new animal house in 1970 changed breeding and rearing conditions very markedly. Therefore, the older observations do not refer to a population identical with the present one.

Oxygen consumption (VO_2) was measured by a modified Noyons—Kipp type diaferometer, using an airflow of 0.7 to 1.4 liter/min for the new-born, and 2.0 liter/min for the adult animals. Colonic temperature was recorded by copper–constantan thermocouples introduced to a depth of 5 to 10 cm according to the size of the animal. The strictly standardized thermal conditions and other details of the experimental setup have been described earlier (DONHOFFER et al. 1958; VÁRNAI et al. 1970; FARKAS and DONHOFFER 1973a). After introducing the guinea pigs into a metabolic chamber of appropriate size, this was submerged into a water-bath providing an ambient temperature (T_a) of 35°C for the two youngest age-groups, and either T_a 35°C or 30°C for the two older new-born groups; adult animals were placed into the water-bath providing T_a 30°C. Measurements were started 30 to 45 min after submerging the metabolic chamber into the water-bath. Thirty min thereafter the chamber was transferred from T_a 35°C to T_a 30°C, and after another 30 min to T_a 20°C, as were those of the older animals in which exposure to T_a 35°C had been omitted. Most of the adult animals were ultimately exposed to T_a 10°C as well. The effect of hypoxia (8% O_2 in N_2) and hypercapnia (6% CO_2 in air) was observed for 30 min without changing the T_a.

Figures for VO_2 represent averages of readings taken every minute during the last 10—15 min of each 30 min period. Figures for T_c represent values attained at the end of each period. The recording galvanometers have been read, however, every minute throughout the experiment. Pearson's product–moment correlation coefficient r was used for statistical evaluation. Body surface was calculated according to the formula $10 \cdot kg^{0.67}$.

Results

Table I demonstrates that, with the exception of the group aged 10 to 20 days, no significant relationship could be established between BW and ΔT_c subsequent to transfer to T_a 20°C both in the new-born and the adult animals, nor in the latter when transferred to T_a 10°C.

Since the correlation was significant in one age-group only, the question arose whether this might have been due to chance, however, improbable this event appeared to be. Therefore, the results of the observations from the years 1963 to 1967 were calculated for the same age-group, animals aged 7 to 20 days having been lumped into one group earlier. As shown by Part B of Table I, the relationship between BW and ΔT_c was significant statistically in the age-group of 10 to 20 days in the older series also, whereas no relationship between the same variables could be established in the younger animals. The number of observations on new-borns aged 7 to 9 days was too small in the older series for statistical evaluation, and from those years data for adult guinea pigs were not available.

The possibility could not be excluded that the lack of correlation between BW and ΔT_c might have been due to a greater increase in heat production in the smaller animals. Therefore, the relationship between body mass and

Table I

Relationship between body weight (BW) and the changes in colonic temperature (ΔT_c) in response to transfer from T_a 30°C to T_a 20°C. In brackets, number of observations, range of BW and ΔT_c. Part A: observations dating from 1971 and 1972; Part B: observations dating from 1963 to 1967. Experiments on adult animals were performed from 1970 to 1973

Variables	Age		r	t	p
	BW, g (M ± S.E.)	$\Delta T_{c(30-20°)}$ (M ± S.E.)			
Part A (1971 and 1972)					
< 2 days (46)	91±3 (55–131)	−0.51±0.09 (+0.54 to −2.64)	+0.168	1.132	>0.2
3 to 6 days (24)	88±4 (57–136)	−1.12±0.14 (+0.34 to −2.52)	+0.087	0.410	>0.6
7 to 9 days (21)	104±5 (67–155)	−1.22±0.13 (−0.20 to −3.09)	+0.228	1.019	>0.3
10 to 20 days (29)	130±7 (85–229)	−1.37±0.12 (−0.22 to −2.62)	+0.491	2.926	<0.01
Adult (48)	662±15 (525–850)	−0.66±0.08 (+0.44 to −2.61)	+0.024	0.164	>0.8
Adult (44)	663±15 (525–850)	−0.98±0.13* (+0.55 to −2.94)	+0.180	1.188	>0.2
Part B (1963 to 1967)					
< 2 days (49)	89±2 (58–115)	−0.50±0.08 (+0.22 to −2.39)	+0.038	0.258	>0.7
3 to 6 days (21)	94±4 (70–132)	−1.08±0.14 (−0.14 to −2.35)	−0.042	0.183	>0.8
10 to 20 days (22)	143±6 (105–200)	−1.21±0.13 (−0.06 to −2.11)	+0.442	2.200	<0.05

* ΔT_c in response to transfer from T_a 30°C to T_a 10°C

the change in heat production (ΔO_2) in response to cold exposure was also analyzed. Table II shows that no significant correlation could be demonstrated between these variables. The small correlation coefficient obtained with adult animals between BW and ΔO_2 subsequent to transfer from T_a 30°C to T_a 10°C approached statistical significance, and might indicate that under certain conditions the increase in heat production in response to cold was slightly correlated to BW, the smaller animals tending to increase heat production more than the larger ones.

Tables III and IV contain the data on the relationship between BW and ΔT_c in response to hypoxia and to hypercapnia. At T_a 20°C, no correlation was found between BW and ΔT_c during exposure to 8% O_2 in the newly born, nor could one be demonstrated at T_a 10°C in the adult animals. At T_a 20°C, however, a significant correlation was established in adults between the same

Table II

Relationship between body weight (BW) and the changes in oxygen consumption (ΔO_2) in response to transfer to T_a 20° from T_a of minimum heat production. In brackets, number of observations, and ranges of BW and ΔO_2

Age	Variables		r	t	p
	BW, g (M ± S.E.)	$\Delta O_{2(30-20°)}$ ml/dm² · min (M ± S.E.)			
< 2 days (43)	92±3 (55—131)	+1.04±0.04* (+0.29 to +1.59)	+0.151	0.976	>0.3
3 to 6 days (24)	88±4 (57—136)	+0.66±0.05* (+0.22 to +1.10)	+0.228	1.379	>0.1
7 to 9 days (21)	104±5 (67—155)	+0.76±0.05 (+0.57 to +1.14)	+0.012	0.053	>0.9
10 to 20 days (28)	129±7 (85—229)	+0.78±0.05 (+0.20 to +1.20)	−0.150	0.774	>0.4
Adult (42)	666±16 (525—850)	+0.32±0.05 (−0.40 to +1.13)	+0.147	0.965	>0.3
Adult (42)	666±16 (525—850)	+1.12±0.04** (+0.56 to +1.75)	−0.279	1.879	0.1>p>0.05

* ΔO_2 based on oxygen consumption at T_a 35°C or T_a 30°C, whichever was the lower
** ΔO_2 in response to transfer from T_a 30°C to T_a 10°C

Table III

Relationship between body weight (BW) and the changes in colonic temperature (ΔT_c) in response to exposure to 8% O_2 at T_a 20°C in the newly born, and at T_a 20°C and T_a 10°C in the adult animals. In brackets, number of observations and the range of BW and ΔT_c

Age	Variables		r	t	p
	BW, g (M ± S.E.)	$\Delta T_{c(8\% O_2)}$ (M ± S.E.)			
2 days (32)	91±3 (55—126)	−2.29±0.12 (−0.86 to −3.56)	−0.116	0.642	>0.5
3 to 6 days (22)	89±4 (57—136)	−1.64±0.11 (−0.78 to −2.65)	−0.094	0.422	>0.6
7 to 9 days (21)	99±5 (67—155)	−2.01±0.11 (−1.36 to −3.06)	−0.217	0.970	>0.3
10 to 20 days (26)	133±7 (85—229)	−2.26±0.12 (−0.24 to −3.64)	−0.298	1.529	>0.1
Adult (22)	633±19 (530—850)	−1.16±0.08 (−0.63 to −1.86)	−0.563	2.841	<0.02
Adult (10)	694±35 (540—840)	−2.18±0.10* (−1.75 to −2.69)	−0.270	0.794	>0.4

* Exposed to hypoxia at T_a 10°C

Table IV

Relationship between body weight (BW) and the changes in colonic temperature (ΔT_c) in response to exposure to 6% CO_2 at T_a 20°C in the newly born, and at T_a 20°C and T_a 10°C in the adult animals. In brackets, number of observations and the range of BW and ΔT_c

Age	Variables		r	t	p
	BW, g (M ± S.E.)	$\Delta T_{c(6\%\,CO_2)}$ (M ± S.E.)			
< 2 days (30)	89±3 (55—131)	−1.24±0.10 (+0.02 to −2.80)	+0.052	0.273	>0.7
3 to 6 days (22)	89±4 (57—136)	−1.31±0.13 (−0.30 to −2.50)	+0.251	1.160	>0.2
7 to 9 days (21)	99±5 (67—155)	−1.40±0.11 (−0.38 to −2.31)	+0.459	2.251	< 0.05
10 to 20 days (24)	136±7 (85—229)	−1.53±0.15 (−0.18 to −3.00)	+0.027	0.125	>0.9
Adult (14)	606±21 (525—820)	−0.92±0.04 (−0.46 to −1.23)	+0.463	1.807	0.1 > p > 0.05
Adult (14)	685±31 (540—840)	−1.19±0.11* (−0.58 to −2.09)	+0.420	1.601	>0 1

* Exposed to hypercapnia at T_a 10°C

variables. The negative sign of the coefficient indicates, however, that the smaller the body mass, the smaller tended to be the reduction in core temperature.

In hypercapnia, the correlation between the same variables (BW and T_c) was found to be significant statistically in the group aged 7 to 9 days, and correlation coefficients of similar magnitude were obtained with adult animals, although the latter failed to reach statistical significance, the number of observations being comparatively small. The positive coefficients indicated that in hypercapnia the fall in T_c tended to be smaller in the larger animals, as would be expected on the basis of the mass–surface ratio.

Discussion

The results have confirmed and extended the earlier observations showing that body mass is not an important determinant of the change in core temperature subsequent to transfer from a thermoneutral, or near thermoneutral, environment to a moderately cold one. The fact that in guinea pigs aged 10 to 20 days statistically significant coefficients of correlation of similar magnitude were obtained in two series of observations separated by an interval of a few years, and made on animals bred and reared under very different conditions, lends weight to the conclusion that in this age-group the relationship between BW and ΔT_c, although not a dominant one, is never-

theless real, although no corresponding relationship was revealed by the other age-groups of the newly born or by the adult animals.

No significant relationship could be demonstrated in any age-group between BW and ΔO_2 subsequent to transfer from a thermoneutral environment to a moderately cold one (Table II). The increase in heat production was significantly highly greater in the youngest age-group than in any of the others, while mean BW and the range of BW were practically identical in the groups aged less than 2 days and 3 to 6 days, the difference in ΔO_2 being reflected in the greater reduction of T_c in animals aged 3 to 6 days (Table I). Evidently, age and not body mass was the main determinant of thermoregulatory heat production in response to the lower T_a.

In the new-born, the fall in colonic temperature during exposure to 8% O_2 showed no correlation with BW. In adult guinea pigs exposed to 8% O_2 at T_a 20°C, a significant correlation was, however, established between the same variables. The negative sign of the coefficient r indicates that the greater the body weight, the more the core temperature tended to fall in hypoxia (Table III). On the basis of the body mass–body surface ratio the opposite would be expected.

Attention has to be paid to the difference in the relationship between the same variables in hypoxia and in hypercapnia. In hypercapnia (Table IV), a significant correlation between BW and ΔT_c was observed in the group aged 7 to 9 days, and coefficients of similar megnitude were obtained with adult animals. The coefficients were, however, positive and indicate therefore that in hypercapnia a greater BW tended to be associated with a smaller reduction in T_c. The difference between hypoxia and hypercapnia is rather impressive: in the adult animal at T_a 20°C, for instance, $r = -0.563$ in hypoxia, and $r = +0.463$ in hypercapnia. These results are in complete agreement with earlier ones obtained on new-born rats (VÁRNAI et al. 1970), new-born rabbits (VÁRNAI et al. 1971), and adult guinea pigs (FARKAS 1973; FARKAS and DONHOFFER 1973a, b), which showed that the mechanisms of the thermoregulatory effects of hypoxia and hypercapnia are not identical and that the ratio in which mechanisms of heat loss and of heat production participate in the response was greater in hypercapnia than in hypoxia.

On the whole, the relationship between body mass and core temperature was remarkably feeble whether changes in ambient temperature, hypoxia, or hypercapnia were applied as stimuli, and when statistically significant correlation was observed, this was confined to one or the other age-group. Body mass is, therefore, at most a minor determinant of the change in deep body temperature. This naturally refers not to environments leading to progressive hypothermia, under which conditions body mass is evidently an important determinant of the rate of fall of core temperature, as it is in the rate of cooling of the dead animal.

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THERMOREGULATORY HEAT PRODUCTION AND THE REGULATION OF BODY TEMPERATURE IN THE NEW-BORN AND THE ADULT GUINEA PIG

By

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The responses of colonic temperature (T_c) and heat production (VO_2) to changes in ambient temperature (T_a) were studied in new-born guinea pigs aged less than 2 days, 3 to 6 days, 7 to 9 days, and 10 to 20 days, as well as in adult animals. In the newly born, VO_2 expressed per unit body weight (BW) decreased with age at T_a 35°, 30°, and 20°C. Using instead of unit BW, unit body surface ($10 \cdot kg^{0.67}$) as the frame of reference, VO_2 was identical in the four new-born groups at T_a 35° and T_a 30°C, whereas at T_a 20°C a highly significant reduction in VO_2 was observed between the group aged less than 2 days and that aged 3 to 6 days. This reduction was followed by a progressive, significant increase in thermoregulatory heat production with age. T_c decreased with age significantly at all three T_a-s, and within the same age-group T_c decreased with decreasing T_a. The decrease in T_c in response to transfer from T_a 35° to T_a 30°C was of a similar magnitude in all age-groups, whereas the decrease in response to transfer from T_a 30° to T_a 20°C was highly significantly smaller in the youngest age-group than in any other of the new-born groups, and even smaller, although not significantly so, than that observed in adult animals. Distribution of the changes in T_c in response to transfer from T_a 30° to T_a 20°C ranged over a fairly wide domain even in the adult guinea pig. Taking as the dividing line changes in T_c not exceeding −0.5°C, or −1.0°C, the χ^2 test revealed highly significant differences between the group aged less than 48 hours and the older new-born groups. No significant difference was found between the youngest new-born group and the adult animals, whereas the differences between the older new-born groups and the adult animals were significant. It has been concluded that the variability in the responses of T_c to a reduction of T_a was due neither to "immaturity" or "inability" to increase heat production, nor to an unfavourable mass–surface ratio, but to a functional variant of the cybernetic system controlling body temperature and thermoregulatory heat production.

After studying the regulation of body temperature and thermoregulatory heat production in response to changes in ambient temperature in the new-born rat (VÁRNAI 1961; VÁRNAI and DONHOFFER 1970), and in the new-born rabbit (VÁRNAI et al. 1970), similar observations on new-born guinea pigs were of special interest, since guinea pigs are born, in contrast to the rat and rabbit, at an advanced stage of maturity, and are generally held to maintain deep body temperature when exposed to moderate cold. The observations of BRÜCK and WÜNNENBERG (1965, 1966) demonstrating a profound change during the first weeks of life in the source of thermoregulatory heat production, i.e. in the ratio of non-shivering and shivering thermogenesis, added to the interest of the problem.

Methods

Newly born and adult guinea pigs of both sexes, bred and reared in the Institute's animal house and fed a standard diet, were used. The observations on new-born guinea pigs were made throughout 1971 and 1972, those on adult animals from 1970 to 1973. For the sake of comparison, observations on new-born guinea pigs from the period 1963 to 1967 have been included (Tables III to VII). Between 1967 and 1970 the guinea pig colony of the Institute had been reduced in number, and moving the animals into a new animal house changed housing and rearing conditions profoundly. Therefore, the older observations cannot be held to originate from a population identical with the present one.

Oxygen consumption (VO_2) was measured by a modified Noyons-Kipp diaferometer using an air flow of 0.7 to 1.4 litre/min for the newly born, and of 2.0 litre/min for the adult animals. Colonic temperature (T_c) was recorded by copper–constantan thermocouples introduced to a depth of 5 to 10 cm, according to the size of the animal. The strictly standardized thermal conditions and other details of the experimental setup have been described earlier (DONHOFFER et al. 1958; VÁRNAI et al. 1970). After introducing the guinea pigs into a metabolic chamber of appropriate size, this was submerged into a water-bath ensuring an ambient temperature (T_a) of 35°C for the two youngest groups, and of either 35° or 30°C for the two older new-born groups. Adult animals were always placed into the water-bath providing T_a 30°C. Measurements were started 30 to 45 min after the metabolic chamber had been submerged into the water-bath. Thirty min thereafter the chamber was transferred from T_a 35° to T_a 30°C, and from there, after another 30 min, to T_a 20°C, as were those of the older animals not exposed initially to T_a 35°C. Most of the adult animals were exposed ultimately also to T_a 10°C.

Figures for oxygen consumption represent averages of readings taken every minute during the last 10—15 min of each 30 min period. Data on T_c represent the values attained at the end of each period. The recording galvanometers were, however, read every minute throughout the experiment. Student's two-tailed t test and the χ^2 test with Yates' modification for small numbers were used for statistical evaluation. Body surface was calculated according to the formula $10 \cdot kg^{0.67}$.

Results

Table I, Part A shows that at T_a 35° and T_a 30°C, VO_2 per unit body surface did not change with age. At T_a 20°C, VO_2 was, however, significantly lower in the groups aged 3 to 6 and 7 to 9 days than in animals aged less than 48 hours. The progressive increase in mean VO_2 at T_a 20°C between the groups aged 3 to 6 and 10 to 20 days approached, but failed to reach, statistical significance, as did the difference between mean VO_2 of the youngest and the oldest group.

In Part B of Table I, VO_2 was expressed per unit body weight (BW) instead of per unit body surface. Per unit BW, VO_2 decreased progressively with age from the youngest to the oldest age-group at T_a 35° and T_a 20°C, and a statistically significant decrease was observed also at T_a 30°C between the group aged 3 to 6 days and the two older age-groups.

Part C of Table I is based on the means of the changes in individual observations. The changes in VO_2 subsequent to transfer from T_a 35° to T_a 30°C were practically identical in the three younger age-groups, and the difference between the youngest and the oldest group only approached statistical significance. The change in VO_2 (ΔVO_2) subsequent to transfer from T_a 30° to T_a 20°C was, however, significantly greater in the youngest group than in any other age-group. In contrast to the differences between mean VO_2

Table I

Oxygen consumption (VO_2) at T_a at 35°, 30°, and 20° C in ml/dm²·min (Part A), and in ml/kg·min (Part B), and the change in heat production (ΔVO_2) in response to transfer from T_a 35° to T_a 30°, and from T_a 30° to T_a 20° C (Part C). In brackets, number of observations

A
VO_2 ml/dm² · min
(M ± S.E.)

Age	T_a 35°C	T_a 30°C	T_a 20°C
< 2 days	1.14±0.03 (40)	1.13±0.03 (42)	2.05±0.05 (44)
	p > 0.1		p < 0.001
3 to 6 days	1.07±0.04 (12)	1.15±0.02 (23)	1.76±0.05 (24)
		0.1 > p > 0.05	p > 0.2
7 to 9 days	1.05±0.07 (8)	1.08±0.03 (21)	1.84±0.04 (21)
	p > 0.2	p > 0.1	
10 to 20 days	1.17±0.07 (11)	1.16±0.04 (27)	1.91±0.05 (31)
Youngest vs. oldest	p > 0.6	p > 0.5	0.1 > p > 0.05

p < 0.01

0.1 > p > 0.05

B
VO_2 ml/kg · min
(M ± S.E.)

< 2 days	25.3±0.6 (40)	25.3±0.8 (42)	44.9±1.1 (44)
	0.1>p>0.05		p < 0.01
3 to 6 days	23.1±1.0 (12)	26.2±0.7 (23)	39.9±1.4 (24)
		p < 0.01	
7 to 9 days	22.3±1.2 (8)	23.4±0.7 (21)	39.4±1.1 (21)
			p > 0.4
10 to 20 days	21.7±1.3 (11)	23.5±0.9 (27)	38.1±1.2 (31)
Youngest vs. oldest	p < 0.02	p > 0.1	p < 0.001

< 0.05

< 0.05

p < 0.001

> 0.3

Table I (continued)

Age	C ΔVO_2 ml/dm² · min (M ± S.E.) T_a 35°–30°C	T_a 30°–20°C
< 2 days	+0.07±0.04 (34)	+0.94±0.04 (46)
		p < 0.001
3 to 6 days	+0.04±0.06 (11)	+0.61±0.05 (23)
		p < 0.05
7 to 9 days	+0.07±0.12 (8)	+0.76±0.05 (21)
10 to 20 days	−0.05±0.05 (10)	+0.77±0.04 (27)
Youngest vs. oldest	0.1 > p > 0.05	p < 0.01

< 0.02

< 0.05

(Table I, Part A), the difference between the means of ΔVO_2 was statistically significant also between the 3 to 6-day-old and the 7 to 9-day-old and between the former and the 10 to 20-day-old group.

Table II, Part A contains the mean T_c-s measured at T_a 35°, 30°, and 20°C; Part B the means of the changes in core temperature (ΔT_c) subsequent to transfer from T_a 35° to T_a 30°C, and from T_a 30° to T_a 20°C. T_c declined highly significantly with T_a in all age-groups. The only exception was the difference between T_a 35° and T_a 30°C in the oldest group, which was not significant. Calculated on the basis of ΔT_c (Part B of Table II), the fall in core temperature was, however, highly significant ($p < 0.001$) in this age-group also.

Comparison of different age-groups showed at T_a 35° and T_a 30°C that T_c was significantly lower in the 10 to 20-day-old animals than in the younger ones. At T_a 20°C, T_c was significantly lower in all three older age-groups than in animals aged less than 48 hours. The differences between T_c of either of the two youngest groups and that of the oldest group were highly significant at all three T_a-s.

Part B of Table II shows that, although T_c was significantly lower in the oldest age-group than in the youngest at both T_a 35° and T_a 30°C, no significant difference could be established between ΔT_c observed subsequent to transfer from T_a 35° to T_a 30°C (ΔT_c 35°—30°). ΔT_c subsequent to transfer from T_a 30° to T_a 20°C (ΔT_c30°—20°) was, however, highly significantly greater in the three older age-groups than that in the youngest one.

In adult animals, ΔT_c following transfer from T_a 30° to T_a 20°C (Table II, Part C) did not differ significantly from that in the animals aged less than 48 hours, and was significantly smaller than that in the other age-groups of the newly born. The fall of T_c in the adult animals after transfer to T_a 10°C

Table II

Colonic temperature (T_c) of new-born guinea pigs at T_a 35°, 30°, and 20°C (Part A) and the change in colonic temperature (ΔT_c) following transfer from T_a 35° to T_a 30°C (ΔT_c 35°−30°), and from T_a 30° to T_a 20°C (Part B). Part C contains for the sake of comparison ΔT_c of adult animals following transfer from T_a 30° to T_a 20°, and from T_a 30° to T_a 10°C. (M±S.E.). In brackets, number of observations

A

Age	T_c at T_a 35°	T_c at T_a 30°	T_c at T_a 20°
< 2 days	39.83±0.10 → < 0.001 ←	39.24±0.06 → < 0.001 ←	38.73±0.09
	(46)↓	(46)↓	(48)↓
	>0.2	>0.3	< 0.01
	↑	↑	↑
3 to 6 days	40.01±0.11 → < 0.01 ←	39.35±0.12 → < 0.001 ←	38.19±0.13
	(12)↓	(24)↓	(24)↓
	>0.1	>0.3	>0.2
	↑	↑	↑
7 to 9 days	39.78±0.13 → < 0.01 ←	39.19±0.11 → < 0.001 ←	37.97±0.14
	(10)↓	(21)↓	(21)↓
	< 0.02	0.1 > p > 0.05	< 0.05
	↑	↑	↑
10 to 20 days	39.23±0.18 → < 0.1 ←	38.92±0.10 → < 0.001 ←	37.57±0.13
	(13)	(29)	(29)
Youngest vs. oldest p	< 0.01	< 0.01	< 0.001

B

Age	ΔT_c 35°−30°	p vs. youngest	ΔT_c 30°−20°	p vs. youngest
< 2 days	−0.45±0.6 (46)	—	−0.51±0.09 (46)	—
3 to 6 days	−0.60±0.14 (12)	> 0.3	−1.12±0.14 (24)	< 0.001
7 to 9 days	−0.53±0.06 (10)	> 0.3	−1.22±0.13 (21)	< 0.001
10 to 20 days	−0.50±0.10 (13)	> 0.6	−1.37±0.12 (29)	< 0.001

C

		p vs. 2 days	p vs. 3 to 6 days	p vs. 7 to 9 days	p vs. 10 to 20 days
Adult ΔT_c (30°−20°)	−0.66±0.08 (48)	> 0.2	< 0.01	< 0.001	< 0.001
Adult ΔT_c (30°−10°)	−0.98±0.13 (44)	< 0.001	> 0.4	> 0.1	< 0.05

was, however, significantly greater than that of the youngest group at T_a 20°C, but significantly smaller than that in the oldest new-born group at T_a 20°C.

Table III demonstrates the corresponding data on body temperature originating from the years 1963 to 1967. Core temperatures of the younger age-groups agreed with those of the newer series (Table II) at all three T_a-s: there was no significant difference between the T_c-s of the two younger groups at T_a 35° and T_a 30°C, whereas at T_a 20°C, T_c was significantly lower in the group aged 3 to 6 days than in the group aged less than 48 hours. The 10 to 20-day-old group of the older series resembles the recent one in that T_c declined progressively with T_a. In contrast, the two series differ significantly at all three T_a-s in their relationship to the T_c-s of the younger age-groups. Whereas in the recent observations (Table II, Part A) T_c at the age of 10 to 20 days was significantly lower than the T_c of the two youngest groups, in the older series (Table III, Part A) it was significantly higher than that of the youngest group at T_a 35° and T_a 30°C, and almost significantly higher than that of the 3 to 6-day-old at T_a 20°C.

Part B of Table III shows that ΔT_c between T_a 35° and T_a 30°C was practically identical in the three age-groups, and even more so than in the recent series (Table II, Part B). The differences between the two series were, however, not significant. Following transfer from T_a 30° to T_a 20°C, ΔT_c was almost identical in the two series. It should be noted that despite the highly significant difference between the T_c-s of the 10 to 20-day-old groups of the two series, ΔT_c-s were practically identical in this age-group also.

Since averages tend to obscure the variety of individual observations, the distribution of T_c around the mean at T_a 30° and T_a 20°C, as well as the distribution of ΔT_c subsequent to transfer from T_a 30° to T_a 20°C have also been analyzed. Table IV and V show that both at T_a 30° and at T_a 20°C, core temperature varied within fairly wide limits in all age-groups of the newly born, and even in the adult animals. Approximately 70% deviated, however, only within $\pm$ 0.5°C of the mean at T_a 30°C and — excepting the adult animals — between 55 and 62% varied within the same limits also at T_a 20°C. No essential difference emerged between the older and the more recent series, except that in the older series the deviation from the mean exceeded $\pm$ 1.0°C in none of the animals aged 10 to 20 days.

Table VI demonstrates the distribution of ΔT_c in response to transfer from T_a 30° to T_a 20°C. In the newly born, ΔT_c increased with age, at least during the first three weeks of life. Accordingly, ΔT_c of the group aged less than 48 hours resembled much more closely that of the adult animals than that of any of the older groups of the newly born. Another feature deserving attention was the concordant distribution of ΔT_c in the older and in the recent series of observations.

Table III

Colonic temperature (T_c) of new-born guinea pigs at ΔT_a 35°, 30°, and 20° (Part A), and ΔT_c in response to transfer from T_a 35° to T_a 30°C, and from T_a 30° to T_a 20°C (Part B), based on observations made from 1963 to 1967. In brackets, number of observation ($M \pm$ S.E.)

A

Age	T_c at T_a 35°	T_c at T_a 30°	T_c at T_a 20°
< 2 days (49)	39.40 ± 0.05 → < 0.01	← 39.07 ± 0.07 → > 0.001	← 38.60 ± 0.11
	↓ >0.4 ↑	↓ >0.2 ↑	↓ < 0.05 ↑
3 to 6 days (21)	39.49 ± 0.10 → 0.1 > p > 0.05	← 39.19 ± 0.10 → < 0.001	← 38.11 ± 0.19
	↓ < 0.001 ↑	↓ < 0.01 ↑	↓ 0.1 > p > 0.05 ↑
10 to 20 days (22)	40.02 ± 0.09 → < 0.05	← 39.68 ± 0.10 → < 0.001	← 38.47 ± 0.09
Youngest vs. oldest p	< 0.001	< 0.001	> 0.3

B

Age	ΔT_c(35°−30°)	p vs. youngest	ΔT_c (30°−20°)	p vs. youngest
< 2 days (49)	-0.33 ± 0.05	—	-0.50 ± 0.08	—
3 to 6 days (21)	-0.30 ± 0.18	>0.8	-1.08 ± 0.14	< 0.001
10 to 20 days (22)	-0.30 ± 0.06	>0.7	-1.21 ± 0.13	< 0.001

Table IV

Distribution of colonic temperature around the mean at T_a 30°C. In brackets, number of observations

Age		T_c, °C M ± S.E.	ΔT_c, °C				
			−1.0 to −1.5	−0.5 to −1.0	±0.5	+0.5 to +1.0	+1.0 to +1.5
			%				
< 2 days	1971−72 (46)	39.24±0.06	2.2	6.5	76.1	15.2	—
	1963−67 (49)	39.09±0.08	4.1	12.2	65.3	16.3	2.1
3 to 6 days	1971−72 (24)	39.28±0.09	—	12.5	70.9	8.3	8.3
	1963−67 (21)	39.20±0.12	9.5	9.5	66.7	14.3	—
7 to 9 days	1971−72 (21)	39.19±0.11	4.8	14.3	61.8	14.3	4.8
10 to 20 days	1971−72 (31)	38.92±0.10	—	9.7	70.9	9.7	9.7
	1963−67 (22)	39.69±0.10	4.5	9.1	72.8	13.6	—
Adult	1970−73 (48)	39.19±0.07	—	12.5	72.9	12.5	2.1

Table V

Distribution of colonic temperature around the mean at T_a 20°C. In brackets, number of observations

Age		T_c, °C M ± S.E.	ΔT_c, °C						
			−2.0	−1.5 to −2.0	−1.0 to −1.5	−0.5 to −1.0	±0.5	+0.5 to +1.0	+1.0 to +1.5
			%						
< 2 days	1971–72 (50)	38.67±0.09	2.0	4.0	2.0	8.0	62.0	20.0	2.0
	1963–67 (49)	38.60±0.11	4.1	—	2.0	16.3	53.1	20.4	4.1
3 to 6 days	1971–72 (24)	38.19±0.13	—	—	8.3	12.5	58.4	12.5	8.3
	1963–67 (21)	38.11±0.19	—	9.5	9.5	9.5	42.9	14.3	14.3
7 to 9 days	1971–72 (21)	37.97±0.14	—	—	9.5	9.5	57.2	19.0	4.8
10 to 20 days	1971–72 (29)	37.57±0.13	—	—	6.9	17.2	55.3	17.2	3.4
	1963–67 (22)	38.47±0.09	—	—	—	9.1	81.8	9.1	—
Adult	1970–73 (47)	38.51±0.08	—	—	6.4	8.5	74.5	6.4	4.2

Table VI

Changes in colonic temperature (ΔT_c) in response to transfer from T_a 30°C to T_a 20°C. In brackets, number of observations

Age		$\Delta T_{c(30°-20°)}$, °C					
		+0.5 to +1.0	±0.5	−0.5 to −1.0	−1.0 to −1.5	−1.5 to −2.0	−2.0 to −3.0
		%					
< 2 days	1971–72 (46)	2.2 (1)	56.5 (26)	23.9 (11)	13.0 (6)	2.2 (1)	2.2 (1)
	1963–67 (49)	—	61.3 (30)	16.3 (8)	14.3 (7)	6.1 (3)	2.0 (1)
3 to 6 days	1971–72 (24)	—	25.0 (6)	16.7 (4)	20.8 (5)	33.3 (8)	4.2 (1)
	1963–67 (21)	—	28.6 (6)	14.3 (3)	33.3 (7)	14.3 (3)	9.5 (2)
7 to 9 days	1971–72 (21)	—	4.8 (1)	28.6 (6)	42.8 (9)	19.0 (4)	4.8 (1)
10 to 20 days	1971–72 (30)	—	16.7 (5)	20.0 (6)	13.3 (4)	30.0 (9)	20.0 (6)
	1963–67 (22)	—	13.6 (3)	27.3 (6)	22.7 (5)	31.9 (7)	4.5 (1)
Adult	1970–73 (48)	—	43.7 (21)	35.4 (17)	12.5 (6)	4.2 (2)	4.2 (2)
Adult	1970–73 (44)*	2.2 (1)	31.9 (14)	18.2 (8)	15.9 (7)	15.9 (7)	15.9 (7)

*ΔT_c in response to transfer from T_a 30°C to T_a 10°C

Table VII contains the statistical analysis (χ^2) of the data presented in Table VI. Taking as the dividing line ΔT_c −0.5°C and of −1.0°C, respectively, the differences between the youngest age-group and the older new-born groups were significant in both series, whereas no significant difference was found between the newly born aged less than 48 hours and the adult animals.

Table VII

Differences between the group aged less than 48 hours and the older groups in distribution of ΔT_c in response to transfer from T_a 30° to T_a 20°C, according to whether ΔT_c did, or did not exceed -0.5°C ($\Delta T_c < -0.5$° vs. $\Delta T_c > -0.5$°), and whether ΔT_c did or did not exceed -1.0°C ($\Delta T_c < 1.0-$° vs. $\Delta T_c > -1.0$°). In brackets, distribution and number of observations. YATES'S *correction for small numbers was used for calculating* χ^2

1970—1973

$\Delta T_c < -0.5°$ vs. $\Delta T_c > -0.5°$			χ^2	p	$\Delta T_c < -1.0°$ vs. $\Delta T_c > -1.0°$			χ^2	p
< 2 days (27 : 19; 46)	vs.	3 to 6 days (6 : 18; 24)	5.863	< 0.02	< 2 days (38 : 8; 46)	vs.	3 to 6 days (10 : 14; 24)	10.452	< 0.01
< 2 days (27 : 19; 46)	vs.	7 to 9 days (1 : 20; 21)	15.639	< 0.001	< 2 days (38 : 8; 46)	vs.	7 to 9 days (7 : 14; 21)	13.697	< 0.001
< 2 days (27 : 19; 46)	vs.	10 to 20 days (5 : 25; 30)	11.485	< 0.001	< 2 days (38 : 8; 46)	vs.	10 to 20 days (11 : 19; 30)	14.778	< 0.001
< 2 days (27 : 19; 46)	vs.	Adult 30°—20° (21 : 27; 48)	1.543	>0.2	< 2 days (38 : 8; 46)	vs.	Adult 30°—20° (38 : 10; 48)	0.026	>0.8
3 to 6 days (6 : 18; 24)	vs.	Adult 30°—20° (21 : 27; 48)	1.667	>0.1	3 to 6 days (10 : 14; 24)	vs.	Adult 30°—20° (38 : 10; 48)	8.508	< 0.01
7 to 9 days (1 : 20; 21)	vs.	Adult 30°—20° (21 : 27; 48)	5.559	< 0.02	7 to 9 days (7 : 14; 21)	vs.	Adult 30°—20° (38 : 10; 48)	11.601	< 0.001
10 to 20 days (5 : 25; 30)	vs.	Adult 30°—20° (21 : 27; 48)	4.936	< 0.05	10 to 20 days (11 : 19; 30)	vs.	Adult 30°—20° (38 : 10; 48)	12.351	< 0.001
< 2 days (27 : 19; 46)	vs.	Adult 30°—10° (15 : 29; 44)	4.320	< 0.05	< 2 days (38 : 8; 46)	vs.	Adult 30°—10° (23 : 21; 44)	8.132	< 0.01
3 to 6 days (6 : 18; 24)	vs.	Adult 30°—10° (15 : 29; 44)	0.250	>0.5	3 to 6 days (10 : 14; 24)	vs.	Adult 30°—10° (23 : 21; 44)	0.341	>0.5
7 to 9 days (1 : 20; 21)	vs.	Adult 30°—10° (15 : 29; 44)	5.106	< 0.05	7 to 9 days (7 : 14; 21)	vs.	Adult 30°—10° (23 : 21; 44)	1.357	>0.2
10 to 20 days (5 : 25; 30)	vs.	Adult 30°—10° (15 : 29; 44)	1.936	>0.1	10 to 20 days (11 : 19; 30)	vs.	Adult 30°—10° (23 : 21; 44)	1.173	>0.2

1963—1967

< 2 days (30 : 19; 49)	vs.	3 to 6 days (6 : 15; 21)	5.035	< 0.05	< 2 days (38 : 11; 49)	vs.	3 to 6 days (9 : 12; 21)	6.530	< 0.01
< 2 days (30 : 19; 49)	vs.	10 to 20 days (3 : 19; 22)	11.991	< 0.001	< 2 days (38 : 11; 49)	vs.	10 to 20 days (9 : 13; 22)	7.535	< 0.01
3 to 6 days (6 : 15; 21)	vs.	10 to 20 days (3 : 19; 22)	0.680	>0.3	3 to 6 days (9 : 12; 21)	vs.	10 to 20 days (9 : 13; 22)	< 0.001	>0.98

The distribution of ΔT_c of the older new-born groups differed significantly from that of the adults, as they did from that of the youngest group. The distribution of ΔT_c of adult animals in response to transfer from T_a 30° to T_a 10°C differed, however, significantly from the distribution of ΔT_c of the youngest group in response to transfer from T_a 30° to T_a 20°C, and when —0.5°C served as the dividing line also from the distribution of ΔT_c following transfer to T_a 20°C of the group aged 7 to 9 days, however, in the opposite direction as it did from the youngest group.

Discussion

Heat production. The same basic data, i.e. measurements of VO_2 of the whole new-born guinea pig, as those of other newly born mammals, lend themselves to different interpretations when the results are expressed per unit BW and when expressed per unit body surface. However, even within the same frame of reference, contradictory results have been obtained. For instance, ADAMSONS et al. (1969) found per unit BW a progressive increase with age in heat production in a thermoneutral environment: during the first 5 hours after birth 16.4 $\pm$ 0.22 ml O_2/kg · min, 8 to 24 hours after birth 18.8 $\pm$ 0.17 ml/kg · min, and at the age of 7 days 20.1 $\pm$ 0.31 ml/kg · min. BRÜCK and WÜNNENBERG (1965) observed a rather steep increase between the day of birth and the following three days from 15.1 $\pm$ 1.0 ml/kg · min to 20.4 $\pm$ 1.2 ml/kg · min, followed, however, by a progressive decline to 15.7 ml/kg · min by the age of three weeks. Our observations showed per unit BW, a progressive fall in VO_2 with age at all three T_a-s, whereas using unit body surface, i.e. 10 · $kg^{0.67}$, as the frame of reference, no change with age was observed in VO_2 at T_a 35° and T_a 30°C. Even when the animals aged less than 48 hours were divided according to age into two groups, VO_2 failed to show an increase between the first and the second 24 hours of life.

BRÜCK and WÜNNENBERG (1965) explained the progressive fall with age of VO_2 per kg BW, with the rapid increase in BW and the consequent reduction in heat loss per unit BW, stating, however, that during the first three weeks of life the reduction in heat production in response to cold was greater than the reduction in heat loss per unit BW. Our observations demonstrated that, at T_a 20°C, the increase in BW is not an important determinant of the reduction in VO_2, since VO_2 was significantly lower at the age of 3 to 6 days than in animals aged less than 2 days, whereas BW-s were practically identical (FARKAS and DONHOFFER 1974).

At T_a 20°C, VO_2 per unit BW declined progressively with age, the greatest reduction occurring between the two youngest groups. Per unit body surface, however, the highly significant fall in VO_2 between the two youngest groups was followed by a gradual increase with age, approaching statistical

significance when mean VO_2 values were compared (Table I, Part A), and reaching statistical significance when the means of ΔVO_2 were compared (Table I, Part C).

Differences in the responses of highly complicated systems, like the regulation of heat production and of body temperature are due often, but not necessarily always, to differences in the experimental procedure. In our older series from 1963 to 1967, for instance, the experimental setup was exactly the same as that used in the recent observations, and so were all the details of the experimental procedure. Housing and rearing conditions differed, however, considerably, and genetical differences between the two populations could neither be excluded. In contrast to the recent observations, VO_2 was not only lower at T_a 35° and T_a 30°C in the two younger age-groups of the older series, but at T_a 35°C a progressive increase with age was observed, whereas at T_a 30°C there was no difference between the two younger age-groups. VO_2 at the age of 10 to 20 days was similar to that in the recent series at T_a 35°C and at T_a 30°C. At T_a 20°C, no change with age in VO_2 (in $ml/dm^2 \cdot min$) was noted; VO_2 was, however, significantly lower than in the recent series in every age-group (FARKAS et al. 1972).

Core temperature. At T_a 35°C, mean T_c values for the two younger age-groups were significantly higher in the recent series than in the older observations ($p < 0.001$, and $p < 0.01$, respectively), whereas at T_a 30° and at T_a 20°C, no significant difference was found between the two series. The most impressive difference between the two series was observed at the age of 10 to 20 days. In the recent series (Table II, Part A), mean T_c was at all three T_a-s significantly lower than in the groups aged less than 2 days and 3 to 6 days, whereas in the older series mean T_c of the 10 to 20-day-old was higher than that of the younger groups (Table III, Part A), and at all three T_a-s higher than T_c in the same age-group of the recent series ($p < 0.001$).

In view of these very marked differences in T_c between the two series, ΔT_c-s following changes in T_a were remarkably similar in the two series (Tables II and III, Part B). Subsequent to transfer from T_a 35° to T_a 30°, ΔT_c was of similar magnitude in all age-groups in both series, the small differences not being significant. ΔT_c-s subsequent to transfer from T_a 30° to T_a 20°C were practically identical in the two series, and demonstrated convincingly that, on the average, guinea pigs aged less than 48 hours respond to a change from T_a 30° to T_a 20°C with a highly significantly smaller decrease in T_c than any of the other new-born groups, and even with a smaller decrease than the adult animals, the difference, however, not being significant. In contrast, ADAMSONS et al. (1969) found that the fall in T_c subsequent to transfer from a thermoneutral environment to T_a 21—22°C decreased with age in the new-born guinea pig. Previous observations of BRÜCK and WÜNNENBERG (1965) agree with our recent series as far as in the course of much more severe cold

exposure (8°C), T_c decreased with age up to 21 days, and with our older series as far as in the course of more moderate cold exposure (16° and 26°C) T_c was higher at the age of 21 days than at the age of 4 to 8 days. Data for T_c prior to exposure to cold were not given, so that no comparison was possible with our observation that, notwithstanding the differences in T_c prior to, and after 30 min of cold exposure, ΔT_c-s were similar in the two series.

Tables IV and V show that both at T_a 30° and at T_a 20°C, T_c was distributed around the mean within wider limits as appears to be generally assumed. The distribution of ΔT_c following transfer from T_a 30° to T_a 20°C (Table VI), demonstrated that sweeping statements about the "stability" of T_c in the new-born, and even in the adult guinea pig, are hardly justified. The shift with age towards a greater decrease in core temperature in the newly born strikes the eye at the first glance and, as demonstrated by Table VII. is highly significant statistically: the distribution of ΔT_c in both the older and the recent series of the group aged less than 48 hours differs much more from all the other age-groups of the newly born than from the distribution of ΔT_c in the adult animals.

In some aspects, ΔT_c subsequent to transfer from a thermoneutral, or near thermoneutral, environment to a moderately colder one, resembled in the new-born guinea pig that observed under similar conditions in new-born rabbits (VÁRNAI et al. 1970). Some of the newly born of both species maintained core temperature within narrow limits, while others responded under the same conditions with a marked fall in T_c. The range of ΔT_c and the frequency distribution of ΔT_c differed, however, in the two species.

The most striking difference is that guinea pigs aged less than 48 hours responded not only with a highly significantly smaller fall in T_c than any other of the older new-born groups, but resembled in their responses those of adult guinea pigs closely. The increases in heat production in response to exposure to T_a 20°C having been significantly greater in the group aged less than 48 hours than in the older ones, the impression might arise that the greater reduction in T_c beyond the age of 2 days could be due to the decreasing role of non-shivering (brown fat) thermogenesis (BRÜCK and WÜNNENBERG 1965, 1966). The greater reduction in T_c is, however, not due to a decrease in the capacity for heat production, since oxygen consumption increased also in the older new-born guinea pigs to considerably higher levels when exposed to more severe cold. The difference in the response between the group aged less than 48 hours and those beyond that age, although highly significant, was nevertheless a statistical one, and animals maintaining T_c within a few tenths of a centigrade and others responding with a very marked fall in T_c when transferred from T_a 30° to T_a 20°C, were found in every age-group.

The fact that in response to exposure to T_a 20°C, a fall in T_c of more than 2.0°C has been observed even in some adult guinea pigs, constitutes

additional evidence that the greater decrease in T_c in response to moderate cold is neither the consequence of "immaturity" nor of an "inability" to increase heat production to a higher level, but has to be regarded rather as the manifestation of a functional variant of the cybernetic system, in which changes in core temperature are only part of the highly complex input driving the central regulating mechanism, or modifying its function. In other words, it has to be kept in mind when interpreting changes in core temperature that these depend, on the one hand, on the output of the regulatory system, one branch of which governs thermoregulatory heat production and, on the other hand, that they are simultaneously an important part of the input of the system.

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PLASMA RENIN-LIKE ACTIVITY IN THE SPLANCHNIC AREA OF THE RAT

By

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Plasma renin activity was found to be higher in portal than in aortic blood of both sham-operated and bilaterally nephrectomized male rats. This seems to point to the presence of a structure producing renin-like enzyme in the rat's splanchnic area.

The kidneys had long been considered the exclusive site of renin production (TIGERSTEDT and BERGMAN 1898), but recent research has demonstrated a number of extrarenal renin or renin-like enzyme sources (DENGLER 1956; STAKEMAN 1960; WERLE et al. 1962; FERRIS et al. 1967). Renin or renin-like enzyme activity was found to be higher in the portal vein than in systemic blood in both man (BARNARDO et al. 1969) and the dog (GANTEN et al. 1970).

The purpose of this work was to investigate the plasma renin activity (PRA) of blood taken from the aorta and the portal vein of rats.

Methods

Male Wistar rats of 220—240 g body weight were used in the experiments. All were kept on standard laboratory diet. Under light ether anaesthesia bilateral nephrectomy was performed through a dorsal incision in 12 animals (Group 1) and sham operation in 10 animals (Group 2). Methodological problems were solved in preliminary experiments on 34 animals.

Twenty-four hours after operation, under light ether anaesthesia from the animals of both groups blood samples for PRA determination were obtained through a ventral incision, simultaneously from the portal vein at the height of the gastroduodenal vein, and the abdominal aorta at its division into the iliac arteries. In the control group the renal blood vessels were clamped immediately before the blood samples were taken.

The samples were prepared for PRA determination by the method of KANEKO et al. (1967). PRA determination and bioassay were done as described earlier (SONKODI et al. 1970); the values were expressed in ng angiotensin per ml plasma (ng A/ml/24 hr); statistical analysis of the results was performed with Student's *t*-test on coupled values.

Results

Mean PRA in aortic blood of the control animals (Group 2) was $8.21 \pm \pm 1.30$ ng A/ml, while in the portal vein, 15.23 ± 1.81 ng A/ml. The difference was significant statistically ($p < 0.01$).

In the bilaterally nephrectomized animals (Group 1), with the exception of three cases, measurable PRA values were found. The mean in aortic blood was 1.76 ± 0.44 ng A/ml, while in the portal vein, 9.06 ± 2.03 ng A/ml, significantly higher ($p < 0.002$) than in aortic blood. The pressor substance was heat stable, dialyzable and destroyed by trypsin and chymotrypsin. Its pressor effect in the rat was identical with that of synthetic angiotensin II.

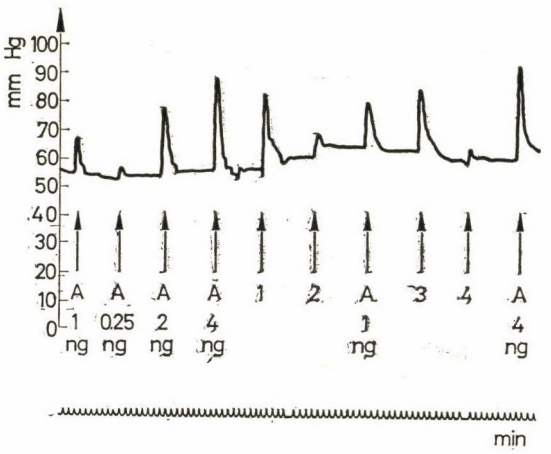

Fig. 1. PRA in portal and aortic blood of two bilaterally nephrectomized rats. "A" shows the standard angiotensin II doses. 1: plasma from the portal vein of animal 1; 2: plasma from aorta of animal 1; 3 and 4: plasma of animal 2 from portal vein and aorta, respectively

Fig 1 shows the PRA of portal venous and aortic blood of the nephrectomized rats as compared with dilutions of standard angiotensin II (Hypertensin, Ciba). All plasma samples and angiotensin dilutions were injected in a volume of 0.1 ml.

Discussion

The fact that in the control group PRA was significantly higher in portal blood than in aortic blood makes it probable that a renin-like enzyme is produced in the splanchnic area of the rat.

In our experiments the renal and other known extrarenal renin sources were excluded as far as possible. Male rats were used because this excluded the uterus as a possible extrarenal renin source. Bilateral nephrectomy abolished the possibility of renal origin.

The low PRA value measured in the aortic blood of nephrectomized rats, similarly as in the observations of WORCEL et al. (1969), is an indirect evidence of extrarenal renin-like enzyme production. The high PRA value

in the portal vein seems to prove that a renin-like enzyme is produced in the splanchnic area of the rat, and that this renin-like enzyme source is fairly significant.

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THE EFFECT OF CHRONIC PHYSICAL OVERLOAD ON SKELETAL MUSCLE METABOLISM AND ADRENOCORTICAL ACTIVITY

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The effect of chronic physical overload of various duration on white and red skeletal muscle water and electrolyte metabolism, Na^+K^+-ATPase activity and plasma corticosterone content was investigated. The most regular change was the sodium uptake and potassium loss by both red and white fibres. Tissue water content and its distribution between extracellular and intracellular compartments were essentially unaltered. The above mentioned disturbances in the tissue electrolyte content were accompanied by the decrease in red muscle Na^+K^+-ATPase activity and fall of blood plasma corticosterone content. When overtrained animals were exercised until exhaustion, a slight increase in the skeletal muscle potassium and decrease in sodium content were observed. In red muscles the ionic shifts correlated with the increase of Na^+K^+-ATPase activity. The additional exertion also increased the adrenocortical activity. Thus, the changes in red skeletal muscle electrolyte metabolism were reversible. The obtained results suggest that the mechanism of active transport of ions in red skeletal muscle has a great adaptive capacity and cannot completely be disturbed by a short period of "overtraining". The possible role of the adrenal cortex in the regulation of that mechanism is discussed.

A large scale of biochemical and hormonal indices, characteristic of a well-trained organism or of the state of over-training was established by the fundamental studies of YAKOVLEV (1955, 1970), HOLLOSZY (1967) and several other investigators (DEREVENCO et al. 1967; FRENKL and CSALAY 1962; HULTMAN et al. 1967; KEUL et al. 1969; VENDSALU 1960; VIRU 1971). The investigations allowed to formulate a theory about the specific character of biochemical adaptation to various training regimes (YAKOVLEV 1955, 1970) and undoubtedly improved the practical management of the training process. However, in spite of the great success in the biochemical estimation of the state of fitness and exhaustion, the investigations presented in the literature are far from offering an integral picture of the biochemical mechanisms responsible for the increase or decrease in physiological capabilities of the organism.

The present study was undertaken to assess the effect of chronic muscular overload of various duration on the transmembrane shifts of water and electrolyte, and on Na^+K^+-ATPase activity in red and white skeletal muscles.

It has been demonstrated that corticosteroids participate in the regulation of water and electrolyte distribution between intracellular and extracellular compartments (KOLPAKOV 1967). Furthermore, determination of

Na^+K^+-ATPase activity in the renal microsomal fraction of adrenalectomized rats and of rats treated subsequently with gluco- and mineralocorticoids have revealed the relationship between adrenocorticosteroids and enzyme activity (JORGENSEN 1968, 1969; SUZUKI and OGAWA 1969). During extreme exertion, the decrease in adrenocortical activity is accompanied by a characteristic and pronounced decrease of the Na/K ratio in the blood plasma, water and sodium shifts into the heart cells, and a decrease in myocardial Na^+K^+-ATPase activity (KÔRGE et al. 1973a). In order to evaluate the role of the adrenal cortex in the genesis of water and electrolyte disturbances, the plasma corticosterone content was determined.

Methods

Material

A total of 59 male Wistar rats with an initial body weight of 190—250 g was used in the experiments. The animals were maintained and fed under constant conditions. Eighteen hours before the experiments, food was removed from the cages, while free access to water was allowed. "Overtraining" was accomplished by prolonged swimming daily. The initial load was gradually increased up to 10 hours. The characterization of "overtraining" regimes are presented in Table I.

Table I

Characterization of experimental groups

Group	Subgroup	n*	Duration of overtraining (week)	Total swimming time (hour)	Duration of final swim-test
1st	A. sedentary controls	4			
	B. exhausted** controls	6	1	43	—
	C. "overtrained animals" immediately after swimming	6	1	43	until exhaustion
2nd	A. sedentary controls	4			
	B. Exhausted** controls	5	1	53	—
	C. "overtrained animals" immediately after swimming	4	1	53	until exhaustion
3rd	A. sedentary controls	5			
	B. exhausted** controls	5	1	53	
	C. "ovedrtraine animals" immediately after swimming	4	1	53	until exhaustion
	D. "overtrained animals" immediately after swimming	4	1	53	10 hours
4th	A. sedentary controls	4			
	B. exhausted** controls	4	2	123	—
	C. "overtrained animals" immediately after swimming	4	2	123	until exhaustion

* number of animals used in biochemical studies
** investigated 20 hr after the last exertion

The animals were divided into four groups and each group, except for the 3rd, was further divided into three subgroups as shown in Table I. Each group consisted of 4—5 sedentary controls (subgroup A) and "overtrained" animals. At the end of the "overtraining" period, half of the rats were sacrificed 20 hours after the last exercise and used "exhausted controls" (subgroup B). The other "overtrained" animals were subjected to additional swimming until exhaustion and tissue samples were taken immediately after the exertion (subgroup C). In the 3rd group, part of the animals was killed immediately after the 10-hour swim (subgroup D).

The animals were sacrificed under light ether anaesthesia by bleeding from the abdominal aorta into a heparinized syringe. Skeletal muscle samples were excised from the hind leg immediately after the blood sampling. In view of the different electrolyte composition of white and red fibres (SRETER and WOO 1963),the muscle sample was divided into predominantly white (m. sartorius) and red (m. quadriceps femoris) fibres. Staining with Sudan black B revealed that admixture of fibres of other type were 20—30%.

Water and electrolyte estimation

The muscle tissue was prepared, the electrolytes were extracted and H_2O, Na and K distributions between tissue compartments calculated as described previously (KÔRGE and VIRU 1971). Na, K and Ca in plasma and tissue extracts were determined with a Zeiss III type flame-photometer; Cl by potentiometric titration; Mg, Zn and Cu with Unicam atomic absorption spectrophotometry.

Mg^{2+}-and Na^+K^+-ATPase activity determination

The tissue was homogenized in a glass homogenizer with 9 vol of 0.32 M sucrose containing 5 mM Na_2 EDTA, adjusted to pH 7.4 with tris buffer. Homogenization time was approximately 8 min and during this period the homogenizer was immersed in ice. The homogenate was centrifuged in a refrigerated centrifuge at 0°C for 15 min (700 g). The supernatant was centrifuged at 12,000 g for 30 min and the supernatant from the second centrifugation at 30,000 g for 1 hour to obtain a microsomal fraction. The 30,000 g pellet was stored at —5°C overnight and then suspended in 0.32 M sucrose containing 0.1% sodium deoxycholate, using a glass homogenizer. The microsomal suspensions were stored at —5°C for approximately 2 hours and then used for enzyme assay.

Enzyme activity was determined in a reaction mixture containing 3 mM $MgCl_2$, 100 mM NaCl, 10 mM KCl, 3 mM ATP*, 40 mM tris, and microsomal protein 0.1—0.2 mg. The final pH was 7.4 and the total volume, 1.5 ml. The reaction was started by the addition of the microsomal protein and terminated by the addition of 0.5 ml percholoric acid after 30 min incubation at 37°C. Protein was determined by the method of LOWRY et al. (1951) and inorganic phosphate by the method of FISKE and SUBBAROW (1925), using thiourea as the reducing agent.

Na^+K^+-ATPase activity was defined as the difference between values obtained without Na and K (Mg^{2+}-ATPase) and those obtained in the presence of Na and K. Results were expressed as micromoles of P_i liberated per mg of protein during 30 min.

Corticosterone estimation

1.5 ml of plasma washed with 15 ml petroleum ether was diluted with 3.5 ml of bis-distilled water and extracted with 15 ml of methyl chloride by shaking gently for 3 minutes. After washing the methyl chloride extracts with 1.5 ml of 0.1 n NaOH, 12 ml of extract was evaporated to dryness under vacuum. The dry residue was concentrated at the bottom of the test tube, dissolved in a minimum volume of ethanol and spotted into plates about 2 cm from the end under warm air stream. For thin-layer chromatography, "Silufol" UV_{254} plates (KAVALIER, Czechoslovakia) were used. On both sides of the plates 1 μg of corticosterone (Merck) was dropped for the detection of samples. The solvent systems used were (1) hexane–chloroform (1 : 3), (2) chloroform–ethanol (85 : 15). The R_f of the corticosterone was 0.50. The position of the standards was marked under an UV lamp and the appropriate areas and the blank sample were scraped off into glass centrifuge tubes. After shaking the samples with 2 ml of methanol, the aqueous phase was removed and evaporated to dryness under vacuum. The corticosterone content was determined on the basis of the fluorescence recorded 30 min after the addition of the ethanol–sulphuric acid reagent (1 : 3) to the dry residue.

* ATP (Reanal) was made Na-free by passing it through a Dowex 50 × 8 column.

Results

The effect of "overtraining" and additional acute exertion on skeletal muscle water content is presented in Fig. 1. "Overtraining" altered the tissue water content and distribution neither in white nor in red muscle except for a slight decrease in total and intracellular water content in red muscle after the one-week "overtraining" (lst group). Acute extreme exertion increased water content in both red and white muscle due to water accumulation in the cells in all investigated groups. At the same time, water content decreased in the extracellular compartment.

The effect of "overtraining" on sodium content and distribution in white skeletal muscle is presented in Fig. 2 and that in red muscle in Fig. 3. "Overtraining" increased the skeletal muscle sodium content significantly in the lst, 3rd and 4th groups. The increase was apparently due to Na accumulation in the cells. However, no changes were observed either in Na content or distribution in rats of the 2nd group, treated and investigated similarly to those of the 3rd group. In the 2nd group "exhausted controls" had also some minor peculiarities in skeletal muscle K content and Na^+K^+-ATPase activity. Why the one-week "overtraining" failed to alter the skeletal muscle sodium content in the 2nd group, is a matter of speculation. Nevertheless, the unidirectional changes in the other groups clearly demonstrate that the general effect of "overtraining" on the electrolyte content of both tissues was to increase Na and to decrease K.

Acute exertion regularly decreased the tissue sodium content caused by the decrease of sodium in the extracellular compartment (Figs 2 and 3).

The effect of "overtraining" on skeletal muscle potassium content and Na^+K^+-ATPase activity was the greatest in red muscle where enzyme activity and cellular K content had significantly decreased in all investigated groups (Fig. 4). Na^+K^+-ATPase activity and cellular K content tended to decrease also in white muscle but the changes were not significant statistically, except for the lst group, where K values had decreased.

Acute extreme exertion increased Na^+K^+-ATPase activity and cellular potassium content in red skeletal muscle as compared with the values obtained in the "exhausted controls". The increase in cellular K content was significant in the lst and 4th groups and that in Na^+K^+-ATPase activity in the 1st and 2nd groups. Enzyme activity was increased in the 4th group. However, as compared to the unexercised controls, the potassium and Na^+K^+-ATPase values had either slightly decreased or remained unchanged after exertion (Fig. 4).

In general, during physical exertion, the changes in Na^+K^+-ATPase activity and cellular K content had the same direction in red but not in white skeletal muscle.

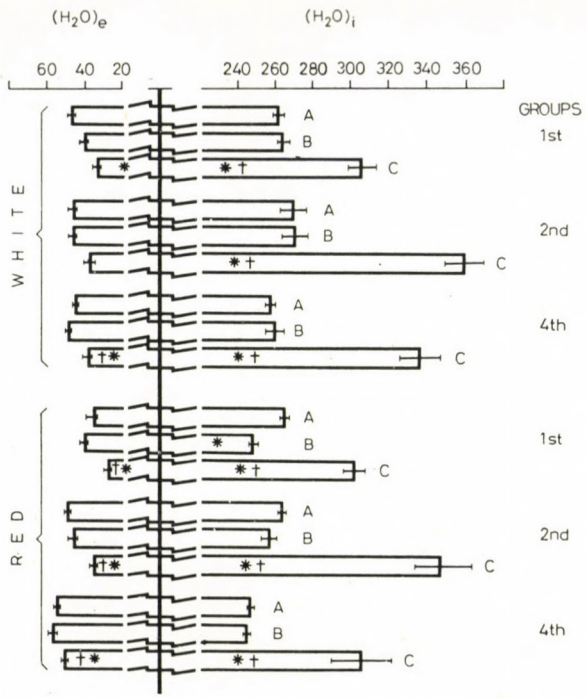

Fig. 1. Effect of "overtraining" and acute exertion on skeletal muscle water content and distribution (in grams per 100 g fat-free solids, FFS). In Figures 1, 4 and 5, subgroups are marked as indicated in Table I. A: Sedentary controls; B: "exhausted controls"; C: "overtrained" animals immediately after extreme exertion; D: "overtrained" animals immediately after 10 hours exertion. Asterisk (*) indicates a significant difference from sedentary controls and cross (†) from exhausted controls

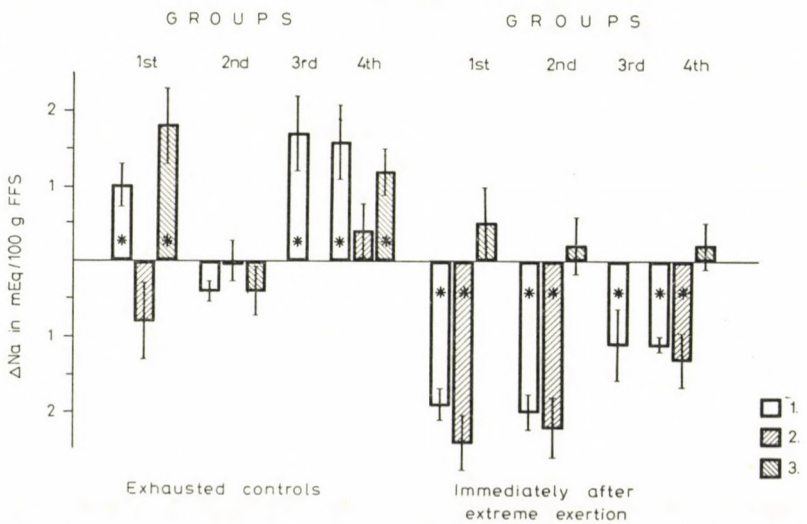

Fig. 2. Effect of "overtraining" and acute exertion on white muscle sodium content and distribution. 1: total; 2: extracellular; 3: intracellular. Asterisk (*) indicates a significant difference from sedentary controls

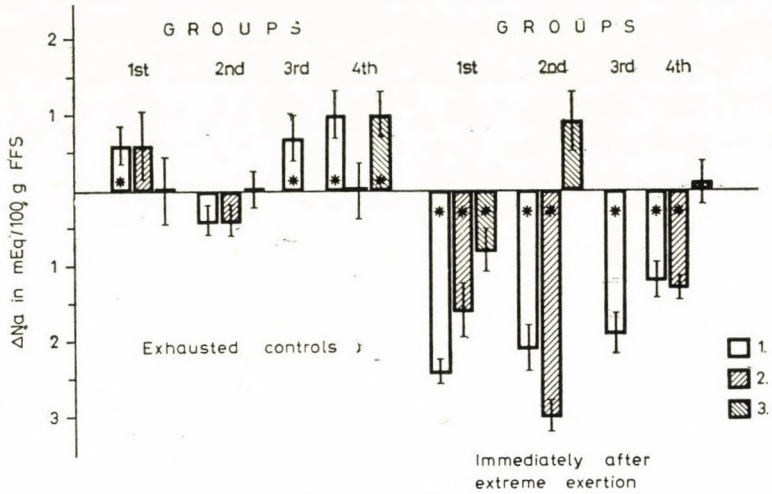

Fig. 3. Effect of "overtraining" and acute exertion on red muscle sodium content and distribution. For explanation of symbols, see Fig. 2

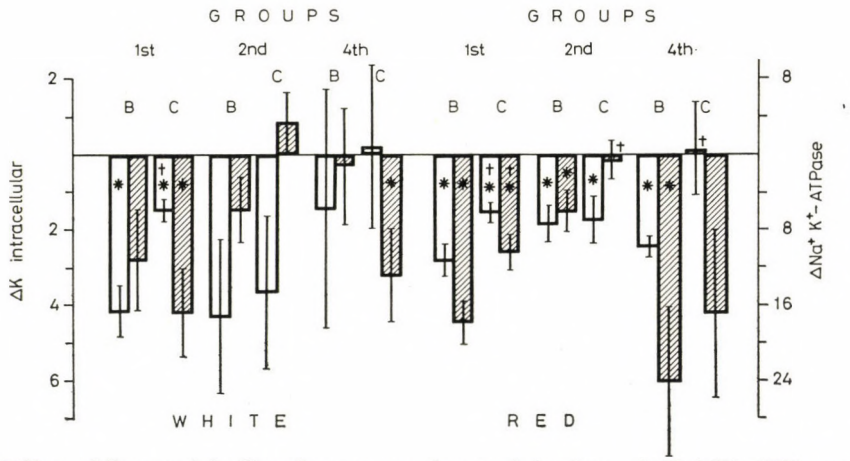

Fig. 4. Effect of "overtraining" and acute exertion on skeletal muscle Na^+K^+-ATPase activity and intracellular K content. □: K content; ▨ Na^+K^+-ATPase activity. Na^+K^+-ATPase activity in μmoles of P_i/mg protein/30 min. K content in mEq per 100 g FFS. For explanation of other symbols, see Fig. 1

Mg^{2+}-ATPase revealed no characteristic alterations on "overtraining", while the extreme exertion increased the enzyme activity in red skeletal muscle.

In most cases, the "overtraining" slightly increased the Ca, Zn and Cu content of skeletal muscles and acute exertion decreased the Ca and Zn values.

In the group of exhausted controls, the plasma corticosterone content had significantly decreased. Both additional 10 hours exertion and extreme

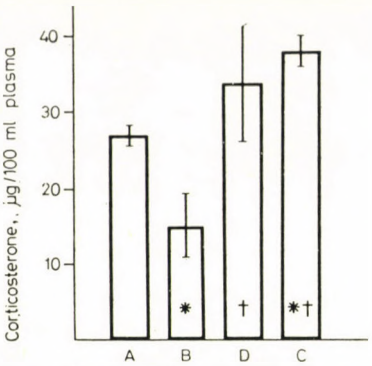

Fig. 5. Effect of "overtraining" and acute exertion on blood plasma corticosterone content (3rd group). For explanation of symbols, see Fig. 1

exertion increased the blood corticosterone content as compared with the value for the "exhausted controls" (Fig. 5).

After one-week "overtraining" (3rd group) the body weight reduction was 28.6 $\pm$ 4.27 g. The severity of "overtraining" was also characterized by a mortality of approximately 50% in all "ovetrained" groups.

Discussion

The results revealed a good accordance between the changes of Na^+K^+-ATPase activity and potassium content in red fibres, on the one hand, and adrenocortical activity, on the other. The concomitant decrease in all indices was the most pronounced and regular effect of "overtraining". When animals "overtrained" for one week were subjected to acute extreme exertion, a significant increase in enzyme activity, intracellular potassium and corticosterone content occurred. Acute exertion after more prolonged overtraining induced a less pronounced increase in enzyme activity and cellular potassium content. On the other hand, Na^+K^+-ATPase activity in white muscle was not affected either by overtraining or by additional acute exertion. It was remarkable that adrenalectomy decreases myosin ATPase activity only in red skeletal muscle and the myocardium, while activity in white muscle remains unchanged (ROVETTO et al. 1971). Thus, the contractile properties of red muscle are under a more close control of the adrenal cortex than those of white muscles. The close relationship between red muscle (cardiac) myosin ATPase activity and adrenocortical function is demonstrated also by the fact that glucocorticoid administration to adrenalectomized animals completely prevented the decrease in myosin ATPase activity (ROVETTO et al. 1970). In our studies, the decrease in blood corticosterone content correlated with

the fall of myocardial Na^+K^+-ATPase activity during extreme exertion (KÔRGE et al. 1973a).

The effect of adrenocortical hormones on Na^+K^+-ATPase has extensively been investigated using, kidney as the target organ.

Adrenalectomy decreased renal Na^+K^+-ATPase activity, while combined treatment with gluco- and mineralocorticosteroids prevented the decrease in enzyme activity (JORGENSEN 1969; SUZUKI and OGAWA 1969). However, according to JORGENSEN (1968), Na^+K^+ activated ATPase is not primarily under the control of the adrenal cortex as the corticosteroids are regulating enzyme activity by changing the ionic environment. In addition, experiments with purified plasma membrane preparations suggested that the increase in Na^+K^+-ATPase activity induced by glucocorticoids was due to an increase in the amount of plasma membrane per cell rather than to the increase in enzyme activity per unit of plasma membrane (MANITUS et al. 1968). The possible effect of corticosteroids on sarcoplasmic Na^+K^+-ATPase has also been assumed by the said authors as probable explanation of the results. The electronmicroscopic evidence of the continuity of the transverse tubules with the plasma membrane allows to suggest the participation of sarcoplasmic reticulum in the cation exchange between cell and extracellular space (FORSSMANN and GVIARDIER 1970). On the other hand, several studies of the membrane fragments of sarcoplasmic reticulum and plasma membrane demonstrated that Na^+K^+-ATPase was associated with the plasma membrane (BOEGMAN et al. 1970). These studies therefore do not allow to assume the existence of a direct relationship between adrenocortical activity and skeletal muscle microsomal Na^+K^+-ATPase.

Red and white fibres differ in ultrastructure (BENNET 1960), enzyme activity (BASS et al. 1971), myosin properties (KATZ 1970) and in biochemical adaptation to training (YAKOVLEV and YAKOVLEVA 1971). According to YAKOVLEV and YAKOVLEVA (1971), the biochemical adaptation of both white and red muscles to regular exercise depends on the character of training regimes, although the extent of biochemical changes in the studied muscles was different.

When comparing our results obtained in the groups of sedentary controls with those obtained 20 hours after the termination of "overtraining", it became evident that the biochemical changes caused by "overtraining" had the same direction in both white and red fibres. Therefore, our results concerning the electrolyte shifts and changes in Na^+K^+-ATPase activity confirm the findings of the above-mentioned authors also in the case of overtraining.

Ca-ATPase activity of the sarcoplasmic reticulum sedimented at 30.000 g was found to be higher in red than in white muscles (SRETER 1968). According to our results, the same is true for Mg^{2+}ATPase which is suggested to control the passive permeability of excitable cells (BOWLER and DUNCAN 1967).

However, the difference between Na^+K^+-ATPase and Mg^{2+}-ATPase activity was clearly more in white muscle. White muscles were also found to contain more potassium and less sodium than red ones. The difference is due to the different minute amounts of acetylcholine (SRETER and WOO 1963).

Our present and earlier studies (KÔRGE et al. 1973b, 1974) revealed a decrease of the biochemical response to acute exertion of exhausted animals as compared with previously unexercised animals. Similarly to the effect of "overtraining", training decreased the extent of alterations of various homeostatic indices during physical exertion (YAKOVLEV 1955, 1970; KÔRGE et al. 1974).

In contrast to the effect of training which was found to increase the potassium content of the cells of skeletal muscle (NÖCKER 1959) and myocardium (KÔRGE et al. 1974), "overtraining" decreased the transmembrane gradient of potassium by causing a loss of intracellular potassium. In view of the important role of potassium in the regulation of cell metabolism, potassium deficiency obviously leads to various metablic disturbances. There is experimental evidence that a restriction of potassium intake is followed by a decrease in muscle strength (STARLINGER and BERGHOFF 1965) and the dystrophic muscle is characterized by a decreased cellular potassium content and resting membrane potential (HAZLEWOOD and GINSKI 1968). In our study the decrease of potassium in the muscle cells of "overtrained" animals was not connected with the terminal exhaustion of the active ion or body potassium transport. This was proved by the increase in Na^+K^+-ATPase activity and cellular potassium content in red skeletal muscle during the additional exertion. For a better evaluation of the effect of "overtraining" on ion fluxes and adrenocortical activity, it is important to note that we have studied only the surviving animals. These, obviously, had a higher working capacity than those which had died from the "overtraining".

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FUNCTIONAL INTERRELATIONSHIP BETWEEN CERVICAL SUPERIOR GANGLIA, PINEAL GLAND AND HYPOTHALAMO-PITUITARY-ADRENAL SYSTEM IN RATS

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In normal animals, hypothalamic serotonin and plasma corticosterone levels showed a reversed diurnal rhythm. In the morning, the plasma corticosterone was low and the hypothalamic serotonin level was high. At the beginning of the dark period, the high plasma corticosterone was associated with a low hypothalamic serotonin content.

Pinealectomy had no effect on diurnal corticosterone and serotonin rhythm, neither on stress-induced adrenal activation.

Removal of the superior cervical ganglia diminished the circadian rhythm of the pineal serotonin level, but did not affect the hypothalamic serotonin and plasma corticosterone rhythm. The response to stress induced by ether or electric shock, was facilitated. It was still elevated 58 days after ganglionectomy.

The data indicated that the pineal gland has no direct effect either on hypothalamic serotonin content or on the hypothalamo–pituitary–adrenal system. The absence of the cervical superior ganglia facilitated the stress response of the hypothalamo–pituitary–adrenal system without influencing the hypothalamic serotonin content.

The effect of the pineal gland on the hypothalamo–pituitary–adrenal system is somewhat contradictory. Some authors found that pinealectomy increased adrenal weight (WURTMAN et al. 1959; FRASCHINI et al. 1968; RELKIN 1972; VAUGHAN et al. 1972), while others observed no differences (DILL 1961; GIROD et al. 1963; KINSON et al. 1968). In pinealectomized animals, corticosterone production increased (KINSON et al. 1967, 1968), but the plasma corticosterone level was not affected (DILL 1961). The increased corticosterone production returned to normal 30 days after pinealectomy (KINSON et al. 1968). Melatonin given intravenously had no effect (BARCHAS et al. 1969) but when administered intraventricularly, it inhibited the stress-induced adrenal activation (MOTTA et al. 1971; VERMES et al. 1972a). Since the pineal gland produces serotonin, and the hypothalamic serotonin level is capable of modifying pituitary–adrenal function (TELEGDY and VERMES 1973; VERMES and TELEGDY 1972; VERMES et al. 1972b), the question arose whether the hypothalamic serotonin level was influenced by the pineal gland, which, in turn, would affect the hypothalamo–pituitary–adrenal system. It has been shown that the superior cervical ganglion controls the pineal serotonin level

(e.g. WURTMAN et al. 1968) but it is not known whether the hypothalamic serotonin content is influenced by the superior cervical ganglion. In the present paper, some observations will be presented concerning these questions.

Methods

The experiments were carried out on 642 adult male R-Amsterdam rats weighing 185—245 g. The animals were kept on an artificial light schedule of 12 hours light and 12 hours dark period. The light period started at 06.00 a.m. The animals were fed a synthetic food and water was given ad libitum.

The superior cervical ganglion was removed bilaterally under pentobarbital anaesthesia (5 mg/100 g b.w.), according to FENDLER and LISSÁK (1965). Pinealectomy was carried out according to DE VRIES and KAPPERS (1971). The animals were tested 2 weeks after pinealectomy and 1, 2, 4, 8 weeks following ganglionectomy. As controls, sham-operated animals were used.

The plasma corticosterone content was estimated by the method of PURVES and SIRETT (1965), hypothalamic and pineal serotonin content by that of SNYDER et al. (1965, 1967).

Removal of the superior cervical ganglia and of the pineal gland was controlled by histology on randomly selected animals.

Statistical analysis was done by Student's *t*-test.

The animals were grouped as follows.

1. Effect of pinealectomy on circadian rhythm of plasma corticosterone and hypothalamic serotonin level (146 animals). The animals were tested at 4.00, 8.00, 12.00, 16.00, 20.00, and 24.00 o'clock.

2. Effect of pinealectomy on stress-induced plasma corticosterone and hypothalamic serotonin level (74 animals). For stressing, the animals were placed for 2 minutes in ether vapour and tested 30 and 90 minutes later.

3. Effect of cervical superior ganglionectomy on circadian rhythm of plasma corticosterone, hypothalamic and pineal serotonin level (241 animals). The animals were tested as group No. 1, at different time intervals.

4. The effect of superior cervical ganglionectomy on stress-induced plasma corticosterone, hypothalamic and pineal serotonin level (125 animals).

The animals were stressed by ether as in Group No. 2, and tested 30 and 90 minutes later.

For electric shock the animals were placed in a box with a grid floor through which 1.5 mA alternative current was delivered for 5 sec in every 15 sec during 2 min. The animals were tested 30 min later.

5. The effect of superior cervical ganglionectomy on stress-induced plasma corticosterone, hypothalamic and pineal serotonin levels at different times after ganglionectomy (56 animals). The animals were exposed to ether 1, 2, 4 or 8 weeks after ganglionectomy, and killed 30 minutes after ether exposure.

Results

The effect of pinealectomy on the circadian rhythm of plasma corticosterone and hypothalamic serotonin content is shown in Fig. 1. In control (sham-operated) animals, plasma corticosterone showed a typical diurnal variation with the minimum at 8.00 a.m., and the maximum at 8.00 p.m. The hypothalamic serotonin content showed an opposite behaviour, with the maximum at 8.00 a.m., and the minimum at 8.00 p.m. There was no difference between the intact control and sham-operated control animals and therefore their values are expressed as one group. Following pinealectomy, no difference was found vs. the controls either in corticosterone or in serotonin diurnal fluctuation.

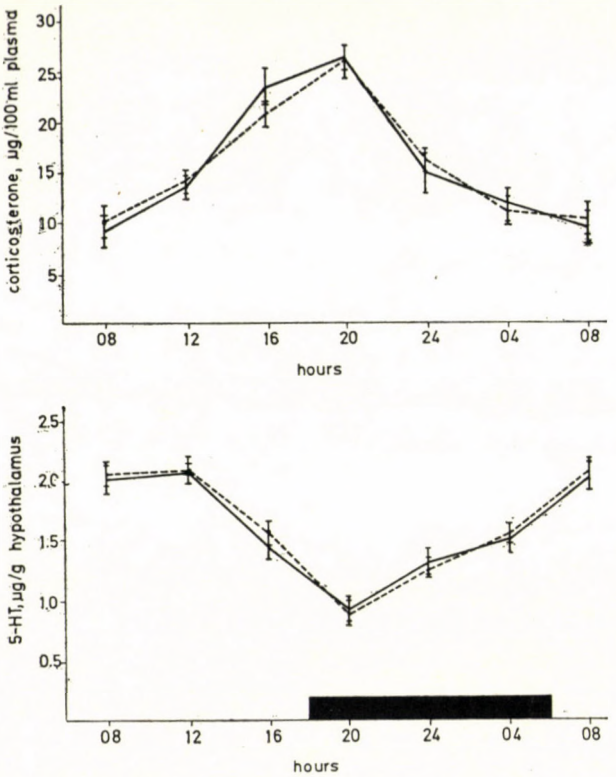

Fig. 1. Effect of pinealectomy on circadian rhythm of plasma corticosterone and hypothalamic serotonin level. Solid line, control; dotted line, pinealectomized. Each point represents the mean and standard error of the mean for 8—22 animals

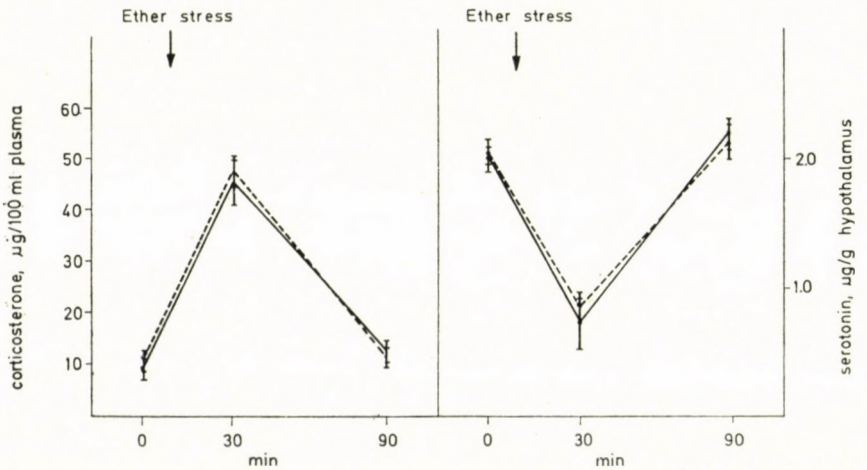

Fig. 2. Effect of pinealectomy on stress-induced plasma corticosterone and hypothalamic serotonin level. Solid line, control; dotted line, pinealectomized. Each point represents the mean and standard error of the mean for 16—23 animals

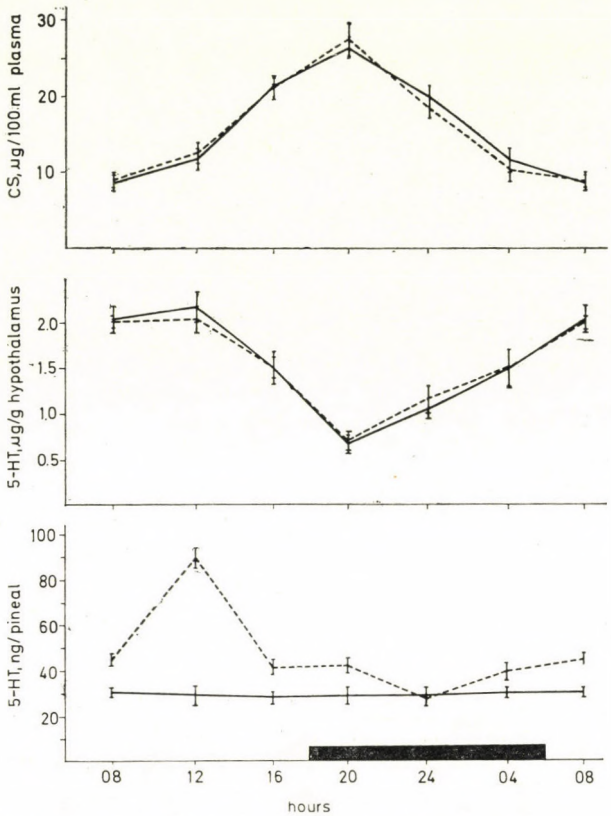

Fig. 3. Effect of superior cervical ganglionectomy on circadian rhythm of plasma corticosterone, hypothalamic and pineal serotonin levels. Solid line, control; dotted line, ganglionectomized. Each point represents the mean and standard error of the mean for 8—46 animals

The stress response after pinealectomy was similar to that in the sham-operated group (Fig. 2). After 30 min, the plasma corticosterone level was high and the hypothalamic serotonin content low. At 90 min, both the plasma corticosterone and the hypothalamic serotonin level returned to normal.

After removal of the superior cervical ganglia, the pineal serotonin level was constantly low, while in the sham-operated animals the maximum was reached at 12.00 a.m. and the minimum at 24.00 (Fig. 3).

Between normal and ganglionectomized animals, there was no difference in hypothalamic serotonin and plasma corticosterone rhythm.

In superior cervical ganglionectomized animals following stress caused by ether or electric shock, the plasma corticosterone level was significantly higher than in the controls, although there was no difference in the hypothalamic and pineal serotonin levels (Table I).

The facilitatory effect of superior cervical ganglionectomy on the ether

Table I

Effect of cervical superior ganglionectomy on stress-induced plasma corticosterone, hypothalamic and pineal serotonin levels

Time (min)	Group	N	Plasma corticosterone		Hypothalamic serotonin		Pineal serotonin	
			μg/100 ml	significance (p)	μg/g	significance (p)	ng/pineal	significance (p)
0	1. Sham-operated	33	9.0±0.93*		2.03±0.06		46.8±1.52	
	2. Ganglionectomized	36	8.6±0.97	NS vs. 1.	2.05±0.17	NS vs. 1.	30.8±1.91	<0.001 vs. 1.
	Ether stress							
30	3. Sham-operated	40	45.1±2.15	<0.001 vs. 1.	0.78±0.05	<0.001 vs. 1.	47.9±2.05	NS vs. 1.
	4. Ganglionectomized	22	64.2±4.10	<0.001 vs. 2. <0.001 vs. 3.	0.86±0.16	<0.001 vs. 2. NS vs. 3.	28.2±2.31	NS vs. 2. <0.001 vs. 3.
90	5. Sham-operated	21	11.5±1.24	NS vs. 1. <0.001 vs. 3.	2.16±0.12	NS vs. 1. <0.001 vs. 3.	44.1±2.50	NS vs. 1. NS vs. 3.
	6. Ganglionectomized	20	10.9±1.05	NS vs. 2. <0.001 vs. 4. NS vs. 5.	2.13±0.11	NS vs. 2. <0.001 vs. 4. <NS vs. 5.	26.0±3.32	NS vs. 2. NS vs. 4. <0.001 vs. 5.
	Electric shock							
30	7. Sham-operated	13	39.4±2.15	<0.001 vs. 1.	0.63±0.16	<0.001 vs. 1.	47.1±3.31	NS vs. 1.
	8. Ganglionectomized	9	59.9±4.15	<0.001 vs. 2. <0.001 vs. 7.	0.74±0.05	<0.001 vs. 2. NS vs. 7.	25.3±3.05	NS vs. 2. <0.001 vs. 7.

* Mean ± S.E.
N: Number of rats
NS: Not significant

Table II

Effect of cervical superior ganglionectomy on ether stress-induced plasma corticosterone, hypothalamic and pineal serotonin levels at different times after ganglionectomy

Time (days)	Group	N	Plasma corticosterone		Hypothalamic serotonin		Pineal serotonin	
			μg/100 ml	significance (p)	μg/g	significance (p)	ng/pineal	significance (p)
7	1. Sham-operated	40	45.1±2.15*		0.78±0.05		47.9±2.05	<0.001 vs. 1.
	2. Ganglionectomized	22	64.2±4.10	<0.001 vs. 1.	0.86±0.16	NS vs. 1.	28.2±2.31	
14	3. Sham-operated	8	47.9±2.25	NS vs. 1.	0.95±0.09	NS vs. 1.	44.1±2.12	NS vs. 1.
	4. Ganglionectomized	10	62.5±3.81	NS vs. 2. <0.01 vs. 3.	0.98±0.12	NS vs. 2. NS vs. 3.	26.3±2.26	NS vs. 2. <0.001 vs. 3.
28	5. Sham-operated	8	44.1±2.83	NS vs. 1.	0.86±0.12	NS vs. 1.	43.1±3.11	NS vs. 1.
	6. Ganglionectomized	8	58.3±2.55	NS vs. 2. <0.001 vs. 5.	0.80±0.07	NS vs. 2. NS vs. 5.	28.0±2.26	NS vs. 2. <0.001 vs. 5.
56	7. Sham-operated	8	45.4±2.41	NS vs. 1.	0.86±0.17	NS vs. 1.	46.8±3.85	NS vs. 1.
	8. Ganglionectomized	10	62.3±2.85	NS vs. 2. <0.001 vs. 7.	0.85±0.05	NS vs. 2. NS vs. 7.	26.2±2.53	NS vs. 2. <0.001 vs. 7.

* Mean ± S.E.
N: Number of rats
NS: Not significant

stress-induced plasma corticosterone level persisted for more than 8 weeks. The hypothalamic and pineal serotonin levels were always the same as in the control group (Table II).

Discussion

The effect of the pineal gland on the hypothalamo–pituitary–adrenal system has always been a matter of controversies. Following pinealectomy, the adrenal gland as well as the gonads show hypertrophy (KITAY 1954; KITAY and ALTSCHULE 1954) and so did the adrenal (WURTMAN et al. 1959; FRASCHINI et al. 1968; HOUSSAY and PAZO 1968). However, adrenal hypertrophy failed to occur in castrated animals (FRASCHINI et al. 1968). The inhibitory role of the pineal gland on gonadal function has been well documented (e.g. FRASCHINI et al. 1971; MESS et al. 1971; MOSZKOWSKA et al. 1971). Increased gonadal weight following pinealectomy would indicate an increase in gonadal function liberated from pineal inhibition. It has also been shown that the oestrogens cause adrenal hypertrophy (TELEGDY et al. 1962; KITAY 1963). The increase in adrenal weight after pinealectomy and the absence of this increase in gonadectomized animals would support the conclusion that the adrenal hypertrophy might be secondary, and be due to gonadal hyperfunction. Other authors could not, however, find differences in adrenal weight following pinealectomy (DILL 1961; GIROD et al. 1963; KINSON et al. 1968).

Corticosterone production in vitro increases after pinealectomy but this increase disappers in 30 days (KINSON et al. 1967, 1968). On the other hand, no difference in plasma corticosterone level was found in rats after pinealectomy (DILL 1961). Keeping the pinealectomized animals in dark, elevated the plasma corticosterone level in 10 days and this increase disappeared by the 30th day after pinealectomy (NIR et al. 1971).

Systemic administration of melatonin had no effect on adrenal function (BARCHAS et al. 1969); but when implanted into the median eminence, the compound caused a decrease in adrenal weight (FRASCHINI et al. 1968) or, when injected into the lateral ventricle, it diminished the adrenal response to stress (MOTTA et al. 1971; VERMES et al. 1972a). The inhibitory action of intraventricularly administered melatonin was blocked by p-chlorophenylalanine treatment (VERMES et al. 1972a), indicating that the action of melatonin was mediated by serotonin.

The role of hypothalamic serotonin in the regulation of hypothalamo–pituitary–adrenal function has been proved (VERMES and TELEGDY 1972; VERMES et al. 1972b). It was suggested that the hypothalamic serotoninergic transmission inhibited the pituitary–adrenal system (TELEGDY and VERMES 1973) and the present findings also supported this concept. The diurnal fluctuation of the hypothalamic serotonin content showed a pattern opposite

to that of the plasma corticosterone. A high plasma corticosterone level was associated with low hypothalamic serotonin contents. Pinealectomy had no effect on the hypothalamic serotonin level, in agreement with the data of GREEN et al. (1973) and in contrast to those of MOSZKOWSKA et al. (1971) who found a decrease in castrated pinealectomized animals. It has been shown that in female animals, ovariectomy by itself decreased the hypothalamic serotonin level (RÓZSAHEGYI et al. 1973).

The role of the superior cervical ganglia in pineal function is well documented (e.g. WURTMAN et al. 1968). The pineal serotonin rhythm (QUAY 1963) diminished following removal of the cervical superior ganglia (SNYDER et al. 1965), and this has been confirmed by the present findings. However, removal of the said ganglion failed to affect the hypothalamic serotonin rhythm. The effect on neuroendocrine processes of the superior cervical ganglia is unclear; their removal has been shown to diminish the compensatory hypersecretion without affecting compensatory adrenal hypertrophy (FENDLER and ENDRŐCZI 1965), and, also, to prevent development of the "miniature neurohypophysis" following transection of the pituitary stalk (FENDLER et al. 1970). It had no action on pituitary blood flow (GOLDMAN 1968) but was able to change the cerebral blood flow (EDVINSSON et al. 1971) and pineal gland flow (GOLDMAN 1967).

In our experiments only the stress response was facilitated without affecting the basal corticosterone level and the diurnal rhythm of the hypothalamic serotonin content. This action cannot be explained by a denervation hypersensitivity since the effect could be observed even 58 days after ganglionectomy and up to then there was no difference in the intensity of the response. Whether the action is due to a change in the blood supply of the brain or of the hypothalamus or some other endocrine glands such as the thyroid, is unclear.

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CONCENTRATION AND IN VITRO RELEASE OF PLACENTAL HUMAN CHORIONIC SOMATOMAMMOTROPIN (HCS) IN THE CASE OF FOETUSES OF NORMAL WEIGHT AND WITH INTRAUTERINE WEIGHT RETARDATION

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Human chorionic somatomammotropin (HCS) concentration of the placenta was determined in the course of parturition of 100 normal-weight foetuses born at term and in 100 dysmature foetuses similarly born at term. In addition, the rate of HCS release from the placenta during 8 hours incubation has been studied. The placentae belonging to dysmature foetuses contained nearly twice as much HCS per g tissue (1399 μg) than those belonging to foetuses of normal weight (684 μg). From normal placentae, 81.2% of the hormone content was released during the 8-hour incubation while from the dysmature placentae only 24.6% of the originally low hormone content. One g of normal placental tissue released, even in absolute terms, more HCS (525 μg) than did dysmature placentae (308 μg). It is assumed that the low maternal serum HCS level in the case of intrauterine retardation accompanying chronic placental insufficiency may be due to inhibited HCS release from the placenta into the maternal circulation.

HCS (Human Chorionic Somatomammotropin) is a proteohormone produced by the placenta. Sciarra (1964) and Grumbach and Kaplan (1964) showed that it was produced by the syncitiotrophoblast and, after entering the maternal circulation, it took part in the regulation of the metabolic processes responsible for the intrauterine development of the foetus (Grumbach et al. 1968). Therefore, the hormone is also called the growth hormone of pregnancy (Kaplan and Grumbach 1964). In the case of foetuses with intrauterine weight retardation, the maternal serum HCS level is considerably lower than in the case of foetuses of normal weight (Bellmann and Lang 1973; Berle 1973; Doszpod and Gáti 1974; Saxena et al. 1969; Zuckerman et al. 1970). Therefore, determination of the serum HCS level is an important endocrine parameter of intrauterine retardation accompanying chronic placental insufficiency.

HCS in maternal circulation is sensitive indicator of placental function, since with its short half-life it immediately calls attention to a change in the function of the placenta. In normal pregnancy, the hormone in maternal serum can be detected with radioimmune methods after the 8th week; from this time on, it exhibits a steady augmentation without diurnal alterations up to the 36th–38th week.

Few data are available on the HCS content of placental tissue. SUWA and FRIESEN (1969) found a value of 308 ± 74μg/g wet weight in six spontaneously delivered placentae. According to GRUMBACH and KAPLAN (1964), the hormone concentration in one g of placental tissue is nearly the same throughout the pregnancy. MACMILLEN (1970) found a low maternal serum level with low placental HCS content in the case of newborns retarded in weight, and a low maternal serum level with normal placental concentration in the case of newborns retarded in length. GÁTI et al. (1970), using a biological method, found the prolactin-like activity of the placenta to be considerably diminished in cases of severe toxaemia.

The present investigations had the aim to study the placental HCS concentration in the case of infants born at term with normal weight and of infants with weight retardation, and to determine the amount of HCS per g of placental tissue, as well as the rate of its release during 8-hour incubation. In addition, we measured the HCS concentration of specimens obtained from various parts of the same placenta and determined the difference in HCS concentration of macroscopically intact and infarcted regions.

Method

The HCS content of one g of placenta was estimated in the course of the delivery of 100 normal-weight foetuses born spontaneously at term (38th–41st week of pregnancy) and of 100 low-weight foetuses with intrauterine retardation, born at term (38th–41st week of pregnancy). One g of tissue, intact at gross examination, was excised from the central part of the placenta in each case, washed in Krebs–Ringer bicarbonate buffer and kept at −20°C. These samples served for determination of the pre-incubation HCS concentration. Hormone concentration of the washing fluid could be neglected, for five ml of it contained less HCS than the lower limit of sensitivity of the method.

A similar one g specimen, taken from the region neighbouring the area of the previous excision, was washed and incubated in 5 ml of Krebs–Ringer bicarbonate buffer pH 7.4 for 8 hours. The incubation medium was changed every hour and stored at −20°C. After incubation, the placental specimen was similarly kept at −20°C; it served for determination of the post-incubation HCS level.

In addition, 10 × 1 g of intact placental tissue was excised from different parts of five normal placentae, in order to study if there was any difference in HCS concentration between the different parts of the placenta.

Forty out of the 100 placentae belonging to dysmature foetuses were studied. In each case, one g pieces were excised from the intact and infarcted region and their hormone content was determined.

The dysmature foetuses were under five percentile according to LUBCHENCO's standard (LUBCHENCO et al. 1963), and the dysmaturity was proved also clinically.

Homogenization of placental tissue. One g (wet weight) of placental tissue was washed in Krebs–Ringer bicarbonate buffer, kept at −20°C for 24 hours and homogenized in 5 ml of a mixture of 0.1 M NH_4OH and 0.1 M NH_4HCO_3 (pH 9.2) in a glass homogenizer. The homogenate was kept at +4°C overnight and then centrifuged at 3000 g. The supernatant was stored at −20°C, the residue was discarded (SUWA and FRIESEN 1969).

Estimation of HCS was performed by means of the "Phadebas" radioimmune kit of Pharmacia, Sweden. The values given refer to one g of wet placental tissue. Sensitivity of the method: 100 ng/ml (LINDBERG et al. 1972). Evaluation of HCS release of placental tissue was done by regression analysis of the pre- and post-incubation HCS concentrations of placentae belonging to normal and dysmature foetuses, by Student's *t*-test.

Results

Table I shows the average weight of the foetuses and placentae, as well as the placental HCS concentrations. The weight of 100 dysmature foetuses averaged 2265 ± 267 g; that of the placentae, 468 ± 98 g. HCS concentration of DP* was 1339.29 ± 24.62 μg/g wet weight; and, for the whole placenta, 654.70 ± 86.12 mg. The weight of 100 foetuses born with normal weight averaged 3464 ± 351 g, that of the placentae 605 ± 135 g. HCS concentration of NP was 684.40 ± 15.47 μg/g wet weight, and, for the whole placenta 413.80 ± 73.66 mg.

Table I

Normal and dysmature newborns, weight and HCS concentration of the placentae

n = 100	Dysmature	Normal
Weight of foetus (g)	2265±267	3464±351
Weight of placenta (g)	468±96	605±135
HCS concentration of placenta (μg/g)	1399.29±23.62	684.40±15.47
Calculated HCS content of placenta (mg)	654.70±86.12	413.80±73.66

One g of DP released 308.58 μg HCS into the incubating medium during an 8-hour incubation. The corresponding value for NP was 525.32 μg/g. The amount of HCS released at one-hour intervals is shown in Fig. 1.

HCS content of one g of DP was 1399.3 μg before incubation and 1055.7μg after incubation. The corresponding values for DM were 684.4 μg and 129.2 μg, respectively. The difference between the pre- and post-incubation values was 343.6 μg for DP and 555.2 μg for NP. The amount of HCS detected in the medium after an 8-hour incubation was 308.6 μg for DP and 525.3 μg for NP. Thus, the difference between the calculated and detected values was 35.0 μg for DP and 29.9 μg for NP (Table II).

In the case of DP, only 24.6% of the HCS originally present in the tissue was released into the medium during the 8-hour incubation; the corresponding value for NP was 81.2%.

The pre- and post-incubation difference in HCS content of one g of DP and NP was highly significant statistically for both DP ($t = 6.781$, $p < 0.001$) and NP ($t = 9.253$, $p < 0.001$).

Table III shows the HCS content of ten one g specimens excised from various parts of five normal placentae. The differences in HCS content were negligible.

* DP means a placenta belonging to a dysmature foetus, and NP one belonging to a normal-weight term foetus.

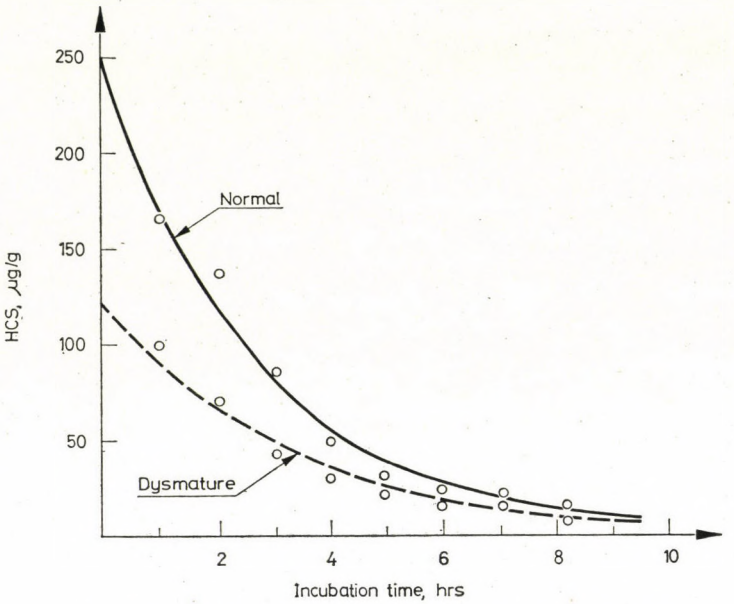

Fig. 1. HCS release from one g of placenta during 8 hours

Table II

HCS concentration of placenta before and after 8-hour incubation and HCS release from placenta during 8-hour incubation

n = 100	Dysmature placental HCS concentration, µg/g	Normal placental HCS concentration, µg/g
Before incubation	1399.3	684.4
After incubation	1055.7	129.2
HCS released during incubation (calculated value)	343.6	555.2
HCS detected in incubation medium (measured value)	308.6	525.3

Table III

HCS concentration of one g specimens from different parts of the placenta

Placenta, No.	HCS, µg/g n = 10	Standard deviation ±	Extreme values
1	451.93	12.63	430—471
2	871.95	22.51	845—915
3	823.50	16.29	796—849
4	654.70	12.58	639.5—683
5	476.65	19.61	446—510

Hormone concentration in 40 DP was 1345.7 $\pm$ 285.2 μg/g in macroscopically intact parts and 176.6 $\pm$ 64.2 μg/g in infarcted parts.

Discussion

VARMA et al. (1971) found a close relationship between maternal serum HCS level and the weight of the placenta. GÁTI et al. (1973) showed a close relationship between the maternal serum HCS level and the weight of the newborn.

On this basis it was thought at the beginning of the present investigations that, in the case of retarded foetuses where the maternal serum HCS level is definitely lower than normal, the placenta too would contain less HCS, i.e. that the cause of the low maternal serum HCS was an inhibited hormone secretion of the injured placenta. The investigations revealed, however, that placentae belonging to dysmature foetuses contained nearly twice as much HCS per g tissue than those belonging to normal-weight foetuses. The total amount of hormone calculated for the whole organ was also considerably higher in the smaller placentae belonging to dysmature foetuses than in the normal placentae of nature foetuses.

As an explanation of these findings a compensatory mechanism of the placenta may be assumed, in other words, an increased hormone production per one g of the placentae of lower weight. However, the high placental hormone concentration was always associated with a low maternal serum concentration, a fact suggestive of a diminished hormone release from the placenta. This is corroborated by SCIARRA'S (1964) finding, according to which the syncitiotrophoblast of the normal placenta produces HCS in large amount but does not store it. On the other hand, the high hormone concentration of placentae belonging to dysmature foetuses speaks for a storage of the hormone. Our incubation studies also indicated an inhibited hormone release into maternal circulation. One g of DP released a considerably lower proportion of its HCS content (24.6%) than did one g of NP (81.2%). The difference between the absolute amounts was also significant: 308.6 μg/g in the case of DP, and 525.3 μg/g in the case of NP.

The HCS level in maternal serum seems to be an important factor in intrauterine development of the foetus. The hormone content per g of placenta, or for the whole organ, seems to be of considerably less importance. It may be assumed that, in chronic placental insufficiency when the vascular disturbance of the placenta is accompanied by a low maternal serum HCS level and intrauterine retardation, the hormone release from the placenta into maternal circulation is disturbed. According to GRUMBACH et al. (1968), HCS passes

from the syncitiotrophoblast into maternal circulation not directly but through a complicated cellular activity. Thus, in cases of chronic placental insufficiency, one has to reckon with some kind of biochemical damage to the placental cells.

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EFFECT OF GONADOTROPHINS ON ELECTRICAL ACTIVITY OF THE BRAIN IN RATS

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Human chorionic gonadotrophin injected into the midline thalamic nuclei or into the lateral ventricle increased the threshold of evoked potential, lengthened the recovery period between two consecutive evoked potentials, and decreased the threshold of spindle-like after-discharges elicited by stimulation of the reticular formation.

Luteinizing hormone given intraventricularly caused spontaneous spindle bursts, decreased the threshold of stimulus-induced after-discharges and lengthened the recovery period between paired evoked potentials.

Follicle stimulating hormone, prolactin or inactivated human chorionic gonadotrophin and luteinizing hormone had no effect.

In the first trimester of human pregnancy there is an increased frequency of psychic and vegetative disturbances. Previous studies have shown that human chorionic gonadotrophin (HCG) facilitates the extinction of conditioned avoidance reflexes and decreases intertrial and exploratory activity during extinction in animal experiments (TELEGDY and RÓZSAHEGYI 1971; TELEGDY et al. 1971).

In the oestrogen- primed rabbit, human chorionic gonadotrophin and luteinizing hormone induce an EEG "after-reaction" characterized by sleep spindles and 8/sec high amplitude synchronous "hippocampal hyperactivity" (SAWYER and KAWAKAMI 1959). In castrated rats, HCG given intravenously (HARTMANN et al. 1971) or administered into the lateral hypothalamus, dorsal hippocampus or the mesencephalic reticular formation decreases the threshold of spindle-like activity elicited by stimulation of the vagina or the mesencephalic reticular formation (HARTMANN et al. 1972).

In the present study the effect of human chorionic gonadotrophin and different pituitary gonadotrophins has been investigated on spontaneous EEG activity, evoked potentials elicited by stimulation of the mesencephalic reticular formation, the stimulus-induced EEG after-reaction as well as on the recovery period of the evoked potential between paired stimuli.

Methods

In the experiments, R-Amsterdam adult female rats were used. The animals were castrated at least two months prior to observation and kept under standard laboratory condition.

For the electrophysiological study the animals anaesthetized with ether were subjected to tracheal cannulation and implantation of electrodes in different regions of the brain. Thereafter the animals were immobilized by curarization (Curarine-Asta, 2 mg/kg) and their respiration was maintained artificially.

The bipolar subcortical recording electrodes were made of stainless steel insulated with enamel except their 0.5 mm tip. For neocortical recordings, silver ball electrodes were used. Electrical activity was registered with a 12-channel Hellige Neuroscript apparatus and dual beam cathode-ray oscilloscope.

Bipolar stainless steel electrodes were used for stimulation of the mesencephalic reticular formation by a Disa Multistim rectangular impulse generator through an isolation unit.

Spontaneous EEG activity, the threshold of evoked potentials in the perietal cortex and various subcortical structures, elicited by stimulation of the reticular formation, the threshold of stimulus-induced spindle-like after-discharges and the duration of spindles have been recorded. In each animal the recovery period between two evoked potentials was also measured in a double stimulation model. Two electric pulses of the same magnitude were applied with a certain latency between them. When the amplitude of the second evoked potential had reached the first, latency time was measured in msec as the recovery period.

The gonadotrophins were injected in 20 μl volume into the medial thalamic nuclei or the lateral cerebral ventricle through a microcannula with an Agla micrometer All-glass syringe (Burroughs Wellcome Co., England).

The following hormones were used: human chorionic gonadotrophin (Choriogonin, G. Richter Co., Budapest or Pregnyl, N. V. Organon Oss, Holland), luteotrophin (Luteinizing Hormone, Calbiochem Equine B grade Lot 71525 or Luteotropin, Versuchs-Präparat Nr. 854 A, Arztneimittelwerk Dresden), follicle-stimulating hormone (NIH-FSH-P-1, Porcine) and prolactin (NIH-P-S-7, Ovine).

The control animals received vehicle or gonadotrophins previously inactivated by boiling for 60 min. Location of the electrodes and the microcannula were controlled in brain sections fixed in 10% formol.

Results

1. Effect of human chorionic gonadotrophin (HCG) on electrical activity of the anterior hypothalamic and preoptic region, dorsal hippocampus and ipsilateral parietal cortex

Localization of the cannula and of the recording and stimulating electrodes is shown in Fig. 1.

a) Effect of HCG injection into the medial thalamic nuclei (parafascicular and paraventricular nuclei). HCG in a dose of 150 I.U. injected in 6 animals in 9 cases caused spike activity for 1—2 min. In some cases this was followed by a seizure activity and "silent period" of a few minutes. In this period, EEG activity was suppressed in every investigated structure. By the tenth min the threshold of evoked potentials was elevated with an average of 30% of the pretreatment level in 3 cases in the hypothalamic-preoptic area, 2 cases in the dorsal hippocampus and 1 case in the parietal cortex.

Following paired stimuli, the amplitude of the second evoked potential was inhibited in the same time interval as after HCG treatment (Fig. 2b vs. a).

The recovery period of the first stimulus as compared to the control value was delayed by 20—60% after treatment in 6 out of 9 cases in the anterior hypothalamic-preoptic region, neocortex and in every case in the dorsal hippocampus (Fig. 2c vs. a).

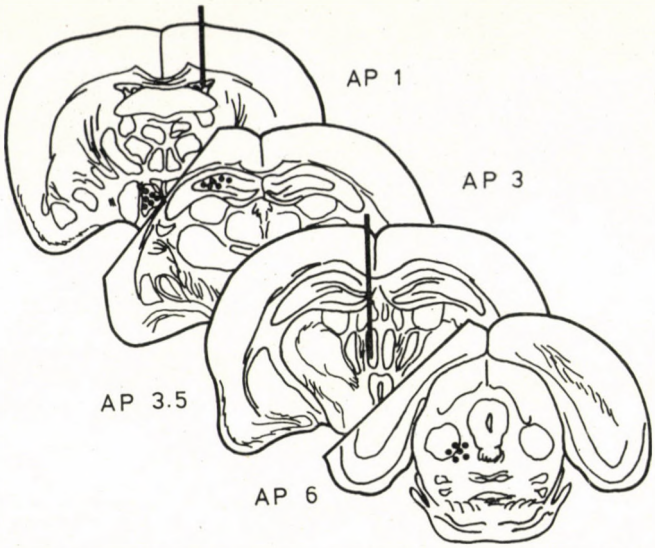

Fig. 1. Schematic localization of cannulae, recording and stimulating electrodes, used in experiment Nos 1a and 1b. The heavy black vertical lines represent the cannulae and the black points the tips of electrodes. AP 1: cannula in lateral cerebral ventricle, recording electrodes in the anterior hypothalamic area; AP 3: recording electrodes in dorsal hippocampus; AP 3.5: cannula in the parafascicular or paraventricular thalamic nuclei; AP 6: stimulating electrodes in mesencephalic reticular formation. Coordinates according to FIFKOVÁ and MARSALA (1967).

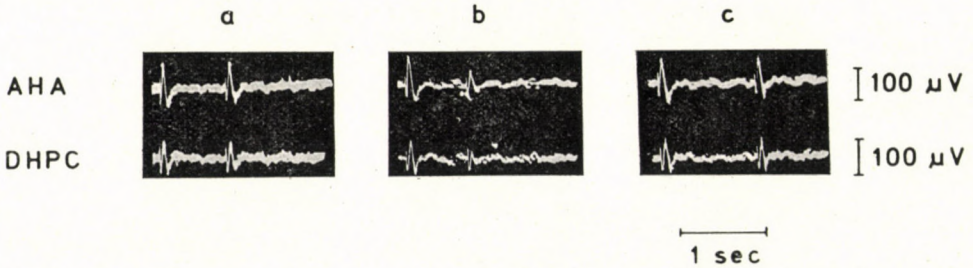

Fig. 2. Effect of human chorionic gonadotrophin (Choriogonin) administered into the medial thalamic nuclei on amplitude and recovery period of paired evoked potentials. The evoked potentials were elicited by stimulation of mesencephalic reticular formation (3 V, 0.15 msec impulse duration). a: prior to treatment; b and c: 20 min after HCG administration. Abbr: AHA: anterior hypothalamic area; DHPC: dorsal hippocampus

The threshold of spindle-like after-discharges followed by stimulation of the reticular formation decreased by 60% in 7 out of 9 cases in the hypothalamic-preoptic area and dorsal hippocampus and in each case in the cortex. After HCG treatment, duration of the spindles was prolonged by about 40%. Spontaneous spindle bursts were never observed.

The changes of the recovery period and the spindle-like after-discharges lasted from 10 to 60 min.

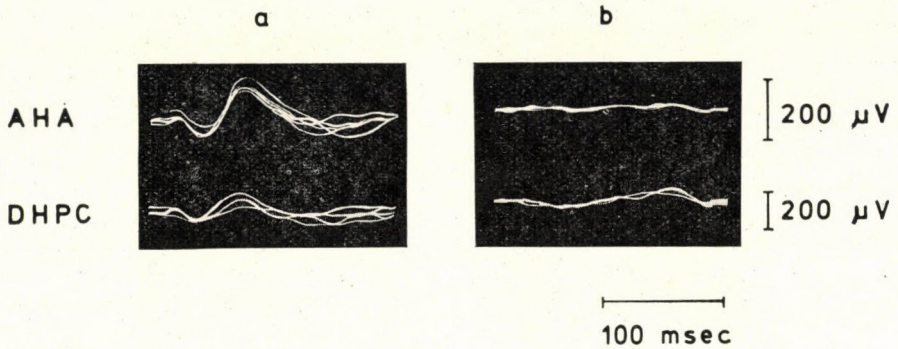

Fig. 3. Effect of human chorionic gonadotropin (Pregnyl, 200 I.U.) administered into the lateral ventricle on the threshold of evoked potentials. The figure shows five superimposed responses. The evoked potentials were elicited by stimulation of the mesencephalic reticular formation (1.6 V, 0.15 msec). a: before treatment; b: 10 min after HCG administration

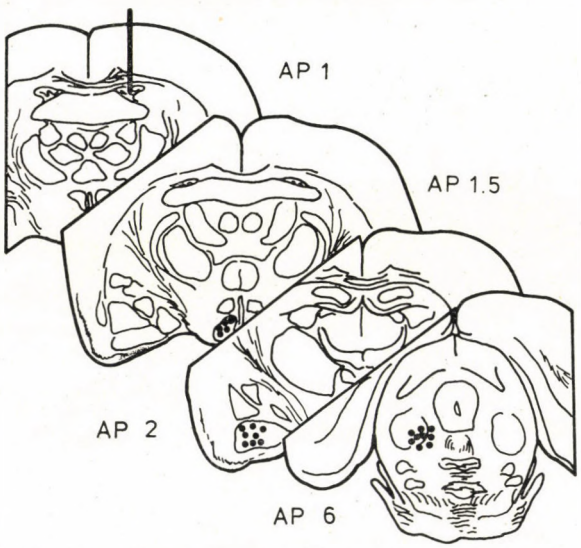

Fig. 4. Schematic localization of cannula, recording and stimulating electrodes, in experiment No 2. AP 1: cannula in lateral cerebral ventricle; AP 1.5: recording electrodes in hypothalamic ventromedial nucleus; AP 2: recording electrodes in basal amygdala; AP 6: stimulating electrodes in mesencephalic reticular formation. For details, see Fig. 1

Inactivation of HCG by 60 min boiling abolished the above effects except the spike activity, which could also be observed in some cases in control animals.

b) Effect of HCG injection into the lateral cerebral ventricle. HCG in dose of 200 to 500 I.U. was given to 3 animals in 6 cases.

Administration of the hormone elicited no spike or seizure activity. The threshold of evoked potentials increased (Fig. 3), and the threshold of

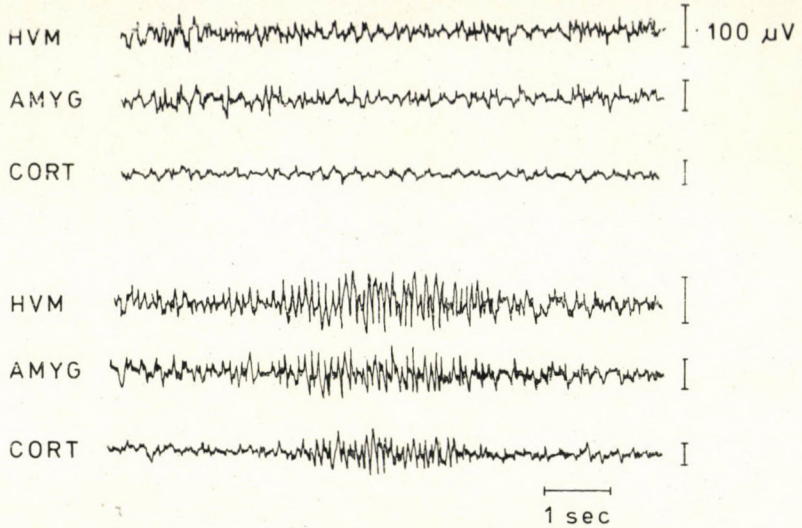

Fig. 5. Effect on spontaneous EEG activity of luteinizing hormone (Luteotropin, 2 I.U.) administration into the lateral ventricle. The first three recordings were obtained prior to treatment, the second three, 35 sec after hormone administration. Abbr: HVM: ventromedial hypothalamic nucleus; AMYG: basal amygdala; CORT: parietal cortex

stimulus-induced spindle activity decreased in each case as in the previous experiment. The recovery period was lengthened in each structure to 150—160% of its pre-injection duration in 5 out of 6 cases. However, the onset of the described changes appeared 5—10 min earlier than after intracerebral administration.

2. Effect of intraventricular administration of luteinizing hormone (LH) on electrical activity of the ventromedial hypothalamus, basal amydala and ipsilateral parietal cortex.

The site of the cannula, the recording and stimulating electrodes is shown in Fig. 4.

LH (Luteinizing Hormone, 200 μg; or Luteotropin, 2—5 I.U.) was applied to 7 animals in 9 cases. The effects of the two different preparations were identical.

No spike or seizure activity could be observed following LH administration. In contrast to previous experiments, the threshold of evoked potentials did not change during the observation period.

Following LH injection, spontaneous spindle bursts appeared with 5—10 min latency (Fig. 5). These changes occurred in 5 out of 9 cases in the subcortex and cortex. Following this period the threshold of the stimulus-induced spindle activity decreased in the ventromedial hypothalamic nucleus

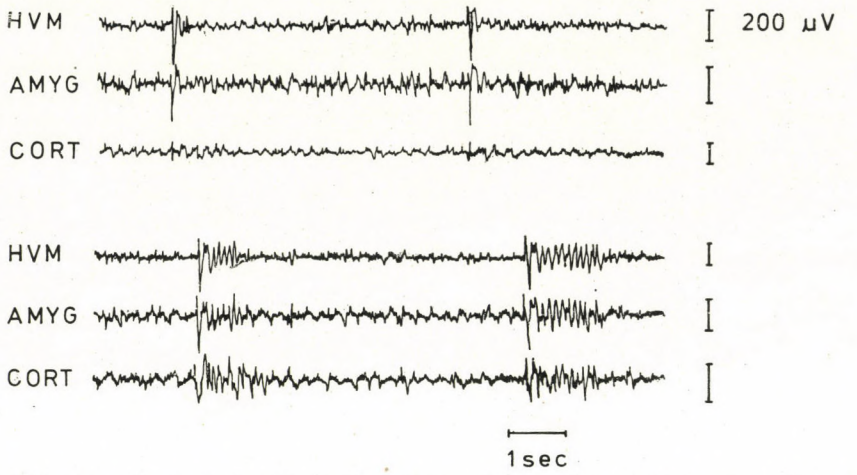

Fig. 6. Effect on stimulus-induced spindle activity of 200 μg luteinizing hormone (Luteinizing Hormone) administered into lateral ventricle. The evoked potentials were elicited by stimulation of the mesencephalic reticular formation (10 V, 0.15 msec). The first three recordings were obtained prior to treatment, the second three 10 min after hormone administration. Abbr: see Fig. 5

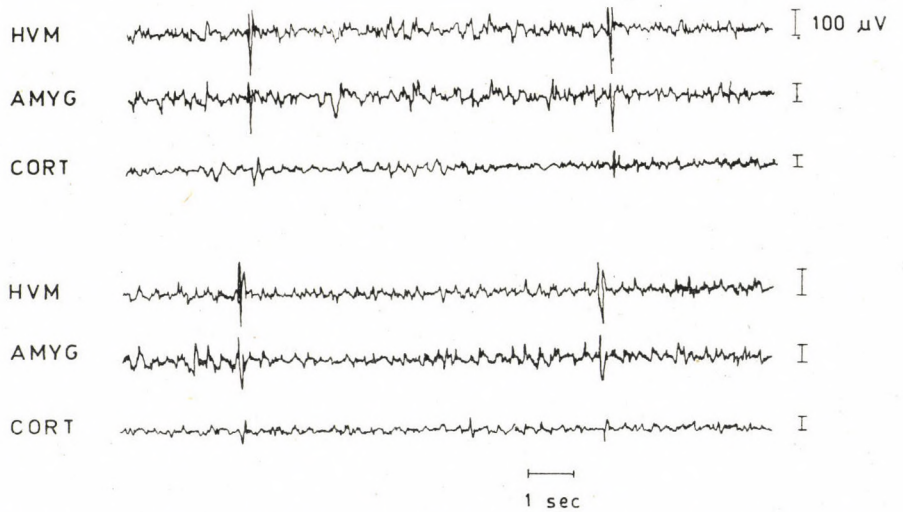

Fig. 7. Effect of inactivated luteinizing hormone administered into the lateral ventricle on stimulus-induced after-discharges. For details, see Fig. 6

and amygdala in 7 out of 9 cases and in the parietal cortex in 6 out of 9 cases (Fig. 6). The changes were in a range of 50—60%.

The recovery period increased by 25—35% in 7 out of 9 cases.

Inactivated LH had no effect (Fig. 7).

Follicle stimulating hormone (100 μg) injected intraventricularly to 5 animals (9 administration) and prolactin (40 μg) to 5 animals (8 administration) were completely ineffective.

Discussion

It has been shown that copulation induced specific changes in cerebral electrical activity in the rabbit (SAWYER and KAWAKAMI 1959). Further experiments showed that these changes were related to the release of luteotrophin, prolactin and posterior pituitary hormones elicited by the coital stimulus (KAWAKAMI and SAWYER 1959). Changes could be detected in single and multi-unit activity which were associated with the secretion of luteotrophin or the administration of gonadotrophin (KAWAKAMI et al. 1970; BLAKE and SAWYER 1972; GALLO et al. 1972; DUFY et al. 1973).

Behavioural studies have shown that HCG facilitates the extinction of conditioned avoidance behaviour and decreases exploratory activity (TELEGDY and RÓZSAHEGYI 1971; TELEGDY et al. 1971). In self-stimulation experiments, after chronic HCG treatment the number of bar pressings decreased (HARTMANN et al. 1971). These behavioural effects are ascribed to the enhanced inhibitory tone of the central nervous processes induced by HCG and LH.

The present study demonstrated that the action of HCG and LH is a general one rather than a locus-specific influence of gonadotrophins on the brain structures. The nature of the effect seems to be an inhibitory one. This was supported by the elevated threshold and lengthened recovery period of evoked potentials.

It has been shown that spindles induced by single or low frequency stimulation of the caudate nucleus are associated with an inhibition of learned behavioural responses (BUCHWALD et al. 1961) and a depression of the firing rate of cells in non-specific thalamic nuclei and the cerebral cortex (BUCHWALD et al. 1967). A correlation between internal inhibition and EEG synchronizing activity of the non-specific thalamo-cortical system (recruiting responses, spindle bursts) has also been shown (SKINNER and LINDSLEY 1967).

In our experiments, the decrease in threshold and increase in duration of stimulus-induced after-discharges are interpreted to indicate an increased tone of central inhibitory processes.

Despite the fact that HCG and LH are almost identical in biological action, pituitary gonadotrophin had no effect on the threshold of evoked potentials, while it was more potent in inducing spindle-like activity. Following LH administration, spontaneous spindle bursts appeared; such bursts have not been observed after HCG treatment.

Since progrestone has been reported to exert a similar action (KOMISARUK et al. 1967; ENDRŐCZI 1969), the question arises whether the action of HCG or LH cannot be brought about by increasing adrenal progesterone secretion. The data published so far seem to exclude this possibility (RESKO 1969).

Other gonadotrophins such as prolactin and FSH were ineffective and

the action of HCG and LH on the brain is related to their gonadotrophic potency. Heat abolishes both the gonadotrophic and the described "neurotrophic" activity.

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Pathophysiologia

BLOOD FLOW AND RELEASE OF FREE FATTY ACIDS IN THE OMENTUM, MESENTERY AND SUBCUTANEOUS ADIPOSE TISSUE OF THE DOG IN HAEMORRHAGIC SHOCK*

By

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Blood flow, release of free fatty acids (FFA) and glycerol were measured in the subcutaneous, mesenteric and omental adipose tissues in chloralose-anaesthetized dogs during a standardized haemorrhagic shock procedure. Resting blood flows were 6.3 ± 1.4 ml/min/100 g (±SEM) in the subcutis, 14.8 ± 3.3 ml/min/100 g in the mesentery, and 5.3 ± 1.2 ml/min/100 g in the omentum. There was a pronounced reduction of blood flow during bleeding to an arterial pressure of 55 mm Hg for 90 min and it remained low during bleeding to 35 mm Hg for an additional 90-minute period. Blood flow in the mesentery was significantly higher than in the other two adipose tissues in both the control and the bleeding periods. There was no increase of FFA release from adipose tissue but glycerol release from the mesentery was significantly increased. The arterial concentration of FFA did not change but there was a significant elevation of the glycerol concentration from 0.21 ± 0.04 mM to 0.95 ± 0.22 mM ($p < 0.05$). Arterial pH decreased from 7.28 ± 0.03 to 7.06 ± 0.04, and the lactate level rose from 3.18 ± 0.38 to 10.66 ± 1.61 mM during bleeding.

It is concluded that the low blood flow in adipose tissue following bleeding may impair the outflow of FFA and glycerol. Regional differences in the intensity of the blood flow reduction may be the explanation for the significant rise in the outflow of glycerol from the mesentery but not from the subcutis or the omentum. The re-esterification of FFA increased following bleeding, presumably due to the high lactate concentration. As a consequence of the low pH, the lipolytic rate diminished in adipose tissue in spite of a presumably high sympathetic neurohumoral activity. The rise in the reesterification rate and inhibiton of lipolysis as well as the diminution in adipose tissue blood flow counteracts the outflow of FFA.

In a previous paper (Kovách et al. 1970) we have shown that in canine subcutaneous adipose tissue during haemorrhage, blood flow decreased approximately to 10% of the resting level and often ceased completely. Hence, this decrease was found to be more pronounced than in other organs including skeletal muscle, myocardium, kidney, liver, hypothalamus and intestine with

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the same haemorrhagic shock procedure (KOVÁCH 1970). Furthermore, there was no elevated rate of FFA release from the subcutaneous adipose tissue despite a presumably high sympathetic neurohumoral activity which promotes lipolysis. It was suggested that a marked restriction of blood flow impaired considerably the outflow of FFA. It is not known, however, whether this holds true also for adipose tissue from other regions. Thus, BALLARD and ROSELL (1969, 1971) found regional differences in the sympathetic neurohumoral control of blood flow and lipid metabolism. The adipose tissue in the mesentery does not seem to be controlled by the sympathetic neurohumoral system (BALLARD and ROSELL 1969). Furthermore, in the omentum during prolonged sympathetic nerve stimulation there was a pronounced escape from the initial vasoconstriction, especially at high (9 c/s) stimulation frequencies (BALLARD and ROSELL 1971). These regional differences in the response to sympathetic nervous activity indicate that the reaction to bleeding in one region may not be representative of adipose tissue in other regions. Therefore, the present series of experiments was initiated in order to compare circulatory and metabolic changes in the canine subcutaneous adipose tissue, omentum and mesentery during standardized haemorrhagic shock. To obtain information concerning the degree of lipolysis and reesterification of FFA, the release of FFA as well as of glycerol has been determined.

Methods

The experiments were performed on 22 female mongrel dogs fasted for approximately 18 hours. They were anaesthetized with 100 mg/kg α-D(+)-glucochloralose (Merck, Darmstadt) and subjected to tracheotomy. Heparin (Richter, Budapest), 5 mg/kg was administered intravenously about one hour prior to the experimental run with no further additions. After this period the effect of heparin on clearing factor lipase seems to be stabilized (FREDHOLM 1970). The subcutaneous tissue in the right inguinal region was isolated from the muscle fascia and the skin as described earlier (ROSELL 1966). The nervous supply to the adipose tissue and the principal artery were left intact. In one series of experiments (10 dogs) a portion of the omentum was also isolated (BALLARD and ROSELL 1971). A branch of the systemic artery, the vein and the nerves accompanying the vessels were dissected free at the level of the greater curvature of the stomach, and a portion of the omental tissue supplied by these structures was separated by ligatures from the remainder of the omentum. The spleen was exteriorized. In another series of experiments (12 dogs) the subcutaneous adipose tissue preparation was combined with a mesenteric preparation (BALLARD and ROSELL 1969). A loop of the small intestine was selected and the superior mesenteric artery, vein and accompanying nerves were isolated from the lymphatic tissue. Branches from the artery and vein other than those supplying the selected loop were ligated. The mesenteric segment was isolated from the small intestine by ligatures.

To record blood flow, the veins from the different adipose tissue preparations were cannulated and the blood directed by polyethylene tubing to drop recorders. The venous blood was then collected in ice-cooled centrifuge tubes for later analysis of FFA and glycerol. Arterial samples were taken from a cannula in the brachial artery for similar analysis. Arterial pressure was measured in the other brachial artery. Cardiac output and three fractions of it were measured according to the thermodilution principle (FRONEK and GANZ 1960; KOVÁCH and MITSÁNYI 1964). Saline at room temperature was injected into the aorta at three different levels: aorta ascendens, at the level of the diaphragm, and below the renal arteries. The thermistor was inserted above the bifurcation of the aorta. Respiratory rate was measured with a thermistor in the tracheal cannula; EEG was recorded from the fronto-occipital leads and ECG from the

standard lead II. These parameters were recorded continuously to establish cerebral and cardiac hypoxic damage during the standardized shock procedure (WIGGERS 1950; KOVÁCH 1961, 1970) FFA in plasma was determined titrimetrically according to DOLE (1956) as modified by TROUT et al. (1960). Plasma was analyzed enzymatically for glycerol according to LAURELL and TIBBLING (1966). Lactate was determined in plasma according to HOHORST (1962), using the tests from Boehringer and Söhne, Mannheim, Germany. The net release or net uptake of the metabolites was calculated from the arteriovenous concentration differences, blood flow, and haematocrits.

During a control period of approximately 60 minutes following completion of the operative procedures, arterial and venous blood samples were taken at 30, 45 and 60 minutes. The dogs were then bled into a reservoir to a mean blood pressure of about 55 mm Hg for 90 min (Bleeding I), followed by further bleeding to about 35 mm Hg for 90 min (Bleeding II). Blood samples were taken every 30 min. Thereafter, the shed blood was reinfused and all parameters were registered for 45 min. Blood samples were taken every 15 min during the reinfusion period.

Statistical analysis was performed according to Duncan's multiple-comparison *t*-test (HARTER 1960).

Results

Arterial concentrations of plasma FFA and glycerol

Mean arterial plasma concentration of FFA was 0.23 ± 0.02 (±SEM) mM (Table I). It did not change significantly during the two bleeding periods or after infusion of the shed blood. The arterial concentration of glycerol, on the other hand, was doubled during Bleeding I and increased further during Bleeding II (Table I). After reinfusion, the glycerol concentration tended to decrease but remained significantly above the prebleeding level.

Table I

Effect of haemorrhage on arterial plasma FFA and glycerol concentrations
(Mean ± S.E.)

	Control	Bleeding I		Bleeding II		Reinfusion 30′
		60′	90′	60′	90′	
FFA (mM)	0.23±0.02 (n = 21)	0.18±0.01 (n = 21)	0.19±0.02 (n = 10)	0.22±0.04 (n = 17)	0.18±0.04 (n = 9)	0.20±0.02 (n = 12)
Glycerol (mM)	0.21±0.04 (n = 21)	0.36±0.05 (n = 19)	0.40±0.1 (n = 9)	0.69±0.12* (n = 16)	0.95±0.22* (n = 8)	0.82±0.12* (n = 9)

* significant difference from control value, $p < 0.05$

Arterial concentrations of plasma lactate and changes in arterial pH

The arterial lactate concentration was around 3 mM during the prebleeding period and rose significantly after bleeding. In Bleeding II the lactate concentration was about 10 mM (Table II). There was a progressive decline in arterial pH from 7.28 during the prebleeding period to 7.06 at the end of Bleeding II. Following reinfusion there was a further decrease.

Table II

Effect of haemorrhage on arterial pH and plasma lactate concentration
(Mean ± S.E.)

	Control	Bleeding I		Bleeding II		Reinfusion 30′
		60′	90′	60′	90′	
Lactate (mM)	3.18±0.38 (n = 10)	6.21±0.54* (n = 10)	6.92±1.05* (n = 5)	10.09±0.69* (n = 8)	10.66±1.61* (n = 4)	8.06±0.63* (n = 6)
pH	7.28±0.03 (n = 7)	7.20±0.04 (n = 7)	7.16±0.04 (n = 5)	7.09±0.04* (n = 4)	7.06±0.04* (n = 4)	7.02±0.08* (n = 3)

* significant difference from control value, $p < 0.05$

Release of FFA and glycerol from the subcutaneous, mesenteric and omental adipose tissues

The rate of FFA and glycerol release from fat depots was estimated in three different regions: the subcutis, mesentery and omentum. The outflow of FFA did not show any significant change following bleeding (Table III). Due to the marked reduction in adipose tissue blood flow during haemorrhage, in many experiments it was not possible to obtain blood samples sufficient for FFA determination. The basal rate of glycerol release was higher than the rate of FFA release. Glycerol release tended to decrease during the first bleeding period and then to rise during Bleeding II and reinfusion. In the case of the mesentery, glycerol release was significantly higher during the second bleeding period than during the control period.

Table III

Release of FFA and glycerol from subcutaneous, omental and mesenteric adipose tissue
(Mean ± S.E.)

	Control 45′	Bleeding I 60′	Bleeding II 60′	Reinfusion 30′
Subcutis FFA (μM/min/100 g)	−0.13±0.19 (n = 15)	0.08±0.05 (n = 9)	0.14±0.17 (n = 6)	0.09±0.15 (n = 5)
Subcutis glycerol (μM/min/100 g)	0.29±0.11 (n = 12)	0.07±0.15 (n = 12)	0.11±0.43 (n = 6)	0.37±0.49 (n = 7)
Omentum FFA (μM/min/100 g)	0.08±0.15 (n = 7)	0.22±0.1 (n = 7)	−0.03±0.17 (n = 4)	0.12±0.12 (n = 3)
Omentum glycerol (μM/min/100 g)	0.22±0.42 (n = 9)	0.08±0.16 (n = 7)	0.13±0.12 (n = 4)	0.67±1.01 (n = 6)
Mesentery FFA (μM/min/100 g)	0.07±0.4 (n = 6)	0.37±0.09 (n = 7)	0.14±0.38 (n = 4)	0.37±0.09 (n = 6)
Mesemtery glycerol (μM/min/100 g)	0.37±0.13 (n = 10)	0.19±0.14 (n = 8)	0.48±0.3* (n = 4)	0.65±0.07** (n = 3)

* significant difference from control value, $p < 0.025$
** significant difference from 60 min BI value, $p < 0.02$
— indicates uptake instead of release

Blood flow in the subcutaneous, mesenteric and omental adipose tissue

Resting blood flow was significantly higher in the mesentery than in the subcutaneous and omental adipose tissue. In all three tissues there was a pronounced decrease in blood flow after bleeding (Table IV). Blood flow in the mesentery remained, however, significantly higher than in the subcutaneous tissue.

Discussion

The present results have confirmed and extended our previous findings in standardized haemorrhagic shock. In spite of a presumably high activity in the sympathetic neurohumoral system, no significant increase in the release of FFA from adipose tissue or elevation of the arterial concentration of FFA could be observed (KOVÁCH et al. 1970). On the other hand, the present results have shown that the concentration of glycerol, another product of lipolysis, increased significantly during the later part of the bleeding period. Concomitantly with this rise, glycerol release from the mesenteric adipose tissue increased.

Usually, arterial blood concentrations of glycerol and FFA run in parallel (CARLSON and ORO 1963; FREDHOLM and ROSELL 1968). However, this was not the case in the present experiments. The rise in arterial glycerol concentration may indicate an augmented lipolysis, at least in some parts of the adipose tissue during and after bleeding, e.g. in the mesentery, whereas the divergence of arterial concentrations of FFA and glycerol with time may be due to an increased reesterification of FFA (STEINBERG and VAUGHAN 1965).

It has been shown that a low pH and high lactate concentrations inhibit the release of FFA from adipose tissue in vitro and diminish their arterial blood concentration in vivo (ISSEKUTZ and MILLER 1962; MILLER et al. 1964; TRINER and NAHAS 1965). Thus, intra-arterial infusions of Na-L(+)-lactate resulting in blood concentrations above 5 mM counteract the release of FFA caused by sympathetic nerve stimulation without affecting glycerol release. Arterial lactate above 10 mM induces a 70% blockade of FFA release without significantly changing glycerol release (FREDHOLM 1970, 1971). Therefore, the inhibition of FFA release under these conditions seems to be due to an increase in the reesterification of FFA rather than to a depression of lipolysis. These experiments were performed on the same type of canine subcutaneous adipose tissue as that used in the present experiments. It was therefore interesting to note that in the present experiments the inhibition of FFA release occurred at arterial lactate concentrations attained during the bleeding and reinfusion periods. It is thus conceivable that the absence of any rise in FFA outflow to a large extent was mainly due to an increased reesterification rate.

Table IV

Blood flow (ml/min/100 g) in subcutaneous, omental and mesenteric adipose tissues
(Mean ± S.E.)

	Control	Bleeding I		Bleeding II		Reinfusion 30′
		60′	90′	60′	90′	
Subcutaneous	#1 n = 20 6.3±1.37	#2 n = 19 1.7±0.4	#3 n = 16 1.3±0.22	#4 n = 16 0.86±0.22	#5 n = 13 0.88±0.20	#6 n = 11 4.0±1.05
Mesenteric	#7 n = 12 14.8±3.28	#8 n = 12 3.3±0.52	#9 n = 12 3.2±0.52	#10 n = 10 2.7±0.49	#11 n = 9 2.6±0.51	#12 n = 9 6.7±1.73
Omental	#13 n = 10 5.3±1.19	#14 n = 9 1.9±0.64	#15 n = 6 2.0±0.41	#16 n = 7 1.6±0.28	#17 n = 4 1.3±0.44	#18 n = 6 3.4±0.75
Significance test*	1— 7 $p < 0.05$ 1—13 $p > 0.05$ 7—13 $p < 0.05$	2— 8 $p > 0.05$ 2—14 $p > 0.05$ 8—14 $p > 0.05$	3— 9 $p < 0.05$ 3—15 $p > 0.05$ 9—15 $p > 0.05$	4—10 $p < 0.05$ 4—16 $p > 0.05$ 10—16 $p > 0.05$	5—11 $p < 0.05$ 5—17 $p > 0.05$ 11—17 $p > 0.05$	6—12 $p > 0.05$ 6—18 $p > 0.05$ 12—18 $p > 0.05$

* Values for mean subcutaneous flow were compared to those for omental and mesenteric flow using Duncan's *t*-test to detect significant changes at various intervals in the procedure. Such comparisons were also made between omental and mesenteric flow and changes at the 5% confidence level are shown

The marked diminution of blood flow may be another reason for the absence of an augmented FFA release in every adipose tissue preparation. The numerical values of peripheral resistance seem to indicate that the decrease in blood flow in the mesentery and omentum was due to a diminished blood pressure rather than to an elevated vasoconstrictor tone. In the subcutaneous adipose tissue, however, there was a rise in the calculated peripheral resistance. This may be a sign of regional differences in the vascular reactions of adipose tissue to standardized haemorrhagic shock, presumably as a consequence of regional differences in the sympathetic neurohumoral control of the circulation in the adipose tissue (BALLARD and ROSELL 1969, 1971; ROSELL 1966).

The primary effect of the severe blood flow restriction may be tissue hypoxia which, in turn, leads to anaerobic glycogenolysis, lactate accumulation and tissue acidification (FREDHOLM and KARLSSON 1970). As a result, the rate of lipolysis may diminish and the rate of FFA reesterification rise. Such a development of metabolic events in adipose tissue may be the reason why we did not find FFA accumulation in adipose tissue during haemorrhagic shock. Thus, in the subcutaneous adipose tissue the FFA concentration was 3.5 ± 0.74 ($n = 13$) in the control period and 2.7 ± 0.76 ($n = 6$) at 60 min during the second bleeding period (unpublished observations). There is ample experimental evidence of a strong activation of the sympathetic neurohumoral system during haemorrhage and one would therefore a priori expect a trapping of FFA in adipose tissue due to an increased lipolysis combined with a low blood flow (KOVÁCH et al. 1970). The present results, however, indicate that factors like lowered pH and lactate accumulation may counteract the trapping of FFA.

In traumatic shock due to hind-limb ischaemia, there is an elevated concentration of plasma FFA interpreted as an augmented lipid outflow from adipose tissue (WADSTROM 1959; STONER 1962). The discrepancy between those findings and the results obtained in the present study may merely indicate that changes in lipid metabolism are much dependent upon the type and degree of the trauma.

In view of the fact that the adipose tissue is the largest energy source of the organism, the difficulty to mobilize FFA may have adverse effects on the energy supply of the organism during haemorrhagic shock. Under such conditions it may be of importance to keep the delivery of FFA at a normal or increased rate. One way of doing so is to administer alpha-receptor blocking agents (KOVÁCH et al. 1970) or betamimetic drugs (STONER 1962; KOVÁCH et al. 1971). The therapeutic value of such a treatment deserves further study.

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EFFECT OF HYPERCALCAEMIA ON RENAL FUNCTION

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Acute hypercalcaemia was induced in anaesthetized dogs by intravenous calcium chloride administration and the effect on renal function has been observed.

1. When plasma calcium level was raised by almost 100%, arterial tension was unaffected but urine excretion C_{PAH}, C_{inulin}, sodium and potassium excretion definitely decreased. There was no change in urinary calcium excretion.

2. The reduction of renal function due to hypercalcaemia is attributed to an intensive constriction of the renal vessels, presumably of the afferent arterioles.

Chronic hypercalcaemia is known to affect the renal parenchyma and thus urine excretion. EPSTEIN et al. (1958), GILL and BARTTER (1961), BANK and AYNEDJIAN (1965) have demonstrated in several species that in chronic hypercalcaemia concentrating ability of the kidney was deteriorated. They attributed this to the lack of the hyperosmotic concentration gradient in the medulla or the effect of calcium ions on the permeability to water of the distal and collecting tubules.

SUKI et al. (1969) observed in acute hypercalcaemia a decrease of the free water clearance which they ascribed to blocked sodium reabsorption in the ascending limb of Henle's loop due to the presence of excess calcium ions.

However, it is not sufficiently known whether in hypercalcaemia the calcium ion excess has a functional influence on renal activity, or modifies it only as a result of morphological changes.

In order to clarify this question, renal function was investigated under conditions of acute hypercalcaemia when morphological changes could not be yet responsible for the occurring changes.

Methods

The experiments were carried out on mongrel dogs of both sexes weighing 9 to 11 kg anaesthetized with 25 mg/kg pentobarbital intravenously.

The ureters were exposed from median laparotomy and catheretized supravesically with polyethylene tubings.

Arterial blood pressure was measured in the femoral artery by a mercury manometer and recorded on sooted paper.

To prevent clotting 0.10 ml/kg of heparin was given intravenously.

After the operation PAH and inulin dissolved in 20 ml of physiological saline in quantities to raise plasma concentration to 2 mg and 20 mg per 100 ml, respectively, were injected through a cannula tied into a femoral vein. Maintenance doses were given dissolved in physiological saline in constant infusion at a rate of 1 ml per minute.

After starting the infusion, 60 minutes were allowed for equilibration, then urine was collected in three 10 minute periods. At the middle of each period an arterial blood sample was taken for plasma PAH and inulin determination.

The three control periods were followed by the intravenous infusion of calcium chloride dissolved in 20 ml of physiological saline. The amount of $CaCl_2$ infused in about five minutes corresponded roughly to the amount of calcium in the extracellular space.

Next, the maintenance infusion of physiological saline was changed for one containing in addition to PAH and inulin, 3 mg/ml of anhydrous $CaCl_2$.

After starting the infusion of $CaCl_2$, 30 minutes were allowed for equilibration and urine was again collected in three ten minute periods. At the middle of each period, arterial blood samples were taken.

After the third period, the animals were killed, their kidney were removed and weighed. On gross inspection they displayed no morphological changes whatsoever.

Another group of animals served as control to establish the responsibility of the experimental method for the changes in renal function.

These animals were given 20 ml of physiological saline free of calcium intravenously after the three urine collection control periods.

The maintenance infusion of physiological saline given to the control group contained no $CaCl_2$ either, while in every other respect the procedure was the same as in the previous series of experiments.

Plasma and urinary PAH concentration was determined by the method of SMITH et al. (1945), the inulin concentration according to LITTLE (1949).

Plasma and urinary sodium, potassium and calcium content was determined by flame photometry.

Clearances were determined as usual and the results calculated for 100 g renal tissue.

Results

In Table I the results obtained in 10 dogs are summarized. Columns 1, 2 and 3 show the average values for the urine collection periods. Since the difference between the various periods was not significant their averages and standard deviations were calculated together (columns 1 to 3).

The hypercalcaemic values are given for each period in columns 4 to 6; these show the averages calculated in the three periods, and their averaged results as well as the standard deviations.

The average changes from the control values are given in percentage. The next column shows them and their standard deviations and the last column their significance.

It appears from Table I that when the plasma total calcium concentration was raised from 6.2 ± 1.9 mEq/l to 9.9 ± 2.9 mEq/l, there was no change in arterial blood pressure (123 ± 13 mm Hg and 122 ± 17 mm Hg, respectively).

Hypercalcaemia caused a drop of PAH clearance (C_{PAH}) from 202 ± 112 ml/min to 104 ± 81 ml/min ($p < 0.001$), while inulin clearance (C_{inulin}) decreased from 76 ± 29 ml/min to 46 ± 28 ml/min. The reduction of glomerular filtration was accompanied by a reduction of urinary output which was 1.20 ± 0.83 ml/min in the control periods and 0.47 ± 0.36 ml/min during hypercalcaemia ($p < 0.001$).

During calcium chloride infusion the excretion of sodium ($U_{Na} \cdot V$) decreased from 168 ± 191 μEq/min prior to infusion to 23 ± 26 μEq/min ($p <$

Table I

Effect of hypercalcaemia on renal function

	1	2	3	$\bar{x}^{1-3} \pm$ S.D.	4	5	6	$\bar{x}^{4-6} \pm$ S.D.	per cent ± S.D.	p
Blood pressure, mm Hg	123	123	123	123±13	123	122	121	122±17	−1±11	>0.70
Plasma total Ca level, mEq/l	6.5	6.1	6.0	6.2±1.9	9.6	10.4	10.1	9.9±2.9	+73±43	<.0001
C_{PAH} ml/min	216	209	182	202±112	100	102	106	104±81	−48±30	< 0.001
C_{inulin}, ml/min	83	78	68	76±29	50	48	40	46±28	−39±28	< 0.01
Urine, excretion, ml/min	1.29	1.18	1.11	1.20±0.83	0.59	0.45	0.36	0.47±0.36	−58±54	< 0.01
$U_{Na} \cdot V$, μEq/min	193	166	149	168±191	43	28	17	23±26	−59±54	< 0.01
$U_K \cdot V$, μEq/min	63	62	63	63±29	35	25	20	27±14	−54±24	< 0.001
$U_{Ca} \cdot V$, μEq/min	5.1	4.0	3.9	4.3±3.2	3.4	2.5	2.1	2.7±2.0	−19±68	>0.40

Table II

Control experiments
(n = 6)

	1	2	3	$\bar{x}^{1-3} \pm$ S.D.	4	5	6	$\bar{x}^{4-6} \pm$ S.D.	per cent ± S.D.	p
Blood pressure, mm Hg	140	139	134	137±9	128	125	125	126±16	−8± 9	>0.05
Plasma total Ca level, mEq/l	6.2	5.9	5.6	5.9±1.5	5.6	6.3	5.6	5.9±1.2	0± 7	> 0.80
C_{PAH}, ml/min	208	208	208	208±74	272	217	209	232±103	+8±15	>0.20
C_{inulin}, ml/min	79	89	85	84±36	93	94	82	89±39	+10±14	>0.10
Urine excretion, ml/min	0.93	1.09	1.01	1.01±0.98	1.43	1.20	0.97	1.20±1.35	+9±19	>0.30
$U_{Na} \cdot V$, μEq/min	157	179	160	165±174	181	194	132	169±187	−11±21	>0 30
$U_K \cdot V$, μEq/min	58	62	62	61±27	75	68	50	68±44	+12±42	>0.50
$U_{Ca} \cdot V$, μEq/min	3.2	4.3	3.9	3.9±2.3	4.4	3.8	3.0	3.7±3.8	−9±22	>0.30

< 0.01). Potassium excretion ($U_K \cdot V$) decreased from 63 ± 29 μEq/min to 27 ± 14 μEq/min, while calcium excretion was unaltered, being 4.3 ± 3.2 μEq/min in the control periods and 2.7 ± 2.0 μEq/min in hypercalcaemia ($p < 0.40$).

In the Table II the results obtained in six dogs are summarized. These experiments were destined to decide the responsibility of the operation technique for the changes observed in the first series of experiments.

Table II which was compiled in the same way as Table I, shows clearly, that no significant change took place in any of the investigated parameters during the experiment.

Comparison of the two tables shows a good agreement between the values of the control periods of the two series of experiments.

The data in Table II support the assumption that alterations observed were due to the hypercalcaemia.

Discussion

The results have confirmed that the urine excreting ability of the kidney is lower in acute hypercalcaemia.

Since no morphological changes could develop in these acute experiments, the results seem to point to the direct effect of the plasma calcium level on renal function.

From the reduced PAH clearance and glomerular filtration in hypercalcaemia it seems evident that high plasma calcium ion levels cause constriction of the renal vessels. This would give a satisfactory explanation of all the observed changes.

In these experiments the total plasma calcium concentration and not the amount of ionized calcium was determined. It might, however, be assumed that a rise in plasma total calcium involves a rise in the level of ionized calcium.

ZSOTÉR and SZABÓ (1958) found an enhanced catecholamine sensitivity of the smooth muscle elements in chronic hypercalcaemia.

HURWITZ et al. (1960) were able to influence the contractility of smooth muscles by altering the calcium level. HADDY (1960) and OVERBECK and HADDY (1960) induced vasoconstriction in the forelimbs of dogs by means of concentrated calcium solutions. KOSCHE et al. (1972) blocked the effect of coronary vasodilators by raising the calcium level in the perfused blood. FROHLICH et al. (1962) caused a rise in renal vascular resistance with hyperosmotic solutions of high calcium content.

These data prove that a higher than normal plasma calcium level causes a constriction of the arterioles of various organs.

In acute hypercalcaemia, no reduction of glomerular filtration was observed by SUKI et al. (1969). Though in their experiments the plasma cal-

cium level was almost the same as in our experiments, they failed to register the vasoconstricting effect of hypercalcaemia. The difference between the two series of experiments might perhaps be ascribed to a difference in the administered substances, since we induced hypercalcaemia with calcium chloride, while SUKI et al. (1969) used calcium lactate.

On the basis of earlier experiments, a systemic vasoconstriction and consequenctly a higher arterial blood pressure ought to have occurred in hypercalcaemia, provided that cardiac output did not decrease owing to the positive inotropic effect of calcium ions on the isolated heart, in hypercalcaemia a higher cardiac output should have rather been found.

It is seen from Table I that in our experiments arterial blood pressure did not rise, perhaps because the various vascular sections in the organism are not equally sensitive to the high plasma calcium level. Our experiments suggest a vasoconstriction in the kidney, when there is no constriction of the other vessels, moreover a vasodilatation might occur in some section of the vascular system. This is the only explanation for the finding that the increased vascular resistance in the kidney was not accompanied by a rise in arterial tension.

The experiments failed to find an answer to the question whether vasoconstriction in the kidney is caused directly or indirectly by the higher level of ionized calcium. By indirect cause we mean that vasoconstriction is brought about not directly by the calcium ions, but by some physiologically occurring vasoconstrictor substance and that the calcium ions enhance the sensitivity of the smooth muscle elements in the vascular wall to that particular vasoconstrictive agent.

It might be possible that somewhere in the organism hypercalcaemia enhances the production of some vasoconstrictive substance or substances, causing thereby a secondary constriction of the renal vessels.

In our experiments we have almost doubled the plasma calcium level and this calcium concentration will already functionally reduce the urine secreting ability of the kidney. Our results do not permit the assumption of a close correlation between plasma calcium concentration and renal blood flow, since this would require the study of the renal function at several plasma calcium concentrations.

Our observation of a decreased urinary sodium excretion in hypercalcaemia suggests an essentially unaltered function of the tubular cells.

We believe that in hypercalcaemia it is the change in renal haemodynamics that induces the observed alterations. Reduced glomerular filtration due to the constriction of the afferent arterioles in itself might result in a deterioration of the concentrating function of the kidneys.

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EFFECT OF HYPERCALCAEMIA ON TUBULAR CALCIUM AND PHOSPHATE TRANSPORT

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In anaesthetized rats, known quantities of $^{45}Ca^{2+}$, $^{32}PO^{3-}$ and ^{3}H-methoxy-inulin were injected by a micropipette into various segments of the tubule (early, mid- and terminal proximal, and distal tubule) during mannitol diuresis and sustained hypercalcaemia. From the amounts excreted in the urine conclusions could be drawn concerning the handling of the injected isotopes in various segments of the tubule. It has been shown that

(1) Ca^{2+} ions were reabsorbed along the entire nephron;

(2) PO^{3-} ions were reabsorbed only in the distal tubule but not in the accessible portion of the proximal tubule;

(3) Hypercalcaemia induced by the infusion of $CaCl_2$ resulted in a diminished reabsorption of calcium along the entire nephron;

(4) Hypercalcaemia resulted in a vigorous reabsorption of phosphate in the proximal tubule and the loop of Henle;

(5) The elevated serum calcium level failed to affect distal tubular phosphate reabsorption.

It has been suggested that the same mechanism is responsible for the regulation of calcium reabsorption along the entire nephron. The obtained results allow the assumption that reabsorption of phosphate in the proximal tubule and loop of Henle is a function of intracellular calcium concentration that changes in parallel with the serum calcium level.

Calcium and phosphate exretion by the mammalian kidney has extensively been studied by clearance techniques, and it has been established that a significant fraction of the filtered calcium and phosphate is actively reabsorbed in the tubules. The permeability characteristics for bivalent cations of the mammalian tubular epithelium have been investigated by CHINARD and ENNS (1955) by labelled substances injected into the renal artery. BRONNER and THOMPSON (1961), SAMIY et al. (1960), as well as SHOUTENS and RAYNAUD (1968) employed the stop-flow technique for the analysis of tubular calcium reabsorption. However, neither of these methods has provided sufficient information concerning the localization and quantitative aspects of tubular transport processes.

LASSITER et al. (1963) and DUARTE and WATSON (1967) studied calcium transport in the mammalian kidney by the micropuncture technique, while STRICKLER et al. (1964), CARNE (1964) and AMIEL et al. (1970) employed the same method in studying tubular phosphate handling, determining thus the site of reabsorption within the nephron.

Selective microperfusion of single nephrons was used by FRICK et al. (1965) and BAUMANN et al. (1966) to investigate the dynamics of tubular calcium transport.

In microperfusion experiments, MURAYAMA et al. (1972) performed a detailed analysis of tubular phosphate, calcium and magnesium transport, using the electron probe analysis developed by MOREL and ROINEL (1969a).

In the present experiments, quantitative aspects of tubular calcium and phosphate reabsorption, and the dynamics of ionic transport have been studied by the intratubular microinjection method described by MOREL et al. (1965) and GOTTSCHALK et al. (1965). Hypercalcaemia was induced acutely and its effect has been investigated on tubular calcium and phosphate reabsorption.

Methods

The experiments were done in female rats weighing 200 g on the average and anaesthetized with sodium pentobarbital (5.0 mg per 100 g body weight, intraperitoneally). Food, but not water, was restricted 24 hours prior to the experiment. Following anaesthesia, the bladder was exposed through a lower midline incision, and both ureters were cannulated supravesically by indwelling thin polyethylene catheters.

The abdominal incision was extended upwards, and the left kidney was exposed, then fixed without decapsulation, by the technique of GOTTSCHALK and MYLLE (1956). The anterior surface of the kidney was protected from drying by a thin layer of oil previously heated to 37° C. The surface of the kidney was illuminated by a strong lamp, the light of which was directed to the desired place by fiber optics. The microinjections were done under a stereomicroscope, by a Fonbrune micromanipulator. The micropipettes used for the injections were made by a Fonbrune device. The external diameter of the micropipettes was 5 microns, and their tips were ground on Arkansas stone. The pipettes were filled with 2 nl of perfusion fluid prior to use.

The animals were tracheotomized, and a thin polyethylene catheter was introduced into the right external jugular vein for infusions, while the cannulated right femoral artery served for the withdrawal of arterial blood samples and the recording of blood pressure by a TELCO electric manometer.

The animals were divided in two groups, the first one serving as control, while in the other hypercalcaemia was induced by constant intravenous infusion.

The control rats after surgery were given 2.0 ml of a 12% mannitol solution in 100 mM/l NaCl intravenously, which was followed by a constant infusion of a 6% mannitol solution in 100 mM/l of NaCl at a rate of 100 μl/min. Sixty min were allowed to elapse for equilibration, then the microinjections were started.

Prior to microinjection, the urine excreted by the left kidney was collected for twice 1-min periods on Whatman GF/C glass fibre paper cut into approximately 2-cm disks. Then the pipettes were filled with a solution containing 145 mM/l NaCl, 2 mM/l $^{45}CaCl_2$ (16 mCi/mg), 1 mM/l $Na_3{}^{32}PO_4$ (20 mCi/mg), ^{3}H-methoxy inulin (New England Nuclear Corp.) and lissamine green.

The tip of the pipette was introduced into the lumen of the tubule, and 2 nl of the solution was slowly injected in 15 sec. Subsequently, the urine excreted by the left kidney was again collected in 30-sec periods for 3 min, then for two 1-min periods.

The site of injection was established by means of the green dye. If the solution appeared in many convolutions before arriving at the loop of Henle, the site of injection was considered to be in the early proximal tubule, while if it appeared in a small number of convolutions, the midportion of the proximal tubule was considered to be the site of puncture. Identification of these tubular segments was facilitated by the recognition of the terminal segment of the proximal tubule and the distal tubule.

After completion of urine collection, the microinjection was repeated in another nephron. In each animal, 6 to 8 nephrons were subjected to microinjection.

In the second group of animals, hypercalcaemia was induced by administering intravenously 2.0 ml of a 12% mannitol solution in 100 mM/l of NaCl immediately after surgery, then giving a constant rate infusion (100 μl/min) of 100 mM/l NaCl containing 6% mannitol

and 30 mM/l of $CaCl_2$. Equilibration was allowed for 90 min after starting the infusion, during which period the plasma calcium level increased nearly twofold and changed only slightly in the remaining part of the experiment. Microinjections were done in the same manner as in the control group.

The fibre paper disks used for urine collection were dried after the experiment, then placed into vials containing 10 ml of modified Bray's solution (BRAY 1960). Radioactivity in the vials was determined by a TRICARB (Packard) 3-channel liquid scintillation counter.

Isotope content of the administered solution was also established by placing 2 nl on a fibre paper disk and spreading it with 1 drop of physiological saline. After drying, counting was performed in the same way as described for the urine samples.

In both groups of animals the changes in renal function were checked by collecting urine separately in 10-min periods, twice during each experiment: between the 60th and 90th min, and between the 90th and 120th min in the control group. Arterial blood samples were withdrawn at the beginning of the experiment and in the 60th and 90th min. In the hypercalcaemic group, urine collections were done between the 60th and 90th, and the 160th and 190th min, and arterial blood samples were withdrawn at the beginning and in the 60th, 120th, 180th and 240th min of the experiment.

Sodium, potassium and calcium in plasma and urine were determined by flame photometry.

Tubular calcium and phosphate reabsorption was estimated by calculating the amount of excreted isotope in per cent of the activity administered.

The ^{3}H-methoxy inulin injected into the tubules could be recovered practically quantitatively in the urine. Whenever there was a difference between the amount of inulin administered and regained, this was considered in the calculations in that particular experiment, using a correction factor for the compution of the amounts of administered $^{45}Ca^{2+}$ and $^{32}PO_4^{3-}$. The amounts of isotope obtained in the different urine fractions were pooled and expressed in per cent of the activity administered. Furthermore, activity for each isotope was determined separately in each fraction as well, and fractional excretion was computed in per cent of the total amount of isotope excreted in the urine.

Since the three labelled compounds were excreted in the urine in different percentile amounts, the dynamics of reabsorption could be established. The excretion rate of inulin was used as reference to which the excretion of $^{45}Ca^{2+}$ and $^{32}PO_4^{3-}$ were compared in each fraction. The differences obtained in the individual fractions were used for statistical calculations, using Student's *t*-test.

Results

Tubular calcium and phosphate reabsorption were studied in 6 rats in mannitol diuresis; the obtained data served as controls. Table I summarizes the results of this series, with the means and their standard deviation (S.D.).

In Table II, the values obtained in the 5 experimental animals during hypercalcaemia have been summarized. It is clear from these data that the infusion of $CaCl_2$ resulted in a marked elevation of the serum calcium concentration to almost twice the initial value, then a further much slower rise could be observed till the end of the experiment.

Comparing the data obtained in these two groups it is evident that although the rate of urine flow was the same in the two groups, there were substantial differences in the urinary sodium, potassium and calcium concentrations, all being higher in the hypercalcaemic group. This holds particularly for the urinary calcium level.

Table III shows the data referring to the tubular reabsorption of $^{45}Ca^{2+}$ and $^{32}PO_4^{3-}$ in the control animals. The amount of isotope excreted in the urine is the fraction which has not been reabsorbed in the tubule during

Table I

Plasma and urinary excretion patterns in control experiments (mannitol diuresis)

(n = 6; mean ± S.D.)

Minutes after onset of mannitol infusion	0	60	120	
Plasma Na, mEq/l	135±7	135±8	132±11	
Plasma K, mEq/l	4.09±0.40	4.43±0.14	4.66±0.22	
Plasma Ca, mEq/l	4.46±0.46	4.49±0.40	4.41±0.37	
Minutes after onset of mannitol infusion	60–90		90–120	
Kidney	left	right	left	right
Urine flow, µl/min	45.09±18.52	41.24±15.96	47.09±23.00	41.12±15.20
Urinary Na, mEq/l	7.72± 5.20	4.74± 1.29	9.75± 3.63	8.40± 2.60
Urinary K, mEq/l	15.32± 5.74	10.48± 6.59	20.50± 7.39	17.04± 6.80
Urinary Ca, mEq/l	0.13± 0.08	0.10± 0.04	0.14± 0.05	0.12± 0.03

n: number of experiments

Table II

Plasma and urinary excretion pattern in hypercalcaemia (mannitol diuresis)

(n = 5; mean ± S.D.)

Minutes after onset of mannitol infusion	0	60	120	180	240
Plasma Na, mEq/l	134±2	132±5	133±3	134±3	134±3
Plasma K, mEq/l	3.10±0.31	3.63±0.07	3.91±0.15	3.81±0.18	3.73±0.30
Plasma Ca, mEq1/	4.87±1.12	7.84±1.66	8.62±2.15	9.11±2.48	10.04±2.97
Minutes after onset of mannitol infusion	60–90		160–190		
Kidney	left	right	left	right	
Urine flow, µl/min	43.25± 7.76	40.40± 8.69	46.40± 5.10	44.50± 9.18	
Urinary Na, mEq/l	51.97±12.48	48.60±18.20	67.97±15.32	58.80±27.65	
Urinary K, mEq/l	26.04±10.68	22.88±11.42	36.00± 2.24	33.00± 5.16	
Urinary Ca, mEq/l	9.19± 2.90	7.71± 3.63	16.35± 3.56	14.25± 7.26	

n: number of experiments

the passage of fluid from the site of microinjection to the end of the nephron. Table III contains the means and standard deviations of the total amount of $^{45}Ca^{2+}$ and $^{32}PO_4^{3-}$ excreted in urine during the whole period following the microinjection, per cents of the amount injected.

Table III

Microinjections in control experiments

Percentage excretion of various substances injected directly into proximal and distal convoluted tubules

(mean ± S.D.)

	^{3}H-inulin	^{45}Ca	$^{32}PO_4$
Early proximal tubule, n = 6	100	0	74.37±18.85
Middle proximal tubule, n = 8	100	0	64.79±15.00
Terminal proximal tubule, n = 7	100	5.48±11.10	69.29± 6.48
Distal tubule, n = 10	100	59.81±13.20	78.39±19.00

n: number of microinjections

These data clearly indicate that calcium is intensely reabsorbed along the entire nephron. It is also evident that in this experimental series phosphate reabsorption occurred only in the distal tubule or the collecting duct. On injecting $^{32}PO_4^{3-}$ into 4 different segments of the proximal tubule, the amount excreted in the urine was practically identical with the amount injected.

Table IV shows the corresponding values obtained in the hypercalcaemic rats; the data are expressed in per cent of the amount injected into the tubule.

Excretion of $^{45}Ca^{2+}$ was substantially higher in the hypercalcaemic than in the control rats. Also, the rate of $^{32}PO_4^{3-}$ excretion was considerably affected by hypercalcaemia. While in the control animals no phosphate reabsorption could be detected in the proximal tubule and the loop of Henle, in the hypercalcaemic rats significant reabsorption of phosphate occurred in both the proximal tubule and the loop of Henle.

Table IV

Microinjections in hypercalcaemia.

Percentage excretion of various substances injected directly into proximal and distal convoluted tubules

(mean ± S.D.)

	^{3}-H-inulin	^{45}Ca	$^{32}PO_4$
Early proximal tubule, n = 13	100	9.65± 5.55	23.19±12.60
Middle proximal tubule, n = 11	100	14.73± 8.36	35.19±26.60
Terminal proximal tubule, n = 13	100	16.09± 4.70	55.19±17.85
Distal tubule, n = 10	100	85.00±13.40	81.87± 7.45

n: number of microijections

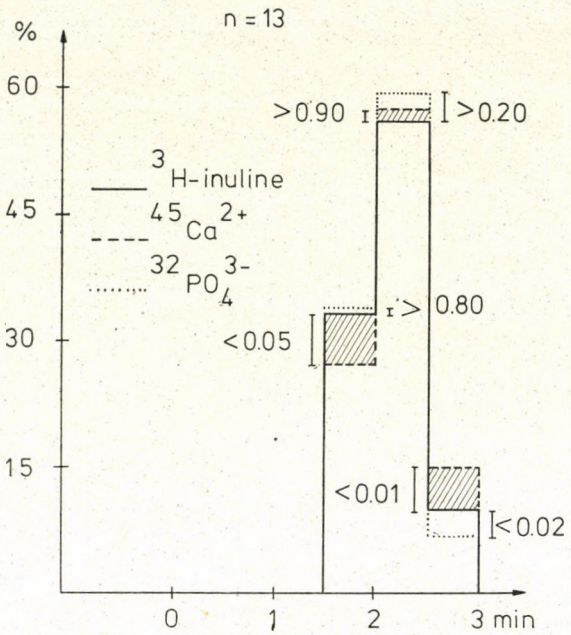

Fig. 1. ^{3}H-Inulin (solid line), $^{45}Ca^{2+}$ (dashed line), $^{32}PO_4^{3-}$ (dropped line) excretion patterns following early proximal microinjection. The data are presented as fractional recovery rates. Microinjection occurred at zero time

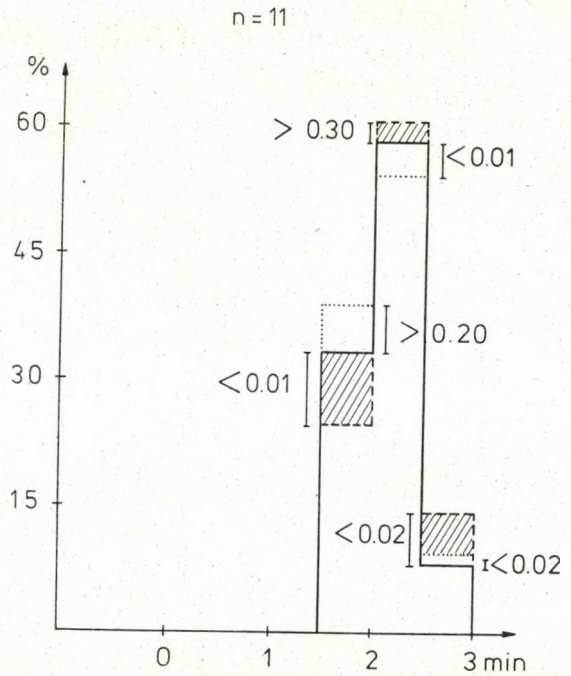

Fig. 2. Excretion patterns following middle proximal microinjection. Symbols, as in Fig. 1

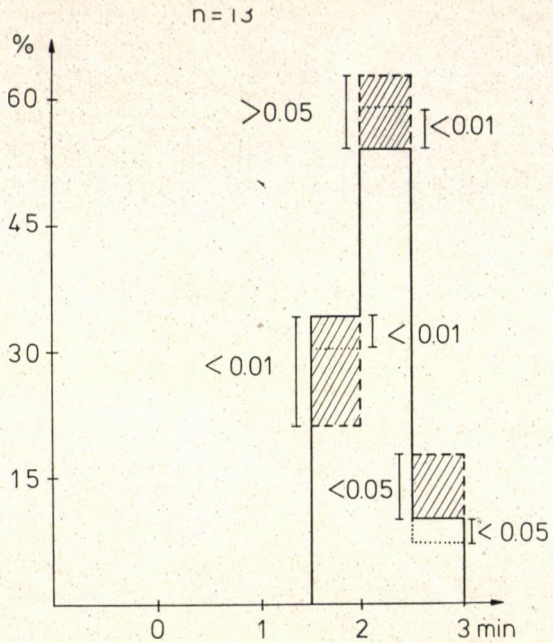

Fig. 3. Excretion patterns following terminal proximal microinjection. Symbols, as in Fig. 1

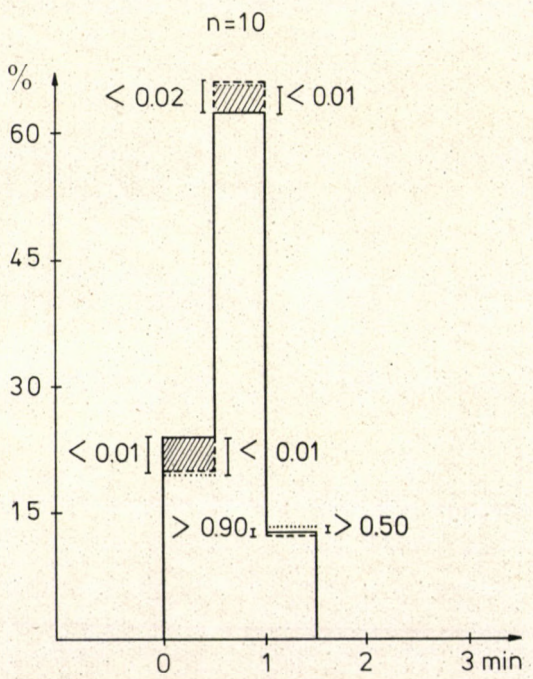

Fig. 4. Excretion patterns following distal microinjection. Symbols, as in Fig. 1

Remarkably, there was no change in the rate of phosphate reabsorption in the distal tubular segments, as in the control rats 78.39 $\pm$ 19.00% of the injected $^{32}PO_4^{3-}$ was excreted in the urine, while in the hypercalcaemic animals this figure attained 81.87 $\pm$ 7.45% of the amount injected into the distal tubule.

There were differences in the amounts of isotope excreted in the various fractions of urine. In the hypercalcaemic series, the fractional excretion of isotopes is expressed in per cents of the total amount excreted in Figs 1, 2, 3 and 4, when injection was done into the beginning of the proximal tubule, mid-portion of the proximal tubule, terminal proximal tubule, and distal tubule, respectively.

All four diagrams indicate unequivocally that the rate of $^{45}Ca^{2+}$ excretion in the first period was somewhat less than that of inuline. The $^{45}Ca^{2+}$ not excreted in the first period could be recovered in the second and third periods. Thus, calcium ions appear in the urine with a delay as compared to inulin. The difference between calcium and inulin excretion is indicated by the hatched area.

The statistical significance of difference is indicated by the p values on the diagrams. The excretion of $^{32}PO_4^{3-}$ was not delayed in every segment of the tubule as compared to inulin: both in the early (Fig. 1) and mid-proximal tubules (Fig. 2) phosphate excretion parallelled that of inulin.

Since in the control animals $^{45}Ca^{2+}$ injected into the proximal tubule was not excreted in the urine, fractional excretion studies were not undertaken in this series.

Discussion

The results obtained in the control animals were in agreement with those obtained for the tubular handling of calcium by CHEN and NEUMAN (1955) and WALSER (1961), using clearance techniques, HOWARD et al. (1959) and GROLLMANN et al. (1963), working with the stop-flow method, and LASSITER et al. (1963) who employed data obtained by micropuncture. These observations unequivocally demonstrate that calcium ions are actively reabsorbed along the entire proximal tubule, in the loop of Henle, most probably its thick segment, and in the distal tubule.

The experiments of WALSER (1961) and WATSON (1966) indicate that the tubular reabsorption of sodium and calcium is interrelated in a way that any increase in sodium clearance involves a rise in calcium clearance. SUKI et al. (1969) demonstrated enhanced natriuresis in acute hypercalcaemia. Our observation that sodium concentration in the urine at identical urine flows was higher in the hypercalcaemic animals than in the controls, is consistent with these data.

In acutely induced hypercalcaemia, calcium is more vigorously excreted by the kidneys. This is partly due to the augmented filtration of calcium as a result of the higher serum-calcium level. In the present experiments, glomerular filtration rate and filterable calcium levels in the plasma have not been measured. LE GRIMELLEC (1971, personal communication) found that in acutely induced hypercalcaemia the plasma concentration of ionized Ca^{2+} failed to rise parallel with the plasma total calcium concentration. Since in the present experiments we measured the plasma total calcium level, it was not possible to establish to what extent the increased glomerular filtration had contributed to the observed enhancement of calcium excretion in the hypercalcaemic animals.

The present results indicate that tubular reabsorption of Ca^{2+} is reduced in hypercalcaemia. Reabsorption was diminished along the entire length of the nephron, proving that the factors affecting tubular calcium reabsorption exert their effect by the same, or almost the same, mechanism in the renal tubule.

The present experiments did not allow an accurate definition of the factor(s) responsible for altered calcium handling during hypercalcaemia.

Parathyroid hormone has been shown by WIDROW and LEVINSKY (1962) to interfere with the renal tubular reabsorption of calcium. It has been concluded from these observations that the hormone enhances the reabsorption of calcium primarily in the distal segment of the tubule. FRICK et al. (1965) failed to affect calcium reabsorption in the proximal tubule either by the administration of parathormone or parathyroidectomy. MASSRY et al. (1968) were unable to decrease the renal calcium clearance by parathormone in hypercalcaemic animals. In parathyroidectomized animals, SAMMON et al. (1970) found no difference in the rate of calcium reabsorption.

Although the observations of CARE et al. (1966) and SHERWOOD et al. (1966) have unequivocally proved that parathyroid hormone release was decreased in hypercalcaemia, it appears that other factors too are involved in the reduction of tubular reabsorption of calcium under hypercalcaemic conditions.

Since the observations of SANDERSON et al. (1960) and COPP et al. (1962) it has become generally accepted that calcitonin participates in the regulation of the serum calcium concentration. TENENHOUSE et al. (1965) found the polypeptide isolated from the parathyroid gland to interfere potently with calcium metabolism. ARNAUD et al. (1967) suggested that tubular reabsorption of calcium and phosphate was affected by calcitonin. More recent data of BELL and STERN (1970) have indicated that in hypercalcaemia the serum calcium concentration is depressed by the resultant release of thyrocalcitonin. It is therefore reasonable to assume that thyrocalcitonin interferes with the tubular reabsorption of calcium.

The localization of phosphate transport in the nephron has been studied by PITTS et al. (1958) and MALVIN et al. (1958) who established that phosphate is reabsorbed predominantly in the proximal tubule. In micropuncture experiments, STRICKLER et al. (1964) succeeded in demonstrating that the bulk of filtered phosphate is actively reabsorbed in the proximal tubule. Tubular secretion of phosphate could not be demonstrated even after considerable phosphate loads and phosphate reabsorption was characterized by a tubular maximum (Tm). On the other hand, AMIEL et al. (1970) have shown a vigorous reabsorption of phosphate in the distal tubule as well.

In the control rats we found a marked reabsorption of phosphate in the distal tubule, but were unable to demonstrate net reabsorption in the proximal segment of the tubule. This finding is in apparent contradiction with the above data, but it should be remembered that earlier investigations concluded to the kinetics of phosphate reabsorption from changes in the concentration of phosphate in the proximal tubular fluid. The present results may be brought into harmony with those of others only by assuming that under normal conditions phosphate is reabsorbed in the earliest convolutions of the proximal tubule inaccessible for micropuncture. If there is an intense reabsorption of phosphate in this segment, the concentration of phosphate in tubular fluid will decrease significantly, rising again in the more distal segments of the proximal tubule where water is reabsorbed in the absence of net phosphate reabsorption. Presumably, the final concentration of phosphate in the urine is determined by the rate of active phosphate transport in the distal, eventually also in the collecting, tubules.

As shown in Table IV, during hypercalcaemia a vigorous reabsorption of phosphate occurs along the entire proximal tubule. This finding indicates that the proximal tubular epithelium is capable of active phosphate transport at any part of the tubule, while only a fraction of this reabsorptive capacity is utilized under normal conditions.

Tubular phosphate reabsorption may be modified by several factors. The role of parathyroid hormone has been demonstrated by HIATT and THOMPSON (1957), showing that renal phosphate clearance is increased in response to parathormone administration. It is conceivable that in the present experiments the increased tubular reabsorption of phosphate was due to a suppression of parathormone release in response to hypercalcaemia.

RASMUSSEN et al. (1967) observed that renal calcium excretion decreased and phosphate excretion increased following the administration of thyrocalcitonin. However, they attributed these changes in the excretion rate of calcium and phosphate to the ensuing hypocalcaemia.

Thus, there is still much controversy about whether tubular phosphate reabsorption is governed by alterations of the blood parathormone level or by the serum calcium concentration.

NIZET (1972) observed in the isolated canine kidney that phosphate reabsorption was reduced when the perfusion fluid was diluted with a solution containing NaCl and KCl but no calcium. From this observation it may be concluded that alterations in the serum calcium level may modify tubular phosphate reabsorption in the absence of parathormone, presumably by evoking changes in the intracellular concentration of calcium in the tubular epithelium.

The present results obtained in hypercalcaemic rats may be explained by assuming that the elevation of calcium concentration in the tubular epithelial cells resulted in an augmented phosphate reabsorption rate. There is reason to believe that changes in intracellular calcium concentration may affect the activity of cellular alkaline phosphatase (SCHMIDT and DUBACH 1969), presumably resulting in an altered phosphate transport in the tubule.

It is remarkable that distal phosphate transport was not affected by hypercalcaemia. This finding seemed to point to the independence of tubular phosphate handling from the serum calcium concentration in this part of the renal tubule.

The obtained results allow certain conclusions concerning the mechanism of tubular ion transport. In the control series, where calcium was injected into the proximal tubule, it was reabsorbed in toto. This means that filtered calcium must be quantitatively reabsorbed when passing along the proximal tubule. Micropuncture experiments have shown that the ratio for calcium of tubular fluid to plasma concentration (TF/P) in the proximal tubule was about 1 (MOREL et al. 1969b). It has therefore to be assumed that in the present experiments the infused $^{45}Ca^{2+}$ reabsorbed from the proximal tubule was continuously replaced by non-labelled Ca^{2+} ions diffusing out of the epithelial cells. There is a continuous bidirectional flux of ions between the tubular fluid and epithelium as well as the peritubular fluid and tubular cells. The net reabsorption will be thus determined by two opposite fluxes

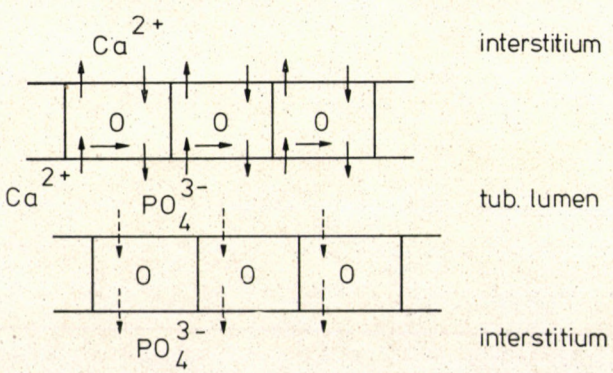

Fig. 5. Ca^{2+} and PO_4^{3-} transepithelial transport in the proximal convoluted tubule

of ions. This intricate mechanism seems to account for the apparent delay of $^{45}Ca^{2+}$ excretion as compared to the simultaneously injected inulin, as shown in Figs 1 through 4.

There being no unidirectional delay of the excretion of $^{32}PO_4^{3-}$ as compared to inulin, the assumption of a bidirectional phosphate flux does not seem to be warranted.

The processes involved in tubular calcium and phosphate transport are schematically summarized in Fig. 5.

Acknowledgement

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EFFECT OF ACIDOSIS AND NORADRENALINE INFUSION ON ^{14}C-NORADRENALINE UPTAKE BY THE RAT MYOCARDIUM

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Earlier studies revealed that the noradrenaline concentration of myocardial tissue is increased during acidosis. The present findings indicate that the uptake of labelled noradrenaline is less in acidosis. An increased concentration of noradrenaline, on the one hand, and a decrease of the uptake, on the other hand, led to assume that the hypernoradrenalinaemia occurring during acidosis is basically involved in the contradictory observations. It was found that the infusion of unlabelled noradrenaline (1.5 μg/min for 20 minutes) produced a significant suppression of ^{14}C-noradrenaline uptake by both ventricular and atrial tissue. These observations clearly suggested that the decreased noradrenaline uptake during acidosis may be attributed to the substitution of binding sites by endogeneous noradrenaline liberated from extracardial sources.

Recently we have studied the catecholamine content in myocardial tissue and the hypothalamus after hydrochloric acid infusion in rats. It was found that the lowering of blood pH is followed by a significant increase of the noradrenaline content in the myocardium (KÁRPÁTI et al. 1973). The present investigations were aimed at studying the mechanism of an increased accumulation of noradrenaline by the heart muscle during acidosis in the rat.

Methods

A total of 98 male albino rats of 160 to 200 g was used in the study. Blood pH determination was performed with a micro-Astrup apparatus (ASTRUP et al. 1960); the blood samples were taken from the tail vein. Acidosis was induced by the injection into the tail vein of 0.3 ml/100 g of 0.4 N HCl. The control animals were given equal volumes of physiological saline solution. The rats were killed 10, 30 and 60 minutes later. Fluorometric assay of myocardial noradrenaline was made according to UDENFRIED and ZALTZMAN-NIERENBERG (1963).

For studying the uptake of ^{14}C-noradrenaline bitartarate (56 mCi/mmol, Amersham, England), the rats were anaesthetized with pentobarbital and the ^{14}C-noradrenaline was infused through a polyvinyl cannula into the jugular vein at a rate of 50 μl/min for 10 minutes in a total dose of 1.8 μCi/100 g except for one experimental series where the total activity administered was 0.5 μCi/100 g. Following the infusion, the animal was killed by decapitation and the atrial and myocardial tissue was dissected and weighed on a torsion balance. The noradrenaline was extracted with 5% trichloroacetic acid; the homogenate was centrifuged and the neutralized supernatant was transferred into counting vials containing 2 ml absolute ethanol and 10 ml liquid scintillation fluid. Radioactivity was measured by a Packard liquid scintillation spectrometer (Model EX 314, 70% efficiency rate).

For studies in vitro, myocardial and atrial tissue slices were incubated in 2 ml Krebs-bicarbonate buffer at pH 7.4 and pH 7.1, respectively. Oxidation of noradrenaline was prevented by adding to the media 0.1% of ascorbic acid. ^{14}C-noradrenaline (0.1 μCi per 2 ml buffer and approximately 100 mg tissue) was added to the media immediately before incubation which

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was performed under oxygen atmosphere with constant shaking at 37°C. After 60 min incubation, the tissue slices were washed with excess buffer solution and then the activity was extracted and measured as described before.

An increase in blood noradrenaline concentration was induced by the infusion of 1.5 μg/min noradrenaline bitartarate for 20 minutes. The combined infusion of unlabelled and labelled noradrenaline was begun in the 10th minute of unlabelled catecholamine infusion.

For studying noradrenaline uptake during acidosis, labelled noradrenaline was infused 10 minutes after the injection of hydrochloric acid. In these experiments, the animals were killed in the 20th minute after hydrochloric acid administration. The control rats were treated with physiological saline and the infusion of labelled noradrenaline was made according to the schedule described above.

Statistical evaluation of the data was done by Student's *t*-test.

Results

A lowering of the blood pH as a result of hydrochloric acid administration produced a significant increase in myocardial noradrenaline content (Fig. 1). After an initial drop of the pH to 7.1, its normalization took approximately 60 minutes after the injection of hydrochloric acid.

Infusion of ^{14}C-noradrenaline during acidosis was followed by a lesser uptake by the myocardial and atrial tissue than in the control animals (see Fig. 2).

Infusion of both unlabelled and labelled noradrenaline resulted in a significant decrease of the uptake of labelled noradrenaline by both the atrial and the myocardial tissues (Fig. 3).

The increased accumulation of noradrenaline and the decrease of labelled noradrenaline uptake during acidosis suggested that the hypernoradrenalinaemia induced by the acidosis was responsible for the suppression of uptake. If it was due to a substitution of the binding sites by endogenous noradrenaline which seemed to be confirmed by double infusion studies, this would exclude the possibility of an increased noradrenaline synthesis in the heart as a result of the acidosis.

A lowering of the pH to 7.1 of the media in which 0.1 μCi noradrenaline was incubated with 80 to 100 mg myocardial and atrial tissue slices for 60 minutes (Krebs-bicarbonate, 37°C, under shaking in oxygen atmosphere) resulted in a significant rise of atrial uptake. Accumulation of radioactivity in the myocardial tissue was also more increased at pH 7.1 than at pH 7.4, although the difference was not significant statistically. The results clearly demonstrated the effect of acidosis on the uptake or noradrenaline. Since the incubation media contained 0.1% ascorbic acid to avoid spontaneous oxidation of the noradrenaline, the difference between the groups of pH 7.1 and pH 7.4 cannot be attributed to a greater degradation of noradrenaline at a higher pH level. By all means, the stronger association constant of the binding sites at the higher H concentration may be involved in the greater accumulation of noradrenaline, and this possibility merits some considerations (Fig. 4).

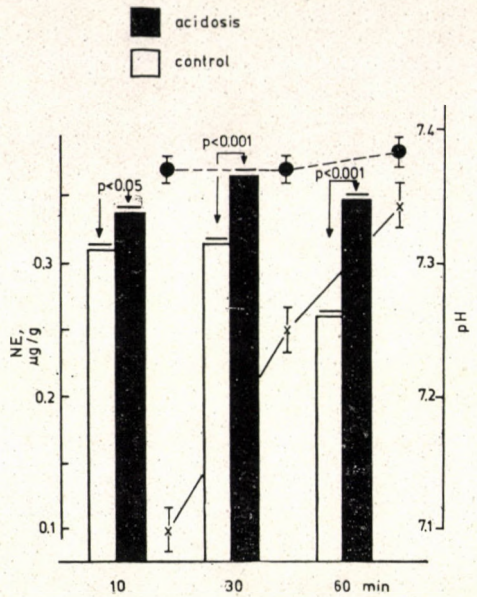

Fig. 1. Myocardial norepinephrine concentration in acidosis. Empty columns: control. Black columns: after hydrochloric acid injection. ×———×: pH in acidosis; ●———●: pH in control rats

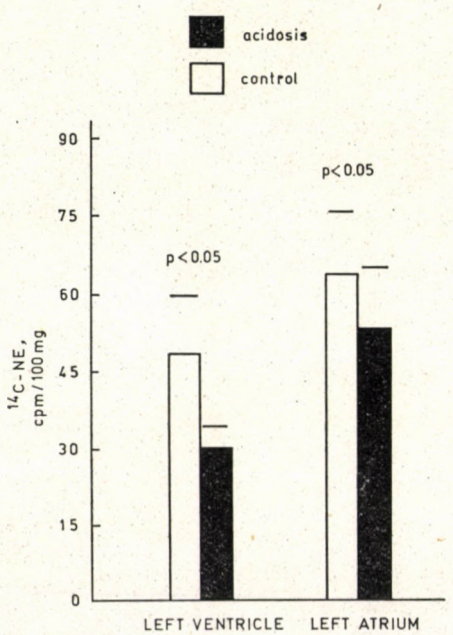

Fig. 2. ^{14}C-norepinephrine uptake in acidosis

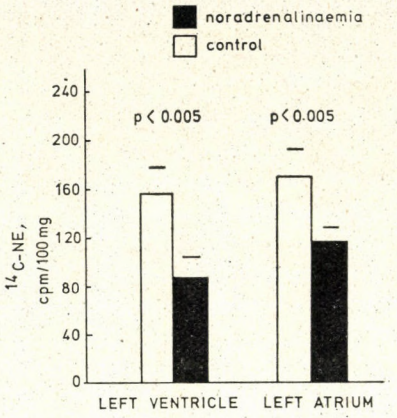

Fig. 3. Influence of noradrenalinaemia on the uptake of ^{14}C-norepinephrine

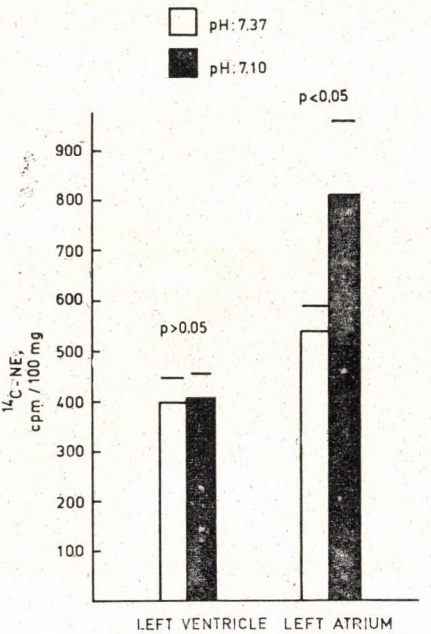

Fig. 4. Effect of pH ^{14}C-norepinephrine uptake by the ventricle and the atrium in vitro

Discussion

Numerous studies have indicated that the blood noradrenaline concentration is increased during acidosis (NAHAS et al. 1961; SMITH and CORBASCIO 1966; NAHAS and POYART 1967; etc.). These findings have been confirmed by us (KÁRPÁTI et al. 1973) and it was moreover found that the acidosis is accompanied by an increased noradrenaline accumulation in the myocardial tissue

(KÁRPÁTI et al. 1973). The mechanism of myocardial noradrenaline uptake is not completely understood. Thus, IVERSEN (1965) reported noradrenaline uptake by the non-terminal axons and cell bodies in the myocardium, and HAMBERGER (1967) showed that the majority of noradrenaline was localized extraneuronally in the muscle.

The seeming contradiction between the increased noradrenaline concentration and the decreased uptake during acidosis was relieved by the finding that hypernoradrenalinaemia which occurs during acidosis results in s competition for binding sites. As a result of the noradrenaline infusion, the uptake of labelled noradrenaline is decreased. Whether this competition is specific for noradrenaline or nonspecific at least for the different monoamines, is a subject for further investigations.

An increased uptake of noradrenaline by the heart tissue at a higher H^+ concentration under in vitro conditions is in accordance with the assumption that the elevated concentration of noradrenaline during acidosis is due to an increased accumulation rather than to an increased biosynthesis. The acidosis- induced catecholamine release from the adrenal medulla and catecholaminergic neurones seems to be responsible for the catecholaminaemia (NAHAS and POYART 1967) which is followed by a redistribution between noradrenaline-target tissues. In our earlier studies (KÁRPÁTI et al. 1973), reserpine pretreatment resulted in a marked catecholamine depletion and a lack of catecholamine accumulation during acidosis, which indicated an active process in the uptake during the course of catecholamine redistribution.

It is generally known that metabolic acidosis is a common complication of cardiac failure and that it is associated with an increased plasma catecholamine level (CEREMUZINSKI et al. 1969). The catecholaminaemia is considered a factor conditioning for arrhythmia (GAZES et al. 1959; STARCICH 1966; HARRIS et al. 1951; BARRERA et al. 1966, etc.). Moreover, it was found that the administration of catecholamine depletors or monoaminergic blocking agents prevented the development of arrhythmia (EBERT et al. 1970). In contrast to the pathological concomitants of the increased catecholamine level as a result of acidosis, the positive metabolic effects of catecholamines on heart performance must also be taken into account.

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Pharmacologia

EFFECT OF CHLOROQUINE PHOSPHATE ON THE ISOLATED NON-PREGNANT AND PREGNANT UTERUS OF DIFFERENT SPECIES

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The action of chloroquine on isolated uteri of pregnant and non-pregnant rats, rabbits and guinea pigs showed species differences. The effect on the rat uterus was either stimulatory or inhibitory depending on whether or not the animal was pregnant and also on the type of bathing fluid used. Chloroquine stimulated the pregnant and non-pregnant uterus of rabbits and guinea pigs in de Jalon's or in mammalian Ringer–Locke's solutions. The stimulatory action of chloroquine was Ca^{+2}-dependent, antagonized by tetracaine and 2,4-dinitrophenol, but unaffected by atropine, lysergide or phenoxybenzamine.

It has been reported by HART and NAUNTON (1964) that chloroquine can cross the placental barrier and may cause injury to the foetus. Recently, chloroquine has been shown to stimulate the non-pregnant and to inhibit the pregnant rat uterus (ABDEL-AZIZ et al. 1971). It was therefore thought of interest to investigate in some detail the effect of chloroquine phosphate on the isolated uterus of pregnant and non-pregnant rats, rabbits and guinea pigs.

Methods

Pregnant uterus. The uterus of pregnant rats, rabbits and guinea pigs was removed in early pregnancy (5th to 10th day) or in late pregnancy (19th to 21st day in rats; 27th to 30th day in rabbits and 40th to 50th day in guinea pigs). The stage of pregnancy in guinea pigs was determined by the length of the foetus as described by BELL (1941).

Non-pregnant uterus. Adult female rats (180—200 g), rabbits (1.75—2.0 kg) and guinea pigs (350—450 g) were used. Some animals were injected subcutaneously with 2.5 mg/kg oestradiol monobenzoate and the uterus was removed 48 hours later. Uteri were also removed from animals not receiving oestrogen.

Post-partum rat uterus. These were removed, as described by ABDEL-AZIZ and BAKRY (1972), 1/2 hour to 6 days after parturition.

Isolated uterus preparation. Non-pregnant and post-partum rat uteri were used in toto or only the upper two thirds of one uterine horn were examined. In the case of non-pregnant

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uteri from rabbits and guinea pigs, only the lower half of the uterine horn was used of pregnant uteri, strips of suitable size were obtained. The uterus was suspended in a 20 ml organ bath and recordings were made on a smoked drum, using an isotonic lever with optimum load and of a magnification of 8 to 10. A mixture of 5% CO_2 + 95% O_2 was used for aeration. The uteri were suspended in mammalian Ringer–Locke's solution at 37°C as used by TOTHILL (1967) or at 30°C in de Jalon's solution which contains one fourth the amount of calcium and half the amount of glucose of that present in Ringer–Locke's solution. Uteri suspended in mammalian Ringer–Locke's solution showed fairly regular rhythmic contractions, while in de Jalon's solution the uteri obtained from rats showed little or no spontaneous contractions and those obtained from rabbits and guinea pigs showed moderate spontaneous contractions. In some experiments, after the action of chloroquine was tested in normal Ringer–Locke's or de Jalon's solution, it was retested in calcium-free media of either solutions.

Results

Results are seen in Table I.

Table I

Summary of the effects of chloroquine on the rat, rabbit and guinea pig uterus

Species	State of uterus	Effect of chloroquine on uterus suspended in	
		de Jalon's solution	Ringer—Locke's solution
Rat	non-pregnant	inhibition	stimulation
	pregnant	inhibition	inhibition
	postpartum (during first 2 days)	inhibition	inhibition
	postpartum (after the first 2 days)	inhibition	stimulation
Rabbits	non-pregnant	stimulation	stimulation
	pregnant	stimulation	stimulation
Guinea pigs	non-pregnant	stimulation	stimulation
	pregnant	stimulation	stimulation

Rat uterus. Chloroquine (20—500 μg/ml) exerted on the non-pregnant rat uterus an oxytocic effect which was associated with an increase in tonus and frequency of spontaneous contractions in mammalian Ringer–Locke's solution. Figure 1 illustrates typical effects of chloroquine on pregnant and post-partum rat uterus suspended in mammalian Ringer–Locke's solution. Chloroquine (50—500 μg/ml) inhibited the pregnant horn of a unilaterally pregnant rat uterus (Fig. 1a) as well as the non-pregnant horn of the same uterus (Fig. 1b). This inhibitory action of chloroquine was found to persist in the post-partum rat uterus during the first 2 days after parturition, but tended to change to a simulatory action from the third to the sixth day following parturition (Fig. 1c, e, f.). The inhibitory action of chloroquine was not blocked

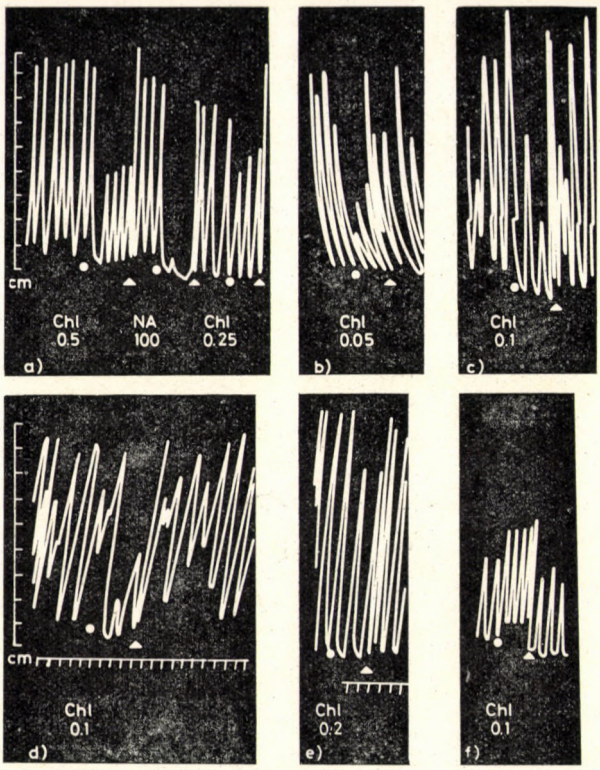

Fig. 1. Action of chloroquine on pregnant and post-partum rat uterus. a: 19-day pregnant uterus; b: non-pregnant horn of the same uterus in a; c: post-partum uterus, 1/2 hour after parturition; d: post-partum uterus, 12 hours after parturition; e: post-partum rat uterus, 24 hours after parturition; f: post-partum uterus, 4 days after parturition. Concentrations of chloroquine (Chl) in mg/ml and of noradrenaline (NA) in nanogram/ml. All uteri were suspended in mammalian Ringer–Locke's solution. At, ▲ washing; time intervals, one min

by 1×10^{-6} propranolol, and the stimulatory action was not effected by 1×10^{-7} atropine, 1×10^{-8} lysergide, or 1×10^{-6} phenoxy-benzamine. On the other hand, chloroquine (50—500 μg/ml) consistently inhibited non-pregnant, pregnant and post-partum uteri suspended in de Jalon's solution. This was observed as a decrease in the tone of uterine muscle and by the antagonism by chloroquine of the stimulatory action of oxytocin and acetylcholine. When de Jalon's solution was exchanged for mammalian Ringer–Locke's solution, the uterus showed marked spontaneous contractions and the stimulatory effect of chloroquine on the non-pregnant and post-partum rat uterus reappeared and then disappeared when the bathing fluid was rechanged to de Jalon's solution. Chloroquine failed to stimulate uteri in calcium-free media. The metabolic inhibitor 2,4-dinitrophenol (DNP) 1×10^{-4} M as well as tetracaine, 50 to 200 μg/ml, antagonized the stimulatory action of chloroquine on non-pregnant and post-partum rat uteri suspended in mammalian Ringer–Locke's solution (Fig. 2).

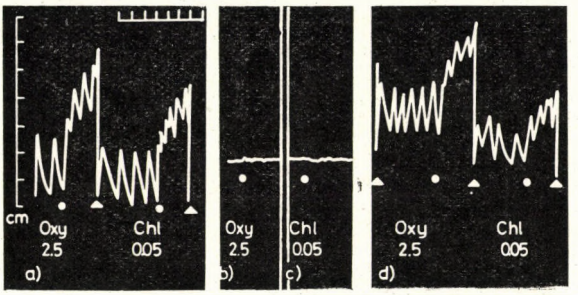

Fig. 2. Effect of tetracaine on the action of chloroquine on the rat uterus 4 days after parturition, suspended in mammalian Ringer–Locke's solution. a: before tetracaine; b and c: in presence of 100 μg/ml tetracaine; d: after washing of tetracaine. Concentrations of chloroquine (Chl) in mg/ml, of oxytocin (Oxy) in μU/ml. At ▲, washing; time intervals, one min

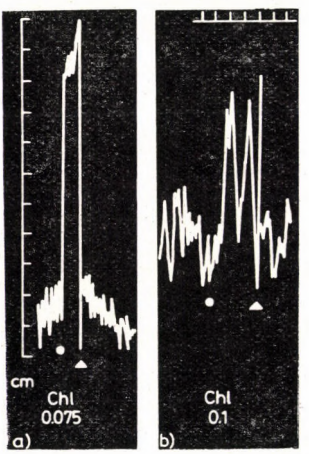

Fig. 3. Action of chloroquine on rabbit uterus in mammalian Ringer–Locke's solution. a: non-pregnant uterus; b: 20-day pregnant uterus. Concentration of chloroquine (Chl) in mg/ml. At ▲, washing; time intervals, one min

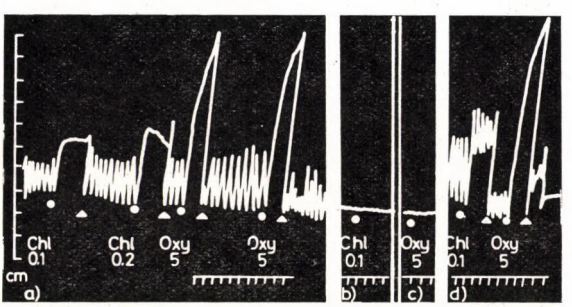

Fig. 4. Action of chloroquine on the non-pregnant oestrogen-treated guinea pig uterus suspended in de Jalon's solution. a: before tetracaine; b: and c: in the presence of 100 μg/ml tetracaine and of oxytocin (Oxy) in μU/ml. At ▲, washing; time intervals, one min

Rabbit uterus. Chloroquine at concentrations of 30 to 500 μg/ml exerted an oxytocic action on pregnant and non-pregnant rabbit uteri suspended in de Jalon's solution or in mammalian Ringer–Locke's solution. The rabbit uterus was generally more sensitive than the rat uterus to the stimulatory action of chloroquine. Figure 3 shows the effect of chloroquine on pregnant and non-pregnant rabbit uteri. The action of chloroquine was not affected by atropine, lysergide or phenoxybenzamine at concentrations similar to those used for the rat uterus. Tetracaine and DNP prevented the effect of chloroquine.

Guinea pig uterus. Chloroquine stimulated the pregnant as well as the non-pregnant guinea pig uterus, whether suspended in de Jalon's or in mammalian Ringer–Locke's solutions (Fig. 4), and this was prevented by tetracaine and DNP but not affected by atropine, lysergide or phenoxybenzamine. Chloroquine failed to stimulate guinea pig uteri suspended in calcium-free solutions.

Discussion

The action of chloroquine phosphate on the isolated uterus of rats, rabbits and guinea pigs showed species differences. The type of response of the rat uterus depends upon the composition of the bathing fluid and whether the uterus is pregnant. On the other hand, chloroquine had an oxytocic effect on the pregnant and non-pregnant uterus of rabbits and guine apigs, whether in de Jalon's or in mammalian Ringer–Locke's solutions. The inhibitory as well as the stimulatory actions of chloroquine on the uterus of the three species studied, seem to be due to a direct action of the drug on the myometrium and confirm the findings of ABDEL-AZIZ et al. (1971).

The results obtained with the post-partum rat uterus were interesting in that the type of chloroquine action depended upon the time elapsed after parturition. Thus, chloroquine caused an inhibitory effect during the first two days following parturition, but after that period the action changed into a stimulatory one, similar to that observed with the non-pregnant rat uterus. The first 2 days following parturition and during which chloroquine inhibited the uterus is, perhaps, the early transitional stage during which physiological adjustment from the pregnant to the non-pregnant state takes place. This qualitative difference in the response of the pregnant as compared to the non-pregnant and post-partum uterus is most probably due to differences in uterine hormonal state which may influence, among others, the degree of binding of calcium and/or the calcium level in the smooth muscle membrane. It was suggested by KNIFTON (1968) that calcium is more firmly bound in the pregnant than in the non-pregnant myometrium and that there is an abrupt decrease in calcium binding on the day of parturition which coincides

with the sudden increase in sensitivity to oxytocin. That calcium is essential to the stimulant action of chloroquine was shown by the finding that chloroquine failed to elicit such an effect on the uteri of the 2 species when suspended in calcium-free media. It is known that the release of calcium and its combination at sites in the cell surface is the final common pathway for excitatory influences acting to contract smooth muscles (CSAPO 1961; DANIEL et al. 1962). It seems, however, that the rat uterus needs a higher calcium concentration in the bathing fluid than the uterus of rabbits and guinea pigs in order to show stimulation in response to chloroquine. KNIFTON (1967) reported on differences between the rabbit uterus and the rat uterus in the effect of oestrogen and progesterone on the tension developed in response to electrical stimulation in the absence of calcium. The antagonistic action of the local anaesthetic tetracaine to the stimulant effect of chloroquine on the uterus was found to occur in all the three species studied and could possibly be linked with the action of tetracaine on calcium as reported by FEINSTEIN (1961) who found that local anaesthetics are antagonizing the calcium exchange of the uterus.

It has been reported by MARSHALL and MILLER (1964) that the metabolic inhibitor 2,4,dinitrophenol can abolish the stimulatory action of oxytocin as well as the rhythmic contractions of the rat uterus. This was confirmed by the present work and it was found that DNP antagonized the stimulant action of chloroquine on the uterus. It has been suggested by MARSHALL and MILLER (1964) that the mechanism by which DNP blocks the action of oxytocin may be associated with an inhibition of ion transport. It is possible that DNP blocks the action of chloroquine by a similar mechanism.

Work is in progress to establish how far the relase of prostaglandins is involved in the qualitative difference observed in the effect of chloroquine on the pregnant and non-pregnant uterus.

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OXOTREMORINE HYPERGLYCAEMIA IN THE RAT

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Oxotremorine after its systemic and intracerebroventricular administration, causes hyperglycaemia in the waking rat. Methylatropine inhibited the effect of intraperitoneally injected oxotremorine but failed to do so after intracerebroventricular injection of the cholinomimetic. The hyperglycaemia evoked by intraperitoneal oxotremorine application was inhibited by adrenalectomy and diminished by hypophysectomy. Pretreatment with aminoglutethimide, a drug inhibiting steroid synthesis, decreased the hyperglycaemic effect of oxotremorine in those animals which exhibited the lowest blood corticosterone level. The hyperglycaemia is peripheral in origin, but it is a central sympathetic excitation, which figures in its development, and the pituitary–adrenocortical system is also necessary for its manifestation.

Tremorine and oxotremorine are known to elevate blood sugar level in the rat, but the mechanism of this action is unclear. According to FRIEDMAN and EVERETT (1964), tremorine hyperglycaemia is central in origin, it manifests itself through the adrenals and cannot be elicited in adrenalectomized animals. OELSSNER et al. (1970) described that oxotremorine elevated the blood sugar level also in adrenalectomized rats, an effect inhibited by methylatropine, acting only peripherally. GUPTA and GANGULY (1969) as well as GUPTA et al. (1970) are of the opinion that oxotremorine does not elevate the blood sugar level through the same mechanism as does adrenaline; according to those authors, oxotremorine would act directly via some liver enzyme.

The present work was aimed at clarifying oxotremorine hyperglycaemia was central or peripheral in origin and what humoral systems are at play in this effect.

Methods

The experiments were performed on waking rats weighing 150—270 g. The animals were deprived of food for 12 hours before the experiment, with free access to water. During the experiments the rats were individually housed in plastic boxes. Blood for blood sugar determination was taken from the tail vein before the treatment and 5—6 times during the first two post-treatment hours. Before experimentation, the rats were kept isolated for at least 60 min.

In one series of experiments the animals were kept in cages under quiet conditions. Then, 25 min after the oxotremorine treatment, they were anaesthetized with ether, and blood was withdrawn from the abdominal aorta for corticosterone and blood sugar determination.

The adrenals were removed 6 days before the experiment under pentobarbital anaesthesia (40 mg/kg intraperitoneally). Following the operation, the rats received to drink 1% sodium chloride solution instead of water.

The pituitary was removed according to GAY's method (1967) one day prior to the experiment. Intracerebroventricular application of the drugs was made according to the method of VERSTER et al. (1971) through chronically implanted cannulae, in a volume of 10 μl.

Blood sugar level was determined according to HAGEDORN and JENSEN (1923), and in some experiments, according to the procedure of HYVÄRINEN and NIKKILÄ (1962) as modified by BAKOS (1966). Corticosterone was determined according to GUILLEMIN et al. (1959).

The drugs used were oxotremorine oxalate (Institute for Experimental Medicine, Hungarian Academy of Sciences, Budapest), methylatropine bromide (Fluka), and aminoglutethimide (Elipten-Ciba). The doses given refer to the corresponding salts.

In each animals, the pre-treatment blood sugar level was determined, and the results obtained were expressed in per cent of these values. For statistical evaluation Student's one-tailed and two-tailed *t*-tests and, in one series of experiments, two-way variance analysis were applied.

Results

The starting blood sugar level was 100 $\pm$ 1.6 mg/100 ml in the control animals (mean of 70 experiments).

Isolation for three hours, or intraperitoneal injection of physiological saline, did not influence the blood sugar level of the rats (Fig. 1). Intracerebroventicular application of sodium chloride caused an insignificant (15 $\pm$ 5%) elevation of the blood sugar level (Fig. 2).

Oxotremorine given intraperitoneally in a dose of 0.5 mg/kg caused hyperglycaemia (Fig. 1). The effect was marked at 15 min (26 $\pm$ 8%), reached the maximum by the 30th min (74 $\pm$ 12%) and disappeared by the 90th min.

The central effects of oxotremorine, for instance its hypertensive action, were also manifest after the intracerebroventricular application of the compound (PHAN and GYÖRGY 1973).

Oxotremorine given intracerebroventricularly also caused hyperglycaemia. In a dose of 5 μg it elevated the blood sugar level by 53 $\pm$ 6%. The maximum was reached by the 15th min, the effect being much less marked by the 30th min (Fig. 2). This small dose, i.e. 5 μg/animal, when injected by the intraperitoneal route, caused insignificant hyperglycaemia (12 $\pm$ 7%).

These results seemed to point to a central nervous origin of oxotremorine hyperglycaemia. Since this conclusion is at variance with the results of OELSSNER et al. (1970), who found that the oxotremorine effect was inhibited by methylatropine, a drug of peripheral action, we investigated ourselves how this cholinolytic agent containing a quaternary nitrogen atom would influence the oxotremorine-induced hyperglycaemia.

Methylatropine, in a dose of 5 mg/kg intraperitoneally, failed to affect the blood sugar level, as compared with the control (Fig. 1). This dose of methylatropine inhibited the effect of intraperitoneally given oxotremorine, a slight (17 $\pm$ 6%) elevation of the blood sugar level occurred only by the 15th min (Fig. 1). However, the activity of oxotremorine applied intracerebroventricularly was not inhibited by methylatropine (an elevation of 39 $\pm$ 6%, Fig. 2).

As mentioned above, data in the literature are contradictory concerning the role of adrenals in oxotremorine hyperglycaemia. In our experiments,

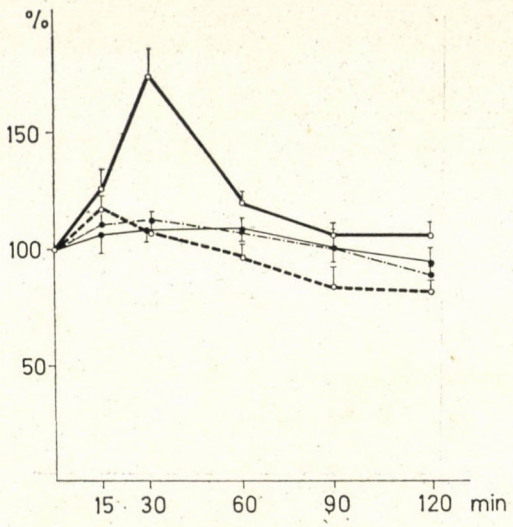

Fig. 1. Effect of systemically administered oxotremorine and/or methylatropine on blood sugar level. ———: 0.9% NaCl intraperitoneally (i.p.); ▬▬: 0.5 mg/kg oxotremorine i.p. (n = 10); —·—: 5 mg/kg methylatropine i.p. (n = 9): — —: 5 mg/kg methylatropine +0.5 mg/kg oxotremorine i.p. (n = 7). Ordinate: blood sugar level in per cent of pretreatment value. The values measured 30 min after oxotremorine significantly differ from those for NaCl-treated animals ($p < 0.01$). The effect of methylatropine and that of methylatropine + oxotremorine are not significant statistically

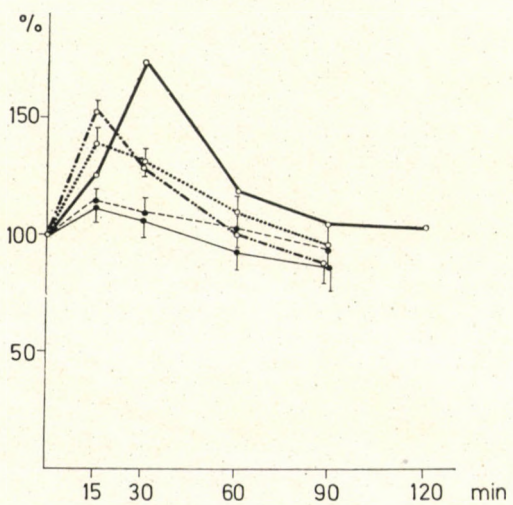

Fig. 2. Comparison of the effect of oxotremorine applied intracerebroventricularly (i.c.v.) and intravenously (i.v.). — —: 0.9% NaCl i.c.v. (n = 8); ———: 0.005 mg/animal oxotremorine i.p. (n = 7); —··—: 0.005 mg/animal oxotremorine i.c.v. (n = 8); ·····: 5 mg/kg methylatropine i.p. + 0.005 mg/animal oxotremorine i.c.v. (n = 9); ▬▬: 0.5 mg/kg oxotremorine i.p. (n = 10). Ordinate: blood sugar level in per cent of pretreatment value. By the 15th min, the difference from controls (NaCl i.v.c.) is significant in animals treated with 0.005 mg/animal oxotremorine ($p < 0.01$) and methylatropine + oxotremorine ($p < 0.05$)

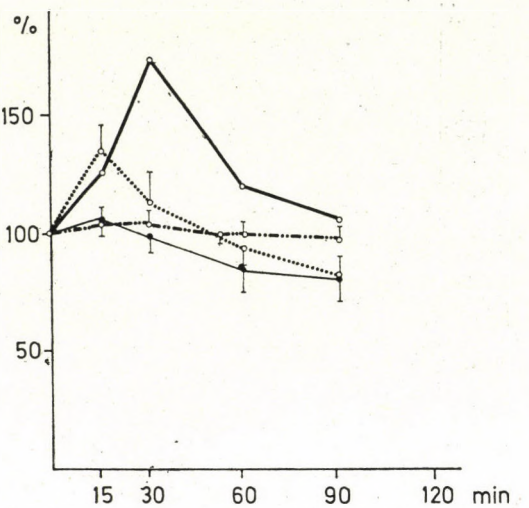

Fig. 3. Influence of hypophysectomy and adrenalectomy on oxotremorine hyperglycaemia. ———: 0.9% NaCl i.p. in hypophysectomized animal (n = 9); ····: 0.5 mg/kg oxotremorine i.p., in hypophysectomized animal (n = 13); —··—: 0.5 mg/kg oxotremorine i.p. in adrenalectomized animal (n = 6); ▬▬: 0.5 mg/kg oxotremorine in intact animal (n = 10). Ordinate: blood sugar level in per cent of pretreatment value. The effect of oxotremorine in hypophysectomized animals significantly differs from that in NaCl-treated animals ($p < 0.05$) by the 15th min

0.5 mg/kg of oxotremorine, administered 6 days after adrenalectomy, failed to influence the blood sugar level (4 ± 5%, Fig. 3). Adrenalin, in a subcutaneous dose of 0.25 mg/kg, caused a considerable increase in the blood sugar of adrenalectomized animals (42 ± 3.7%, n = 6).

Any possible oxotremorine effect manifesting itself through steroid hormones was also prevented by the adrenalectomy. The possibility arose that oxotremorine was activating the pituitary–adrenocortical system. Thus, in further experiments, the effect of the pituitary was investigated on oxotremorine hyperglycaemia.

Hypophysectomy strongly inhibited, but failed completely to abolish, the blood sugar increasing effect of 0.5 mg/kg oxotremorine (Fig. 3). The peak effect (35 ± 11%) was seen at 15 min. At this point of time the activity of oxotremorine was identical with that measured in non-operated animals (26 ± 8%).

It was assumed on the basis of these experiments that corticosteroids might figure in the hyperglycaemic action. Since we had no data concerning the influence of oxtoremorine on the blood corticosterone level, the following experiments were performed.

Under the conditions described in Methods, the blood corticosterone level was 16.6 ± 2.9 μg/100 ml in animals treated with sodium chloride (n = 9) and 47.1 ± 2.6 μg/100 ml in rats treated with 0.5 mg/kg oxotremorine

intraperitoneally ($n = 10$, $p < 0.001$). Oxotremorine elevated the blood sugar level in these experiments by 58% as compared to the controls.

Accordingly, the elevated steroid level seemed to play a part in inducing the oxotremorine hyperglycaemia. It was therefore investigated how the inhibition of steroid synthesis would influence the hyperglycaemic effect of oxotremorine.

Aminoglutethimide was used for inhibiting steroid synthesis (DEXTER et al. 1967; LOSCALZO et al. 1970). The drug was administered in subcutaneous doses of 150 mg/kg on two occasions (10 a.m. and 4 p.m.) on the day before the experiment. On the day of the experiment the rats were kept in plastic boxes, as usual (restraint situation). Blood for blood sugar determination was taken from the tail vein on five occasions. Then, 100 min following the administration of oxotremorine, the animals were bled, and the plasma corticosterone level was determined.

The results are shown in Table I. The corticosterone level was high also in the control group with no further increase upon the effect of oxotremorine (restraint situation). Pretreatment with aminoglutethimide decreased the steroid level which was no longer augmented by oxotremorine in these animals.

It is seen from the data of Table I that aminoglutethimide pretreatment did not inhibit the oxotremorine hyperglycaemia. The pretreatment influenced the corticosterone level to a variable extent. Thus, a correlation was sought for between steroid level and the degree of oxotremorine hyperglycaemia.

Table I

Influence of aminoglutethimide pretreatment on the blood sugar and corticosterone level increasing effect of oxotremorine. The animals received 150 mg/kg of aminoglutethimide (AMG) subcutaneously on two occasions the day before the experiment. Six experimental days, twelve animals in each group

Pretreatment	Treatment	Blood sugar			Time of maximum effect, min ± S.E.	Corticosterone level, 100 min, µg/100 ml ± S.E.
		Initial value, mg/100 ml ± S.E.	Maximal augmentation			
			mg/100 ml ± S.E.	per cent		
1. NaCl	NaCl	96.6±4.15	14.2±3.19	14.7	40.0±8.12	48.8±1.45
2. NaCl	OT	92.9±2.81	46.3±7.64	49.8	29.4±5.57	51.3±2.17
3. AMG	NaCl	96.9±6.38	13.1±5.08	13.5	40.0±7.46	17.9±2.49
4. AMG	OT	103.8±6.88	32.8±8.57	31.6	40.6±7.78	20.1±4.16

OT: 0.5 mg/kg oxotremorine intraperitoneally
Augmentation of blood sugar level after oxotremorine is significant according to two-way variance analysis (1—2 and 3—4, $p < 0.01$). The increases in Groups 2 and 4 do not differ significantly, nor is there any significant difference between the times of maximum effect. There is a correlation in Group 4 between corticosterone level and increase in blood sugar, the correlation coefficient being 0.782, $p < 0.01$
(The authors thank Mr. G. FOLLY for statistical analysis)

The analysis revealed that in animals with a low corticosterone level oxotremorine elevated the blood sugar level in a slight degree (Table I).

Aminoglutethimide pretreatment caused ataxia in most animals, often persisting on the day of the experiment. Since aminoglutethimide decreased the corticosterone level to 18—20 μg/100 ml, three to five repeated treatments were performed instead of administering the drug only twice. However, the animals treated in this way were in an anaesthesia-like condition before the experiment, they did not move, their body temperature decreased. No experiment was performed on these animals.

Discussion

Our finding that adrenalectomy prevented the hyperglycaemia induced by oxotremorine was in accordance with the observation of FRIEDMAN and EVERETT (1964), but in contradiction with that of OELSSNER et al. (1970). These latter authors have studied the metabolic effects of oxotremorine under deep urethane anaesthesia and the discrepancy of the results might have been caused by this factor. It seems also unlikely that oxotremorine hyperglycaemia should be caused by a direct effect on liver enzymes (GUPTA and GANGULY 1969; GUPTA et al. 1970), for such an activity would be present also after adrenalectomy.

Even though the adrenal are necessary for eliciting oxotremorine hyperglycaemia, a direct stimulatory effect of the drug on the adrenals can be excluded. Oxotremorine possesses no ganglionic stimulatory action (HASLETT 1963; GYÖRGY et al. 1971), and its pressor effect in the rat is not influenced by adrenalectomy (PHAN and GYÖRGY 1973).

Tremorine hyperglycaemia is inhibited by splanchnic transection and medullectomy (FRIEDMAN and EVERETT 1964); oxotremorine presumably activates the adrenals through the central nervous system. Stimulation of the cerebral sympathetic centres is well-known to produce hyperglycaemia (BERNARD 1849; FRANKLIN and DISTEFANO 1962; SHIMAZU et al. 1966; KOKKA and GEORGE 1970). The central nervous system is also involved into the regulation of adrenaline secretion (MARLEY 1964), and we could show that oxotremorine stimulates sympathetic centres in the cat (GYÖRGY et al. 1971), as well as in the rat (PHAN and GYÖRGY 1973). We therefore assume this action to play a part in the blood sugar increasing effect of the drug.

Some experimental data, however, are clearly against the exclusive role of a direct effect on the sympathetic centres in the development of oxotremorine hyperglycaemia. This hyperglycaemia is peripheral in origin, as it is inhibited by methylatropine. Accordingly, peripheral factors must play a decisive role in the oxotremorine action, since methylatropine does not penetrate into the central nervous system and does not influence the central effects

of oxotremorine. We assume that oxotremorine stimulates the central nervous system in a reflex way through its peripheral actions inhibited by methylatropine (vasodepression, bradycardia, bronchial constriction etc.). An exclusively peripheral origin of the oxotremorine hyperglycaemia cannot be imagined, as the role of the adrenals is doubtless, but the compound has no direct effect on the adrenals.

Oxotremorine has a direct central effect, too; it causes hyperglycaemia also after intracerebroventricular application, which hyperglycaemia is not influenced by methylatropine. In the case of an intraperitoneal administration the dose used (0.5 mg/kg) is presumably too low for this direct central action to become manifest.

Hypophysectomy markedly diminished the oxotremorine hyperglycaemia. Though also STH may figure in the development of a hyperglycaemic reaction (HOUSSAY 1955), such a role can be excluded in the present case. (The adrenals are necessary for the manifestation of the oxotremorine effect, but not for that of STH.) Our experiments point to a role of the ACTH–adrenal cortex system in the case of oxotremorine.

In freely-moving animals oxotremorine augments the blood corticosteroid level. Under the conditions of our experiments the steroid level was high also in the control rats, due to the restraint situation; in these animals oxotremorine failed further to increase the steroid level. In spite of this, the experiments performed with aminoglutethimide, a drug inhibiting steroid synthesis, also corroborate the role of adrenocortical hormones in the development of oxotremorine hyperglycaemia. The diminution of the steroid level was correlated with the inhibition of the hyperglycaemic response.

Thus, steroids are necessary for the manifestation of the oxotremorine effect. They cannot, however, be assumed to raise the blood, sugar level by a direct action; control animals in restraint situation do not exhibit hypeglycaemia in spite of the high corticosterone level. More probably, steroids augment the hyperglycaemic effect of adrenaline by their permissive action; COLLIP et al. (1936—1937) showed that this adrenaline effect decreased markedly after hypophysectomy in the rat.

According to the above, oxotremorine applied intracerebroventricularly possesses a direct central action on the one hand, and an indirect peripheral action manifesting itself via the central nervous system, on the other. Liberation of adrenaline and the presence of adrenocortical hormones are also necessary for the development of the hyperglycaemia.

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EFFECT OF OXOTREMORINE ON BEHAVIOUR AND EEG OF RESERPINE-PRETREATED RATS

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Oxotremorine in high dose elicits seizure-like manifestations unfrequently in the rat. After pretreatment with reserpine and methylatropine, however, clonic fits develop; at the same time EEG alterations can be observed characterized by 6—7 c/s bursts of 1—3 sec duration appearing at 5—20 sec intervals. A similar pattern is seen in reserpine-pretreated rats after the administration of physostigmine.

The EEG activity elicited by oxotremorine is inhibited by atropine and primidone but left unaltered by mecamylamine.

The convulsant activity of nicotine and other ganglionic stimulatory agents is a well-established fact. According to MOLNÁR et al. (1967) cholinomimetics (oxotremorine, arecoline, etc.) having exclusively muscarinergic activity, do not elicit seizures only tremor, after i. cer. application in the mouse. COX and POTKONJAK (1970) found that oxotremorine in large doses elicited convulsions in the rat. This was a rare occurrence in our experiments in normal rats; however, after pretreatment with reserpine, oxotremorine caused a characteristic, seizure-like behavioural pattern.

In the present experiments, oxotremorine was applied in high doses. The peripheral effects of the drug were inhibited with methylatropine. The aim was to study, at what frequency oxotremorine would induce seizure, whether these were potentiated by reserpine, a drug known to increase the effect of chemical convulsants, and whether the oxotremorine action was associated with any characteristic EEG activity. In addition, the influence of centrally acting anticholinergic agents (atropine, mecamylamine) and the anticonvulsant drug primidone was studied on the symptoms elicited by oxotremorine. Finally, the activity of oxotremorine was compared with that of physostigmine.

The EEG alterations induced by tremorine and oxotremorine were mainly studied in the rabbit (EVERETT 1964; BAN and HOJO 1971) and the cat (BAKER et al., 1960; GEORGE et al., 1964); experiments on the rat were described only by LÉVY and MICHEL-BER (1967). To our best knowledge, the EEG effect of oxotremorine in reserpine-pretreated animals has not been investigated.

Methods

The experiments were performed on albino rats of both sexes, weighing 200—280 g.

Behavioural studies. The symptoms developed after the treatments, to be detailed below, were investigated by observers unfamiliar with the treatment the animals had received.

EEG studies. Cortical screw electrodes made of nickel-plated copper were implanted under pentobarbital anaesthesia (35 mg/kg intraperitoneally) one week before the experiment. Two electrodes were located on the frontal cortex, two further ones on the parietal cortex, at a distance of 2 mm from the coronary and sagittal sutures, respectively. Cortical electrical activity was recorded in freely-moving animals from bifrontal, biparietal as well as right and left frontooccipital leads.

For evaluating the EEG record, we measured the number and duration of the bursts, between 0—5, 6—10, 11—15 etc., min after the injection of oxotremorine, for a total of 30 min. The sum of the total duration of burst activity was determined for each 5-min period. Then arithmetic means were computed and used for comparison.

The various groups of animals were treated at the point of time given in Table I.

Table I

Treatment schedule of various groups of animals

Group	−4 h intraperitoneally	−3 h subcutaneously	−20 min intraperitoneally	−15 min subcutaneously	Zero min intraperitoneally
1.				2 mg/kg methylatropine	2 mg/kg oxotremorine
2.	2.5 mg/kg reserpine			2 mg/kg methylatropine	2 mg/kg oxotremorine
3.	2.5 mg/kg reserpine			10—20 mg/kg atropine	2 mg/kg oxotremorine
4.	2.5 mg/kg reserpine		2.5 mg/kg mecamylamine	2 mg/kg methylatropine	2 mg/kg oxotremorine
5.	2.5 mg/kg reserpine	20 mg/kg primidone		2 mg/kg methylatropine	2 mg/kg oxotremorine
6.				2 mg/kg methylatropine	0.5—1.0 mg/kg physostigmine
7.	2.5 mg/kg reserpine			2 mg/kg methylatropine	0.5—1.0 mg/kg physostigmine
8.			60th min: 20—50 mg/kg tetrabenazine	2 mg/kg methylatropine	2 mg/kg oxotremorine

The drugs used were reserpine (Rausedyl, Richter, Budapest), methylatropine bromide (Fluka), atropine sulphate (Ph.Hg.VI.), mecamylamine (EGYT, Budapest), primidone (Sertan, EGYT, Budapest), oxotremorine oxalate (Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest), physostigmine salicylate (Ph.Hg.VI.), tetrabenazine (Nitoman, Hoffmann La Roche, Basel).

Results

Behaviour

Oxotremorine, in doses from one to two mg/kg or, after methylatropine, in a dose of 4 mg/kg elicited tremor; seizure-like manifestations appeared in about 15% of the cases. After reserpine pretreatment, 2 mg/kg oxotremorine

became highly toxic, caused dyspnoea, asphyxia and often death of the animals. Therefore, in this case the manifestations could not been evaluated. When methylatropine had been given together with reserpine before the injection of 2 mg/kg oxotremorine, all the animals survived and a seizure-like activity appeared in most cases. By the 4th—5th min following the administration of oxotremorine, a tremor of increasing intensity set on, turning gradually into jerking. The rats were standing on their hind limbs, later clonic fits developed in the head forelimbs and the frontal part of the body. They recurred 6—8 times; thereafter, the animals often fell down and lay on their side. The clonic fits were reminiscent of the first phase of the pentetrazole convulsions. Tonic seizures did not develop in any extremity.

In some experiments the effect of tetrabenazine was investigated. One hour after a dose of 20—50 mg/kg, oxotremorine caused symptoms identical with those seen above.

One mg/kg of physostigmine, given after reserpine pretreatment, caused clonic jerks and seizure-like manifestations similar to those seen after oxotremorine; however, no such event was observed without reserpine pretreatment.

The influence of various drugs on the oxotremorine fits of animals pretreated with reserpine and methylatropine was also investigated. Mecamylamine and primidone were found ineffective, while atropine sulphate inhibited their development.

EEG pattern

The monotonous cortical electrical activity of 6—7 c/s was not influenced by 2 mg/kg of oxotremorine in methylatropine-pretreated animals (Fig. 1).

In rats pretreated with reserpine and methylatropine, a fast activity superimposed on high-amplitude waves, was seen (Part 1 of Fig. 2). Under the effect of oxotremorine, four out of five animals exhibited a characteristic EEG pattern: rhythmic basal activity of medium voltage with high and steep bursts of 6—7 c/s appearing at 5—10 sec intervals, simultaneously with the fits (Part 2 of Fig. 2). This activity did not decrease during the 30-min observation period (Fig. 3).

In animals pretreated with reserpine and 10mg/kg of atropine, oxotremorine elicited burst activity of short duration. After 10 min the characteristic waves became rare and shorter and the burst activity practically ceased by the 30th min (Fig. 3). Twenty mg/kg of atropine fully counteracted burst activity in two rats.

The characteristic burst activity appeared in three out of five animals after the combined administration of reserpine, mecamylamine, methylatropine and oxotremorine. The period of bursts was longer than in rats not treated with mecamylamine (2.1 and 1.5 sec, respectively); as to total time, burst activity was increased mainly during the first 15 min (Fig. 3).

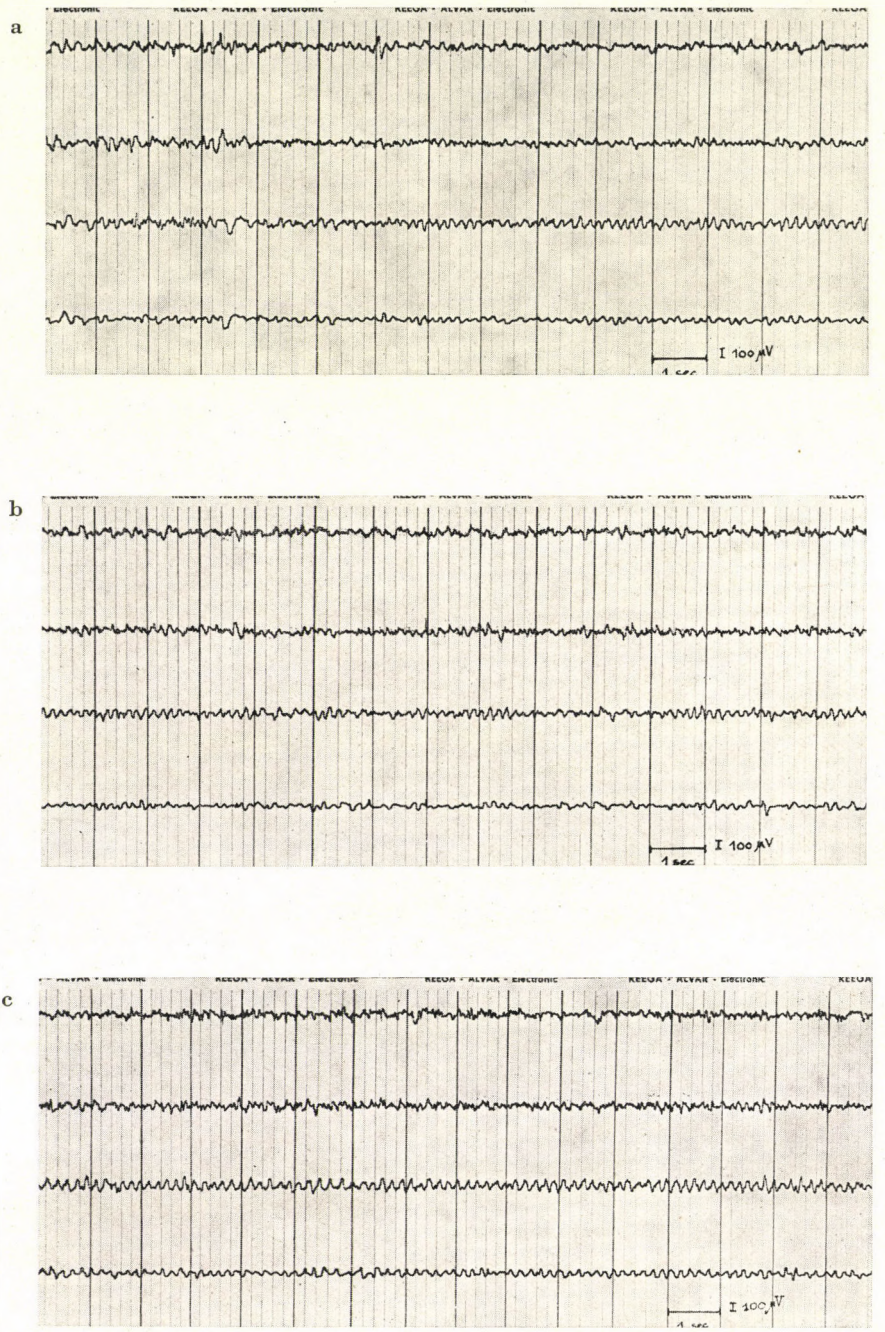

Fig. 1. a: RAT, 270 g, EEG tracing, without pretreatment. Derivations: bifrontal, biparietal, right frontoparietal, left frontoparietal. b: EEG tracing of the same rat after 2 mg/kg methylatropine given subcutaneously, c: EEG tracing of the same rat after 2 mg/kg oxotremorine given intraperitoneally

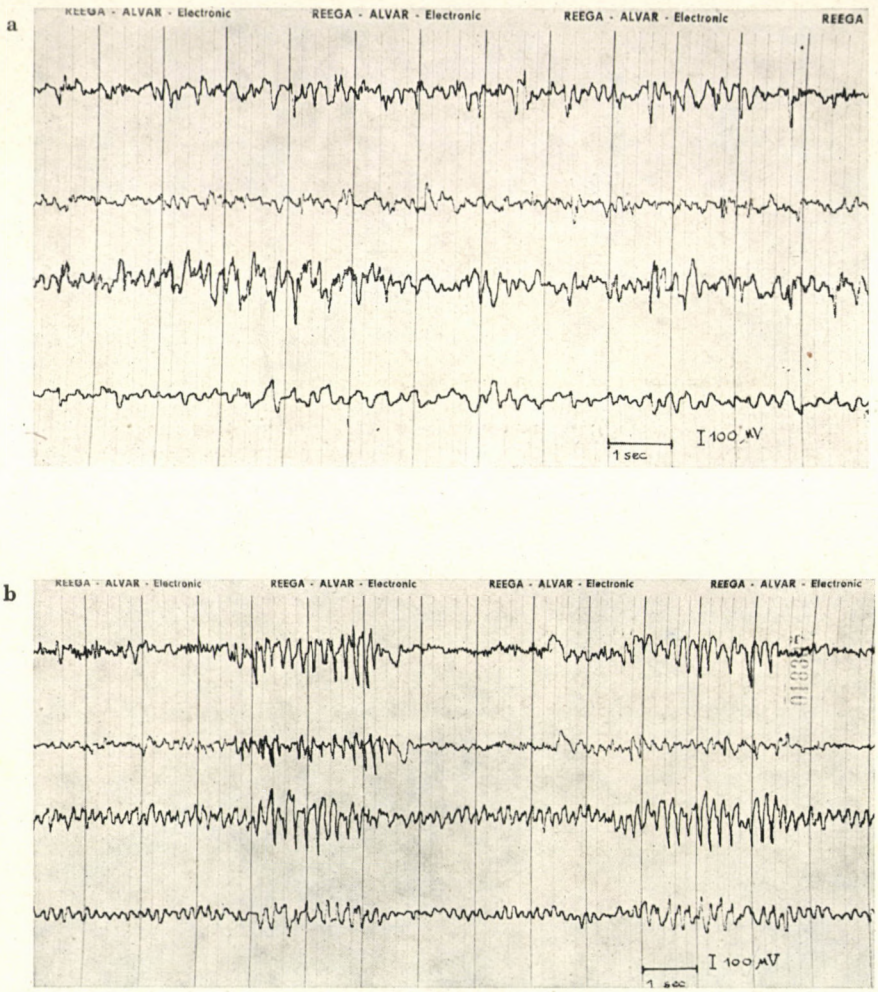

Fig. 2. a: Rat, 210 g, EEG tracing after intraperitoneal pretreatment with 2.5 mg/kg reserpine. Derivations: bifrontal, biparietal, right frontoparietal, left frontoparietal. b: EEG tracing of the same rat after subcutaneous pretreatment with 2 mg/kg methylatropine, and 2 mg/kg oxotremorine given intraperitoneally

In two out of five animals treated with reserpine, primidone and methylatropine, oxotremorine elicited no burst activity; in the remaining three rats it was weak and disappeared quickly (Fig. 3).

Physostigmine did not elicit burst activity in animals pretreated with methylatropine, whereas in rats treated with reserpine + methylatropine it caused a picture reminding of the oxotremorine effect in all respects (Fig. 4).

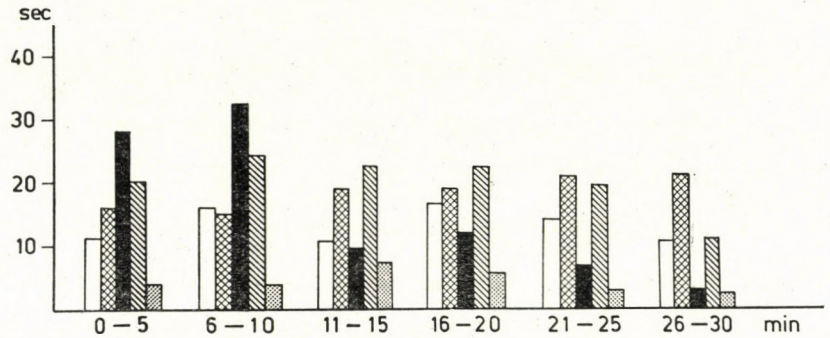

Fig. 3. Burst activity in rats pretreated with reserpine (2.5 mg/kg) and methylatropine (2 mg/kg), after oxotremorine. The height of the columns shows the summarized mean duration of bursts within the given time intervals. □: physostigmine (1 mg/kg intraperitoneally); ▩: oxotremorine (2 mg/kg intraperitoneally); ■: atropine (10 mg/kg subcutaneously) + oxotremorine (2 mg/kg intraperitoneally); ▧: mecamylamine (2.5 mg/kg intraperitoneally) + oxotremorine (2 mg/kg intraperitoneally); ▨: primidone (20 mg/kg subcutaneously) + oxotremorine (2 mg/kg intraperitoneally)

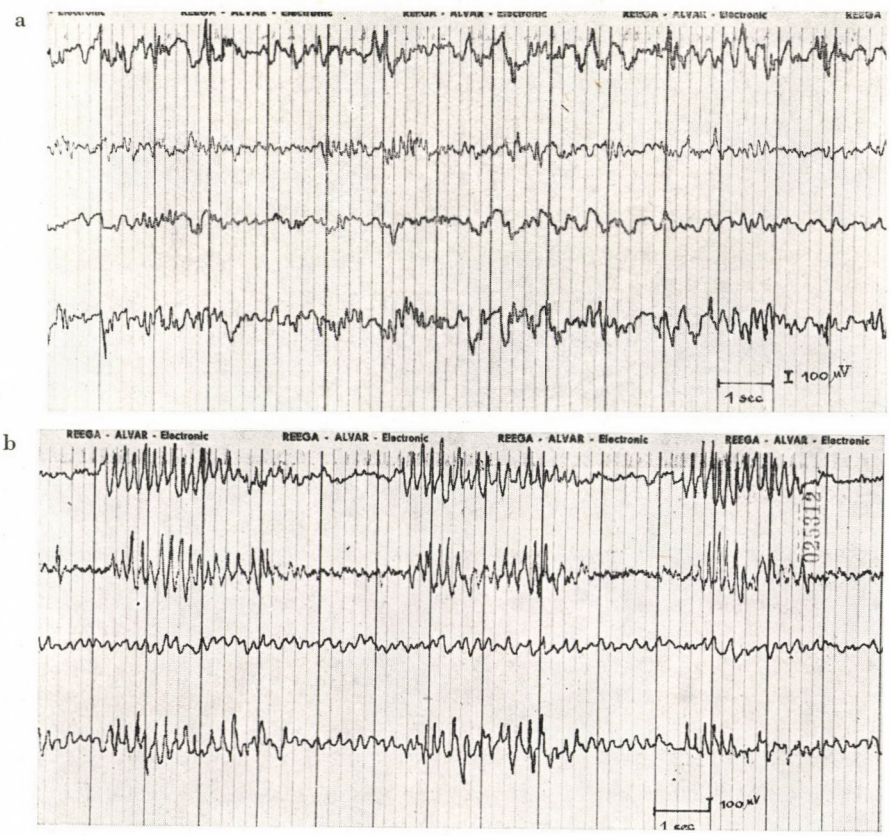

Fig. 4. a: Rat, 250 g, EEG tracing, after pretreatment with 2.5 mg/kg reserpine intraperitoneally. Derivations: bifrontal, biparietal, right frontoparietal, left frontoparietal. b: EEG tracing of the same rat after 2 mg/kg methylatropine subcutaneously and 1 mg/kg of eserine intraperitoneally

Discussion

Cox and Potkonjak (1970) described that 2 mg/kg oxotremorine given intraperitoneally caused convulsions in the rat. In our experiments in non-pretreated animals convulsions were infrequent. However, four hours after reserpine pretreatment, oxotremorine elicited characteristic fits in almost every animal.

Reserpine is known to facilitate the effect of convulsants; thus this drug can be assumed to unmask some "latent" activity of oxotremorine. It should be noted that we call the syndrome clonic jerk or fit, and not convulsion, for it never turns into the generalized fit and tonic extension seen after the administration of nicotine or pentetrazole. The EEG patterns during oxotremorine and pentetrazole fits are also different in that rhythmic spike activity characteristic of the latter drug was never observed after oxotremorine.

The behavioural manifestations and the burst activity are definitely correlated phenomena. Neither of them will develop without reserpine, they appear at the same time and atropine is inhibitory upon both of them.

According to the earlier data of Molnár et al. (1967), muscarinergic stimulants, such as oxotremorine, arecoline and arecoline derivatives do not cause convulsions on intracerebral application in the mouse. Our more recent data have shown that oxotremorine seizures cannot be observed in this species after reserpine pretreatment either. Carbachol, a drug also having a nicotine-like activity, elicits seizures by this route of administration (Decsi et al. 1963) and so do nicotine, DMPP, as well as tetramethylammonium bromide (Molnár et al. 1967). On this basis, we first had to think that the development of carbachol seizures might be due to the nicotinergic action of the molecule. However, carbachol seizures are inhibited by atropine (Decsi et al. 1963) but left unaltered by mecamylamine (György, unpublished); thus the possibility arises that a central "muscarinergic" seizure may also exist. This assumption is corroborated by the finding that atropine inhibits (or, in doses high enough, fully counteracts) the oxotremorine-induced clonic fits and burst activity, while mecamylamine is ineffective in this respect.

Thus, the oxotremorine fit is muscarinic in origin, similarly as the EEG activation elicited by cholinergic stimulants (physostigmine, arecoline) in the cat, a reaction inhibited by atropine but left unaltered by mecamylamine (Yamamoto and Domino 1967).

We cannot explain why primidone, which inhibits the oxotremorine-induced burst activity, has failed to influence the behavioural reaction.

The oxotremorine-induced fits and the accompanying EEG pattern are the consequence of the cholinergic stimulatory property of the molecule; the anticholinesterase drug physostigmine, though having a different site of action, exerts the same effect as oxotremorine.

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Communicatio brevis

BIDIRECTIONAL MODIFICATION OF HYPOTHALAMIC SELF-STIMULATION BY NORADRENALINE IN THE CAT

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It was found in four cats that noradrenaline injected intramuscularly exerts opposite effects on self-stimulation behaviour, depending on the dosage. A small dose of noradrenaline greatly facilitated the lever-pressing behaviour, while a large dose of the drug depressed it. The changes induced in the arterial blood pressure proved to be an excellent indicator of the reversal in the effect of noradrenaline on self-stimulation behaviour.

Since the original description of the phenomenon of intracranial electrical self-stimulation (Olds and Milner 1954), a wide variety of experimental observations has been accumulated to define the central neural mechanism of "reward". In behavioural testing, however, the possible role of the peripheral autonomic mechanisms may not be neglected. There are few experimental data as to whether the peripheral autonomic mechanisms are involved in the neural organization of reward (Malmo 1961; Perez-Cruet et al. 1963, 1965). In an earlier series of experiments marked alterations were found in respiration, heart rate and arterial blood pressure which were continuously recorded during self-stimulation (Ángyán and Rác 1972). It was suggested that the increase in blood pressure to a certain level may contribute to the disruption of lever-pressing behaviour. What is new in the present study is that self-stimulation was tested with the blood pressure elevated by noradrenaline. The choice of noradrenaline for testing was dictated by its well-known pressor effect and its effect on self-stimulation analyzed in many previous studies.

Four adult cats were used in this study. By the conventional stereotaxic technique, bipolar stainless steel electrodes, 1—2 mm apart and bared to 0.5 mm at the tips, were implanted in the mammillary region of the hypothalamus. A week after the operation, the animals were trained to press a lever for receiving a 0.3 sec train of monophasic square wave pulses of 0.3 msec duration at

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100/sec frequency. The training was regarded as successful when the animal began to press the lever spontaneously and repeatedly during at least 20 min on three consecutive experimental days. Both the threshold and the optimum stimulus intensities, i.e. those at which the highest lever-pressing rate was produced, were determined. Following the training for self-stimulation, all subsequent daily sessions were divided into four 20min test sessions, each separated by a 20-min pause. Stimulus intensity was varied systematically from one test session to the next. Whithin each 20-min test session the cats were free to self-stimulate and the frequency of self-stimulation was measured in successive five-minute periods. Five daily sessions were made as control. Then the self-stimulation was tested under the effect of noradrenaline given intramuscularly at the beginning of each 20-min test session during five days. The effect of another dose of noradrenaline was similarly tested after a new control period. Following these experiments, a polyethylene catheter was tied into one of the common carotid arteries and blood pressure was continuously recorded during self-stimulation, both before and after the injection of noradrenaline. Terminally, the location of the stimulating electrodes was verified histologically.

Four different stimulus intensities were used in each cat. The difference between the frequencies of self-stimulation with different stimulus intensities was significant at the 0.05 level or better. A relatively high rate of self-stimulation was found in three animals (1000—1600 presses during 20 minutes at the optimum stimulus intensities). The highest frequency obtained in the fourth cat was 524 presses during 20 min, but this cat pressed the level with its face instead of its leg. It was usual that the highest lever-pressing rate occurred in the first five-minute period of the 20-minute training sessions, then it slowly decreased during the successive five-minute periods.

The effect of noradrenaline depended on its dose. After a dose of 0.1 mg/kg, self-stimulation behaviour was facilitated (Fig. 1). The increase in the frequency of self-stimulation appeared at each stimulus intensity. The results treated intra-individually were significant at the 0.05 level or better. For the group as a whole, facilitation was significant at the 0.01 level. It is important to note, however, that after the injection, the animal was generally excited and visibly unquiet.

In contrast, 0.6 mg/kg doses of noradrenaline seemed to have an opposite effect. The typical effect appeared 2—5 min after the injection. The animal stopped self-stimulating, left the lever and lay down. At the same time, conspicuous vegetative effects appeared including salivation, polypnea and sometimes vomiting. After 10—20 min all these effects disappeared and the animal began to self-stimulate again. The effect of consecutive injections was not always so strong, but the decrease in the frequency of self-stimulation was significant at the 0.01 level for the group as a whole (Fig. 2). The individual

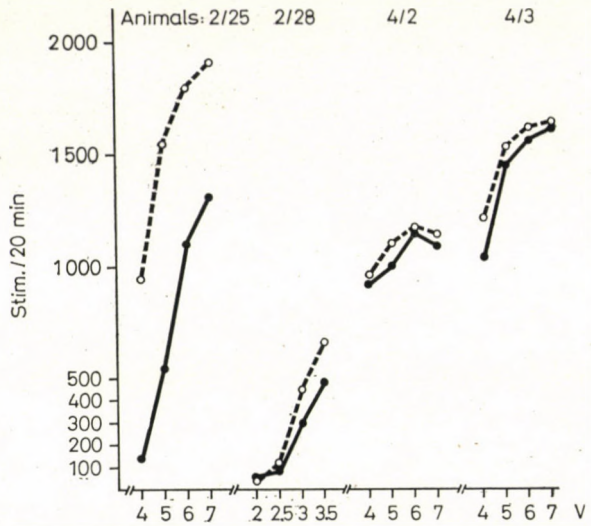

Fig. 1. Under the effect of 0.1 mg/kg noradrenaline a higher lever-pressing rate was obtained in each animal (dotted lines) than in the controls (unbroken lines). The curves were constructed from the mean lever-presses recorded on five experimental days

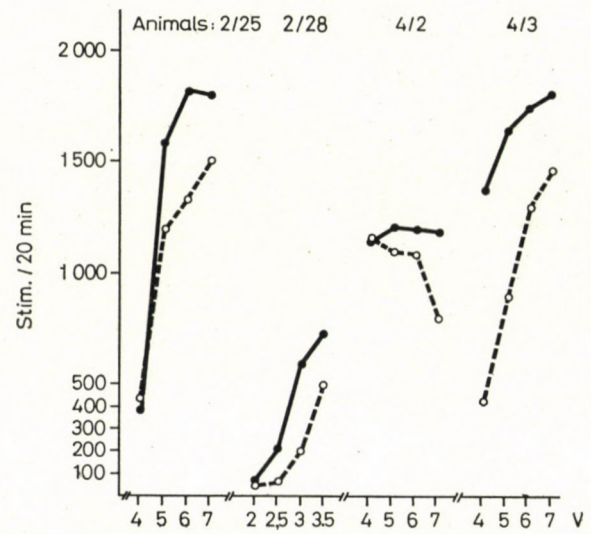

Fig. 2. Frequency of self-stimulation was depressed by 0.6 mg/kg of noradrenaline. Each curve represents mean lever-presses recorded on five experimental days

results were significant at the 0.02 level or better. The suppression of self-stimulation behaviour occurred at each stimulus intensity.

The blood pressure rapidly increased during self-stimulation, and rebound-like after-effects occurred when the animal stopped self-stimulating (Fig. 3A;) according to our earlier results (ÁNGYÁN and RÁC 1972). 2—5 min after

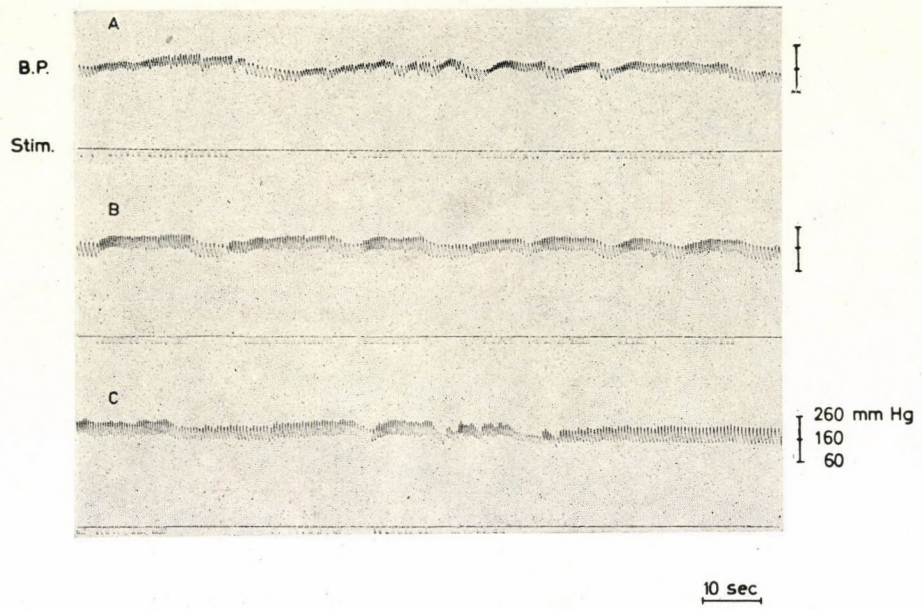

Fig. 3. Changes in blood pressure (B.P.) during self-stimulation (Stim.) by itself (A); and under the effect of 0.1 mg/kg (B), and 0.6 mg/kg (C) noradrenaline, 5 min after its injection

the injection of noradrenaline the blood pressure increased and remained high for several min, then after 10—20 min returned gradually to the control level. However, the rise in blood pressure produced by a small dose of noradrenaline did not exceed the effect of self-stimulation. In this case the animal continued to self-stimulate with a higher frequency (Fig. 3B). The large dose of noradrenaline, however, induced an increase in blood pressure greater than did self-stimulation by itself. After that dose, the cat stopped self-stimulating (Fig. 3C). Thus, the rise in blood pressure produced by noradrenaline showed that the given dose of the drug will facilitate or depress the self-stimulation behaviour according to whether the blood pressure elevation was smaller or higher than that produced by the own effect of self-stimulation.

These results clearly show that noradrenaline may exert opposite influences on self-stimulation behaviour depending on the dosage. Small doses facilitate, while large doses depress the self-stimulation behaviour. The self-stimulation with different stimulus intensities was equally influenced, and general behavioural and vegetative effects were also produced by the drug. These findings seem to be particularly important concerning the specific relationship between noradrenaline and self-stimulation behaviour proposed by many previous studies (Olds 1962; Stein 1964; Olds et al. 1964; Wise and Stein 1969; Poschel 1969; Margules 1969; Poschel and Ninteman

1971; OLDS and ITO 1973; and others). On the basis of the present results it is impossible to rule in or out a specific role of noradrenaline in the central neural mechanism of reward, but under our experimental conditons the general behavioural and vegetative effects of the drug appeared regularly with changes in the lever-pressing rate, showing the importance of peripheral actions in the modulation of self-stimulation behaviour. It is also shown by the finding that the rise in blood pressure produced by noradrenaline proved to be an excellent indicator of the reversal of the drug's effect on self-stimulation behaviour. Namely, when- ever the blood pressure was increased by the drug to more than by the self-stimulation by itself, the frequency of lever-pressings was always depressed. It remains to be studied whether a cardiovascular process itself would produce a similar modulation of self-stimulation behaviour.

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Recensiones

E. SCHUBERT

Seminar der Physiologie

(Seminar of Physiology). VEB Gustav Fischer Verlag, Jena 1973. 215 pages, with 56 figures and 18 tables. Price: M 16.60

This Seminar has been written by Wolfgang HASCHKE, Klaus ECKOLDT, Christfied PFEIFFER and Klaus WILFEST. It aims at giving assistance to student of physiology in medicine, biology and psychology, and to facilitate understanding of the connections of physiology with theoretical biology, mathematics and physics. The usual form of teaching physiology, the lectures and practical laboratory work are insufficient to-day for acquiring the fundamental theoretical knowledge and the way of thinking and about certain calculations unavoidable in modern physiology.

The work includes the following chapters with the seminary themes and exercises. 1. Excitation physiology, nerve and muscle; 2. Receptors and sensations; 3. Functions of the central nervous system; 4. Breathing and circulation; 5. Energy metabolism and homeostatic functions.

The book is a useful manual for students of physiology, particulary recommendable for recapitulation before taking examinations.

K. LISSÁK

H. PEETERS (Ed.)

Proteides of the Biological Fluids

Proceedings of the Twenty-first Colloquium in Brugge, 1973. Pergamon Press, Oxford, New York, Toronto, Sydney, Braunschweig 1974. XVI + 677 pages, with 301 figures and 99 tables. Price: £ 17. —

The volume covers the latest results in the field of membrane proteins, proteinuria research and the new techniques like the automated nephelometric analysis of proteins.

The introductory paper of the conference, the second Arne Tiselius Memorial Lecture, was presented by Frank W. PUTMAN: "Alpha, Beta, Gamma, Omega? The Past, Present and Future of Plasma Proteins"; it is an excellent survey of plasma protein research. The 107 papers by outstanding research workers of the field were presented in three main sections. Section A: Membranes; here were discussed the 1. Solubilization and Characterization of Membrane Proteins, Erythrocyte Membranes, Lymphocyte Membranes, Platelet Membranes and other Membranes; 2. Conformation and Structure of Membranes; 3. Protein-Lipid Interaction in Membranes; 4. Membrane-bound Enzymes and Hormones; 5. Immunological Function of Membranes. Section B: on Urinary Proteins, dealt with 1. Proteinuria, Pathology; 2. Electrophoretic Studies; 3. Plasma and Urinary Protein Levels; 4. Glomerular Clearance of Proteins; 5. Tubular Proteinuria; 6. Secretory Proteins. In Section C, Techniques, 1. Automated Nephelometric Analysis; 2. Radioimmunoassay; 3. New Techniques have been discussed. The volume is complete with an author and subject index.

These annual collection of papers on the progress in the field of plasma proteins will be an indispensable source of up-to-date information for research workers and advanced students of biochemistry, physiology and biological sciences.

K. LISSÁK

Y. KAWAMURA (Ed.)

Physiology of Mastication

Volume I. Frontiers of Oral Physiology. S. Karger, Basel, München, Paris, London, New York, Sydney 1974. XII + 327 pages with 208 figures and 4 tables. Price: SFr 174.—; US $ 53.95; DM 165.—; £ 25.25

Mastication is an important physiological function in man and many animals, essential for the maintenance of health. This excellently edited volume presents the recent concepts of international authorities in some specific fields of the basic biological sciences and clinical disciplines, with emphasis on the significance of mastication to the daily dental practice.

The contents of the volume, after the foreword of the Editor, are: Origin and Evolution of the Oral Apparatus by E. L. DU BRUL; Radiography of the Masticatory Apparatus by F. W. MUSAPH; Neurogenesis of Mastication by Y. KAWAMURA; Action of the Muscles of Mastication by E. MOLLER; An Analysis of Mandibular Movement by C. J. GRIFFIN and R. MALOR; Physiology of Mandibular Positions by N. BRILL and G. TOYDE; Gnathosonics and Occlusion by H. S. BRENEMAN; Mastication in Food Science Technology by B. DRAKE; Bite Force and Chewing Efficiency by G. E. CARLSSON; Bruxism by P. SCHÄRER.

The book is an important reference work for dentists and for all those interested in the physiology of mastication.

K. LISSÁK

I. L. SHANNON, R. P. SUDDICK, F. J. DOWD jr.

Saliva: Compositon and Secretion

Monographs in Oral Science. Vol. 2. Editor: Howard M. MYERS. S. Karger, Basel, München, Paris, London, New York, Sydney 1974. VII + 103 pages, with 25 figures and 17 tables. Price: US $ 18.20; DM 47.—; £ 7.20

The monograph deals with the constituents of human parotid saliva and its changes associated with different levels of glandular activity. The role of salivary constituents is discussed and a new hypothesis is presented regarding the secretion of fluid and electrolyes by salivary glands. Finally, three salivary constituents particularly important in dentistry are discussed: calcium, phosphorus and fluoride.

The chapters of the monograph are: I. Human Parotid Fluid Constituents; II. Calcium and Phosphorus; III. Fluoride; IV. Mechanism of Secretion of Saliva; V. A Refenence Table for Human Parotid Saliva Collected at Varying Levels of Exogenous Stimulation; VI. Summary; VII. References.

The monograph offers interesting and important reading in the fields of dentistry, physiology and biochemistry.

K. LISSÁK

G. JUŽNIČ (Ed.)

Hemodynamic Stress and Relief of the Heart

Bibliotheca Cardiologica, No. 30. S. Karger, Basel, München, Paris, London, New York, Sydney 1973. XIV + 183 pages, with 65 figures and 13 tables. Price: US $ 26.05; DM 80.—

This volume contains the Proceedings of the 8th European Congress on Ballistocardiography and Cardiovascular Dynamics, held in Ljubljana, 5—8 April 1971. Part I: Lectures.

The opening plenary session was followed by a Symposium on Haemodynamic Stress and Relief of the Heart; the moderator was I. STARR. The Symposium strongly emphasized the close interrelationship between heart and blood vessels in cardiovascular function. In the Scientific session, short communications were presented and discussed from the fields of physics, physiology, pharmacology, clinical medicine and medical practice. Each session was introduced by an invited lecturer. On the fourth day of the Symposium, demonstrations and films were

presented. The next session was devoted to the celebration of the Tenth Anniversary of the Society. The text of the lectures presented at the Symposium, at the Tenth Anniversary Session, and the introductory lectures make up this first volume of the Proceedings.

K. LISSÁK

G. JUŽNIČ (Ed.)

Biomedical Science and Cardiovascular Dynamics

Bibliotheca Cardiologica, No. 31. S. Karger, Basel, München, Paris, London, New York, Sydney 1973. XIV + 314 pages, with 130 figures and 50 tables. Price: US $ 35.65; DM 109.—

This is the second volume of the Proceedings of the Lubljana Symposium; it contains the short communications and discussions of the five sessions on biomedical science and cardiovascular dynamics. The 41 papers cover the fields of physics, physiology, pharmacology, clinical medicine and medical practice. The book presents a representative review of the present activity of the members of the Society.

Both this volume and its first part will be welcome by physiologists, pathophysiologists and clinicians interested in the close interrelationships between heart and blood vessels in cardiovascular function.

K. LISSÁK

S. NÉMETH (Ed.)

Hormones, Metabolism and Stress

Recent Progress and Perspectives. Proceedings of an International Symposium, Smolenice, September 17—20, 1972. Publishing House of the Slovak Academy of Sciences, Bratislava 1973. 322 pages, with 45 figures and 23 tables. Price: US $ 6.00

The Proceedings of the Symposium covers five topics: 1. Central Nervous Influences; 2. Production, Secretion and Degradation of Hormones; 3. Peripheral Action of Hormones, Enzyme Induction; 4. Neurophysiological and Psychological Aspects; 5. Whole Body Energetics. The 25 papers are presented by 44 participants, most of them are internationally well-known investigators.

The volume presents an excellent survey of the current state of research in a field which is highly actual and intensively explored. The book will be interesting for endocrinologists, physiologists, biochemists, neurobiologists and for all medical workers interested in the syndrome of stress.

K. LISSÁK

I. H. PAGE, F. M. BUMPUS (Eds)

Angiotensin

Heffter-Heubner's Handbook of Experimental Pharmacology, New Series, Vol. 36. Springer-Verlag, Berlin, Heidelberg, New York 1974. XIX + 591 pages with 70 figures. Price: DM 224.—; US $ 86.30

This volume of the traditional Handbook of Experimental Pharmacology has been written by 52 highly competent investigators. The short history of angiotensin covers not more than fifty years, but its vast literature shows the great theoretical and clinical interest in the compound.

The 29 chapters of the volume contain: 1. The Biological Production of Angiotensin by L. T. SKEGGS, F. E. DORER, J. R. KAHN, K. E. LENTZ and M. LERINE; 2. The Fate of Angiotensin I by J. R. VANE; 3. Converting Enzyme: in vitro Measurement and Properties by Y. S. BAKHLE; 4. The Fate of Angiotensin II by J. W. RYAN; 5. Catabolism of Angiotensin II by J. G. LEDINGTON and W. P. LEARY; 6. Structure-Activity Relationship in Angiotensin II Ana-

logs by M. C. KHOSLA, R. R. SMEBY and F. M. BUMPUS; 7. Tachyphylaxis to Angiotensin by J M. STEWART; 8. Immunogenicity of Angiotensin I and II by M. B. VALLOTTON; 9. Measurement of Renin and of Angiotensin (Extraction and Bioassay) by R. BOUCHER and J. GENEST; 10. Angiotensin Immunoassay by G. W. BODY and W. S. PEART; 11. Bioassay of Angiotensin by P. A. KHAIRALLAH and R. R. SIMEBY; 12. Plasma and Serum Vasopressor Peptides Other than Angiotensins by H. R. CROXATTO; 13. Primary Aldosteronism: Importance of the Level of Plasma Renin as an Adjunct in Diagnosis by J. W. CONN and E. L. COHEN; 14. Secondary Hyperaldosteronism by E. G. BIGLIERI, J. R. STOCKIGT, M. SCHAMBELAN, and R. L. COLLINS; 15. Intermediary Metabolism of Aldosterone by J. C. MELLY; 16. The Renin–Angiotensin System in the Control of Aldosterone Secretion by J. O. DAVIS; 17. Aldosterone Regulation in Sodium Deficiency: Role of Ionic Factors and Angiotensin II by J. R. BLAIR-WEST, J. P. COGHLON, D. A. DENTON and B. A. SCOGGINS; 18. Effects of Aldosterone on Blood Pressure, Water and Electrolytes, by F. GRASS; 19. Adrenal Medulla by M. J. PEACH; 20. Central Neurogenic Effects of Angiotensin by C. J. DICKINSON and C. M. FERRARIO; 21. Peripheral Effects of Angiotensin on the Autonomic Nervous System by J. W. CUBBIN; 22. Effect of Angiotensin on Vascular Smooth Muscle by D. F. BOHR; 23. Circulatory Effects of Angiotensin by R. D. BUNAG; 24. Effects of Angiotensin on the Renal Circulation by L. G. NAVAR and H. G. LANGFORD; 25. Intrarenal Action of Angiotensin by K. THURAU; 26. Morphological Effects of Angiotensin on Arteries by P. KINCAID-SMITH, A. FRIEDMAN, and J. B. HOBBS; 27. Effects of Angiotensin II on the Permeability of the Vascular Wall by A. L. ROBERTSON and P. A. KHAIRALLAH; 28. Biochemical Effects of Angiotensin by T. L. GOODFRIEND, F. FYHROUIST and D. ALLMANN; 29. Some Possible Functions of Angiotensin by I. H. PAGE. Each chapter is completed with references. The volume ends with an author and subject index.

The volume is not intended for the causal reader but for serious students of the problem. It is a very important reference work and should find its place in every medical library.

K. LISSÁK

R. JUNG (Ed.)

Central Processing of Visual Information

A: Integrative Functions and Comparative Data

Handbook of Sensory Physiology, Volume VII/3. Springer Verlag, Berlin, Heidelberg, New York 1973. XI + 775 pages with 208 figures. Price: DM 248.—; US $ 101.70

This volume of the Handbook of Sensory Physiology covers the physiology of the visual system beyond the optic nerve.

The contents of the volume include the integrative functions and comparative data. Research in the field of microphysiology and ultramicroscopy have brought new insights into the neuronal basis of vision. According to the Editor's opinion "Unfortunately, in the present state of our knowledge it is not yet possible to integrate morphology and neurophysiology into a synthesis of structure and function which results in visual perception".

The 12 chapters of the volume were written by 17 highly competent investigators. Chapter 1. Visual Perception and Neurophysiology by R. JUNG; Chapter 2. Neurophysiological Mechanisms in the Visual Discrimination of Form by J. STONE and R. B. FREEMAN, jr.; Chapter 3. Central Mechanisms of Color Vision by R. L. DE VALOIS; Chapter 4. Neurophysiology of Binocular Single Vision and Stereopsis by P. O. BISHOP; Chapter 5. Visual Stability and Voluntary Eye Movements by D. M. MACKAY; Chapter 6. Neuronal Mechanisms of Visual Movement Perception and Some Psychophysical and Behavioral Correlations by O. J. GRÜSSER and U. GRÜSSER-CONEHLS; Chapter 7. Temporal Transfer Properties of the Afferent Visual System: Psychophysical, Neurophysiological and Theoretical Investigations by W. A. van DE GRIND, O. J. GRÜSSER and H. U. LUNKENHEIMER; Chapter 8. Maintained Discharge in the Visual System and its Role for Information Processing by W. R. LEVICK; Chapter 9. Neuronal Changes in the Visual System Following Visual Deprivation by K. L. CHOW; Chapter 10. Principles of the Mosaic Organisation in the Visual System's Neuropil of *Musca domestica* by L. B. V. BRAITENBERGER and N. J. STOCUSFELD; Chapter 11. Comparative Physiology of Colour Vision in Animals by H. AUTRUM and I. THOMAS; Chapter 12. The Evolution of Mammalian Visual Mechanisms by M. SNYDER. The volume is completed with an author and subject index.

This volume of the Handbook of Sensory Physiology, like the other volumes of the series, will be an indispensable reference work in the libraries of anatomy, physiology and neurobiology.

K. LISSÁK

R. F. SCHMIDT (Ed.)

Sinnesphysiologie. Programmiert

Mit Texten von H. ALTNER, J. DUDEL, O. J. GRÜSSER, R. KLINKE, R. F. SCHMIDT. Springer-Verlag, Berlin, Heidelberg, New York 1973. X + 254 pages with 110 figures.

This textbook of sensory physiology close connected with the book "Neurophysiology, programmed" published by Springer-Verlag in 1971 and reviewed in this Journal 42: 315, 1972 is most useful for students of physiology, psychology, biology, zoology and biochemistry who had not studied anatomy and biochemistry, but require knowledge of neurophysiology.

The 7 chapters of the book include 25 lectures: A. General Sensory Physiology by J. DUDEL; B. Somato-Visceral Sensibility by R. F. SCHMIDT; C. Physiology of Vision by O. J. Grüsser; D. Physiology of Hearing by R. Klinke; E. Physiology of the Vestibular System by R. KLINKE; F. Physiology of Taste by H. ALTNER; G. Physiology of Olfaction by H. ALTNER.

K. LISSÁK

E. GENAZZANI, H. HERKEN (Eds)

Central Nervous System: Studies on Metabolic Regulation and Function

Springer-Verlag, Berlin, Heidelberg, New York 1974. VIII + 249 pages, with 121 figures and 55 tables. Price: DM 79,—; US $ 30.50

The book is based on a Symposium, "Metabolic Regulation and Functional Activity in the Central Nervous System" which was held on September 16—17, 1972, at Saint Vincent (Aosta, Italy).

At the Symposium, 62 participants from different countries presented thirty papers in three main sections: I. Biochemical Analysis of Metabolic Pathways; II. Membrane Function and Neurotransmission; III. Hormonal and Pharmacological Studies. The heterogenous subjects of the papers offer a wide survey of recent important research concerning the metabolism and function of the central nervous system.

The book will be of interest for neurobiologists, biochemists, physiologist and clinical neurologists.

K. LISSÁK

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PIO ISTITUTO DI S. SPIRITO ED OSPEDALI RIUNITI - ROMA

ANNOUNCEMENT OF COMPFTITION

for the 1976 "Giovanni Maria Lancisi" International Prize for Medical Science (Lit. 10,000,000)

Art. 1

The "Pio Istituto di S. Spirito" General Regional Hospital Agency, registered in Rome, in collaboration with the Accademia Lancisiana di Roma, hereby announces a competition for the 1976 "G. M. Lancisi" International Prize for Medical Science, of Lit. 10,000,000, to be assigned to the author or authors of an original, unpublished scientific work dealing with the following subject, chosen by the special committee:

"CHRONOBIOLOGY AND MEDICINE"

Art. 2

Those intending to enter for the Prize should send the following to: "Premio Internazionale Scientifico "G. M. Lancisi" presso la Presidenza del Pio Istituto di S. Spirito ed Ospedali Riuniti, Borgo S. Spirito, 3 — 00193 Roma (Italy)" by and not later than 12.00 hours of the 31st December 1976:

1) Application on plain paper to participate in the competition;

2) Nine copies of the work entered for the Prize, in Italian or in English, each copy bearing the signature of the author or authors. All works, accompanied by positive copies of any illustrations, should include a synopsis in Italian or in English, of not more than one thousand words, as well as a full bibliography of the subject dealt with;

3) Certificates testifying to the birth, the residence and the citizenship of the competitor or competitors;

4) A copy of the curriculum vitae and of the scientific activity of the competitor or competitors, with a list of published works;

5) A declaration signed by the author or authors testifying to the original nature of the work, the institute or other place where the work has been carried out, that said work is unpublished and that it has not submitted and will not be submitted, until such time as the "G. M. Lancisi" Prize has been assigned, to other prize competitions.

Art. 3

The Prize is assigned according to the decision of a scientific committee appointed by the Chairman of the Accademia Lancisiana, and said decision is final.

Art. 4

The Prize may not be assigned more than once to the same person, when when said person participates jointly with others.

Art. 5

Further information and clarification may be requested by applying to: "Premio Internazionale Scientifico "G. M Lancisi" presso la Presidenza del Pio Istituto di S. Spirito—Borgo S. Spirito, 3 — 00193 Roma (Italy)".

RESULTS IN NEUROCHEMISTRY, NEUROENDOCRINOLOGY, NEOROPHYSIOLOGY AND BEHAVIOR, NEUROPHARMACOLOGY, NEUROPATHOLOGY, CYBERNETICS

(Recent Developments of Neurobiology in Hungary, V.)

Edited by *K. Lissák*

The fifth volume of this series contains seven review articles from various fields of neurobiology, reflecting the research work carried out at different departments of Hungarian universities and institutes. The main intention of the editors has been to give in this series a possibly full information about the investigations into neurobiological sciences in Hungary by asking outstanding representatives to contribute to these volumes, hoping that a closer acquaintance with the Hungarian trend and results would promote the friendly collaboration of Hungarian colleagues and scientists from abroad.

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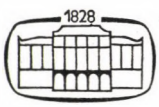

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ACTA PHYSIOLOGIA

ТОМ 45 – ВЫП. 1 – 2

РЕЗЮМЕ

ПУЛЬСОВОЕ СОПРОТИВЛЕНИЕ МОЗГА: МЕТОД ДЛЯ ИЗМЕРЕНИЯ ИЗМЕНЕНИЙ ЦЕРЕБРАЛЬНОГО КРОВОТОКА

З. СЕЛЕНЬИ и М. СЕКЕЙ

Описан метод измерения пульсового сопротивления мозга. Показано действие гиперкапнии и гипоксии на амплитуду пульсового сопротивления мозга.

ВЛИЯНИЕ МАССЫ ТЕЛА НА ТЕРМОРЕГУЛЯТОРНЫЕ ОТВЕТЫ НОВОРОЖДЕННЫХ И ВЗРОСЛЫХ МОРСКИХ СВИНОК ПРИ ИЗМЕНЕНИИ ОКРУЖАЮЩЕЙ ТЕМПЕРАТУРЫ, ПРИ ГИПОКСИИ И ГИПЕРКАПНИИ

М. ФАРКАШ и С. ДОНХОФФЕР

Анализировались связи между весом тела (BW) и изменениями в температуре ободочной кишки (ΔT_c) у морских свинок в возрасте меньше чем 2 дня, от 3 до 6 дней, от 7 до 9 дней и от 10 до 20 дней, а также у взрослых животных, в ответ на перенос их от термонейтрального окружения в умеренный холод (новорожденные: 20°C; взрослые: 20° и 10°C). Впоследствие, животные при этих окружающих температурах (T_a) вдыхали 8% O_2 и 6% CO_2. Не было никакой корреляции между BW и ΔT_c в ответ на перенос в холодное окружение во всех возрастных группах, за исключением группы в возрасте от 10 до 20 дней. В никакой группе не наблюдалось значительной корреляции между BW и увеличением теплопродукции (ΔO_2) в ответ на перенос их в холод. У новорожденных не было найдено связи между BW и ΔT_c при гипоксии, тогда как у взрослых животных, подвергнутых гипоксии при T_a 20°C, коэффициент корреляции был статистически достоверным, но указывал, что уменьшение в T_c стремилось к увеличению с повышением BW. При гиперкапнии статистически достоверная связь была наблюдена между BW и ΔT_c в группе в возрасте от 7 до 9 дней. Коэффициенты сходной величины наблюдались также и у взрослых животных при температурах 20°C и 10°C. Эти данные показали, что тенденция к понижению в T_c была больше у более маленьких животных. Было заключено, что при условиях, не приводящих к прогрессивной гипотермии, BW т. е. отношение массы к поверхности, в большинстве случаев, не является важным определителем изменений в T_c.

ГЕРМОРЕГУЛЯТОРНАЯ ТЕПЛОПРОДУКЦИЯ И РЕГУЛЯЦИЯ ТЕМПЕРАТУРЫ ТЕЛА У НОВОРОЖДЕННЫХ И ВЗРОСЛЫХ МОРСКИХ СВИНОК

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На новорожденных морских свинках возраста меньше 2 дней, в возрасте от 3 до 6 дней, от 7 до 9 дней и от 10 до 20 дней и на взрослых животных исследовали изменения кишечной температуры (T_c) и теплопродукция (VO_2) при изменениях внешней темпера-

туры (T_a). У новорожденных животных VO_2, выраженная в пересчете на вес тела (BW) с возрастом при T_a 35°, 30° и 20°C уменьшалась. Использовав вместо показателя BW показатель площади поверхности тела ($10 \cdot кг^{0{\cdot}67}$) в качестве системы отсчета обнаружили, что VO_2 была идентичной во всех 4-х группах новорожденных при T_a 35° и T_a 30°C в то время, как при T_a 20°C имела место сильно выраженная редукция величины VO_2 между группой новорожденных в возрасте менее чем 2 дня и группой в возрасте от 3 до 6 дней. За редукцией следовало постепенное, отчетливо связанное с возрастом повышение теплопродукции. T_c уменьшалась с возрастом значительно при всех 3-х T_a и внутри одной возрастной группы уменьшалась с уменьшением T_a. Уменьшение T_c в ответ на переход от T_a 35° к T_a 30° было одинаковым во всех возрастных группах, в то время как такое уменьшение в ответ на переход от T_a 30° к T_a 20° значительно меньше в самой младшей возрастной группе чем в любой другой из групп новорожденных и даже меньше, хотя и не значимо, чем у взрослых животных. Распределение величин изменений T_c в ответ на переход от T_a 30° к T_a 20°C варьировало в довольно широких пределах даже у взрослых морских свинок. Приняв за линию разделения изменений T_c не превышающую —0,5°C или —1,0°C и применив критерий χ^2 установили в высшей степени значимые отличия между группой в возрасте менее чем 48 часов и старшими по возрасту группами новорожденных. Не было найдено значительных различий между самой младшей группой новорожденных и взрослыми животными. В то же время различия между старшими группами новорожденных и взрослыми животными были значимыми.

Делается заключение, что описанная вариабильность изменений T_c при уменьшении T_a не была ни следствием »незрелости« или »неспособности« повысить теплопродукцию, ни результатом неблагоприятного отношения массы–поверхности тела, но отражает функциональную вариабильность кибернетической системы, контролирующей температуру тела и терморегуляторную теплопродукцию.

РЕНИНОПОДОБНАЯ АКТИВНОСТЬ ПЛАЗМЫ КРОВИ ОРГАНОВ ЧРЕВНОЙ ОБЛАСТИ У КРЫС

Ш. ШОНКОДИ и П. БОКАИ

На крысах-самцах было обнаружено, что активность ренина плазмы была выше в крови портальной вены, чем в крови аорты, как после ложной операции, так и после двусторонней нефрэктомии.

Кажется достоверным предположение о том, что ренинoподобный энзим вырабатывается у крыс в одном из органов чревной области.

ДЕЙСТВИЕ ХРОНИЧЕСКОЙ ФИЗИЧЕСКОЙ ПЕРЕГРУЗКИ НА МЕТАБОЛИЗМ СКЕЛЕТНЫХ МЫШЦ И АДРЕНОКОРТИКОВУЮ АКТИВНОСТЬ

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Было исследовано действие хронической физической перегрузки различной длительности на метаболизм воды и электролитов белых и красных скелетных мышц, Na^+K^+-АТФазную активность и содержание кортикостерона в плазме. Наиболее регулярно изменились поглощение натрия и выход калия как в красных так и в белых волокнах. Содержание воды в тканях и ее вне- и внутриклеточное распределение были исключительно постоянными. Вышеупомянутое нарушение содержания электролитов в ткани сопровождалось возрастанием Na^+K^+-АТФазной активности и падением содержания кортикостерона в плазме крови. Когда животным давали перегрузку вплоть до полного истощения, наблюдалось некоторое возрастание калия в скелетных мышцах и, одновременно, снижение содержания натрия. В красных мышцах изменение содержания ионов коррелировало с возрастанием Na^+K^+-АТФазной активности. Дополнительное напряжение также увеличивало адренокорковую активность. Таким образом, изменения метаболизма электролитов в красных скелетных мышцах были обратамыми. На основании исследования высказывается предположение, что механизм активного транспорта ионов в красных скелетных мышцах имеет большие возможности к адаптации и не может быть полностью нарушен короткой физической перегрузкой. Обсуждается возможное значение коры надпочечников в регуляции этого механизма.

ФУНКЦИОНАЛЬНАЯ ВЗАИМОСВЯЗЬ МЕЖДУ ВЕРХНИМ ШЕЙНЫМ УЗЛОМ, ШИШКОВИДНОЙ ЖЕЛЕЗОЙ И ГИПОТАЛАМО-ГИПОФИЗАРНО-НАДПОЧЕЧНИКОВОЙ СИСТЕМОЙ

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У нормальных животных уровни серотонина в гипоталамусе и кортикостерона в плазме показали противоположный дневной ритм. Утром уровень сывороточного кортикостерона был высоким, а уровень гипоталамического серотонина — низким. В начале темного периода, высокий уровень сывороточного кортикостерона сопровождался низким содержанием серотонина в гипоталамусе.

Удаление шишковидной железы не оказывало влияния ни на дневной ритм кортикостерона и серотонина, ни на надпочечниковую активацию, вызванную стрессом.

Удаление верхнего шейного узла уменьшало дневной ритм изменения уровня серотонина в шишковидной железе, но не оказывало влияния на ритм изменения гипоталамического серотонина и сывороточного кортикостерона. Стрессовый ответ, вызванный эфиром или электрическим шоком, увеличивался. Он был повышенным и через 58 дней после удаления узла.

Результаты указали, что шишковидная железа не оказывает прямого влияния ни на содержание серотонина в гипоталамусе, ни на гипоталамо-гипофизарно-надпочечниковую систему. Отсутствие верхнего шейного узла стимулировало стрессовый ответ гипоталамо-гипофизарно-надпочечниковой системы, без влияния на содержание серотонина в гипоталамусе.

КОНЦЕНТРАЦИЯ И ВЫХОД IN VITRO ПЛАЦЕНТАРНОГО ЧЕЛОВЕЧЕСКОГО ХОРИАЛЬНОГО СОМАТОМАММОТРОПИНА (HCS) У ПЛОДОВ С НОРМАЛЬНЫМ ВЕСОМ И С ВНУТРИУТРОБНЫМ УМЕНЬШЕНИЕМ ВЕСА

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В течение родов 100 плодов нормального веса, рожденных в срок, и 100 недоразвитых плодов, также рожденных в срок, определяли концентрацию в плаценте человеческого хориального соматомаммотропина (HCS). Кроме того, на протяжении последующих 8 часов инкубации плаценты наблюдали выход HCS. Плаценты недоразвитых плодов содержали приблизительно в 2 раза больше HCS на 1 г ткани (1399 мкг), чем плаценты плодов нормального веса (684 мкг). На протяжении 8 часов инкубации выход гормона из нормальной плаценты составил 81,2%, в то время, как из плаценты недоразвитых плодов только 24,6% от первоначального уровня содержания гормона. Выход HCS с 1 г нормальной плацентарной ткани в абсолютных значениях был больше (525 мкг), чем из плаценты недоношенных плодов (308 мкг).

Предполагается, что сопровождающий хроническую плацентарную недостаточность низкий уровень HCS плазмы кори матери может быть результатом торможения выхода HCS из плаценты в кровь матери.

ДЕЙСТВИЕ ГОНАДОТРОПИНА НА ЭЛЕКТРИЧЕСКУЮ АКТИВНОСТЬ МОЗГА КРЫС

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Инъекция человеческого хориального гонадотропина в ядра медиальной линии таламуса или в боковой желудочек мозга повышала порог вызванных потенциалов, удлинила скрытый период между двумя последовательными вызванными потенциалами и снижала порог веретенообразных разрядов последействия, которые вызывались электрической стимуляцией ретикулярной формации.

Интравентрикулярное введение лютеинизирующего гормона вызывало спонтанные вспышки веретенообразной активности, снижало порог разряда последействия, вызванного стимуляцией и удлиняло скрытый период между двумя последовательными вызванными потенциалами.

Фоликулостимулирующий гормон, пролактин или инактивированный человеческий хориальный гонадотропин, а также лютеинизирующий гормон не имели такого эффекта.

КРОВОТОК И ВЫХОД СВОБОДНЫХ ЖИРНЫХ КИСЛОТ В ПОДКОЖНОЙ ЖИРОВОЙ ТКАНИ, САЛЬНИКЕ И БРЫЖЕЙКЕ У СОБАК В СОСТОЯНИИ ГЕМОРРАГИЧЕСКОГО ШОКА

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У собак под хлоралозовым наркозом измерялся кровоток, выход свободных жирных кислот (FFA) и глицерола в подкожной жировой ткани, в жировой ткани брыжейки и сальника на протяжении стандартизированной кровопотери, приводившей к шоку. Установлено, что кровоток в покое составлял 6,3 $\pm$ 1,4 мл/мин/100 г ($\pm$ SEM) в подкожной, 14,8 $\pm$ 3,3 мл/мин/100 г в брыжейке и 5,3 $\pm$ 1,2 мл/мин/100 г в сальнике. Выраженное уменьшение кровотока наблюдалось при снижении артериального давления до 55 мм рт. ст. в течение 90 мин кровопускания. Он оставался низким также при снижении кровяного давления до 35 мм рт. ст. при дополнительном кровопускании в течение еще 90 мин. Кровоток в ткани брыжейки был выше, чем в других жировых тканях как в контроле, так и при кровопускании. При кровопускании не наблюдалось возрастания выхода FFA из жировой ткани, но было отмечено значительное возрастание выхода глицерола из брыжейки. Артериальная концентрация FFA не изменилась, но имел место значительный подъем концентрации глицерола с 0,21 $\pm$ 0,04 мМ до 0,95 $\pm$ 0,22 мМ ($p < 0,05$). pH артериальной крови уменьшался с 7,28 $\pm$ 0,03 до 7,06 $\pm$ 0,04 и уровень лактата возрос с 3,18 $\pm$ 0,38 до 10,66 $\pm$ 61 мМ.

Делается заключение, что следующий за кровопусканием низкий кровоток в жировой ткани может нарушать выход FFA и глицерола. Значительное возрастание выхода глицерола из жировой ткани брыжейки и отсутствие такой реакции в подкожной жировой ткани и сальнике могут объясняться региональными отличиями снижения кровотока. Повышение реэстерификации FFA на протяжении кровопускания может быть следствием высокой концентрации лактата.

Вопреки возможной высокой симпатической нейрогуморальной активности, низкое значение pH крови была причиной снижения липолиза в жировой ткани. Повышение уровня реэстерификации и торможение липолиза равно как и уменьшение кровотока в жировой ткани противодействует выходу FFA.

ЭФФЕКТ ГИПЕРКАЛЬЦИЕМИИ НА ДЕЯТЕЛЬНОСТЬ ПОЧЕК

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Исследовался эффект острой гиперкальциемии, вызванной внутривенным введением хлористого кальция, на деятельность почек у наркотизированных собак.

1. Когда концентрация кальция в плазме была повышена приблизительно в два раза при неизмененном кровяном давлении, исследованные параметры деятельности почек (C_{PAH}, C_{inulin}, выделение мочи, натрия и калия) значительно уменьшались. Выделение кальция почками не изменялось.

3. По мнению авторов, интенсивная вазоконстрикция, возникающая в сосудах почек, возможно в афферентных артериолах, вызывала уменьшение деятельности почек.

ЭФФЕКТ ГИПЕРКАЛЬЦИЕМИИ НА ТРАНСПОРТ КАЛЬЦИЯ И ФОСФАТА В КАНАЛЬЦАХ

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С помощью микропипетки анестезированным крысам вводили в различные сегменты канальца почки (в начальный, срединный и концевой сегменты проксимального канальца и дистальный каналец) определенные количества $^{45}Ca^{2+}$ а также ^{3}H-метоксиинулина на протяжении маннитового диуреза и при поддерживаемой гиперкальцьмии. На основании их количеств, находящихся в мочи, сделаны следующие заключения о судьбе изотопов, инъецированных в различные сегменты:

(1) Ионы Ca^{2+} реабсорбируются по целому нефрону.

(2) Ионы PO_4^{3-} реабсорбируются только в дистальном канальце и нереабсорбировались в доступной части проксимального канальца.

(3) Вызванная инфузией $CaCl_2$ гиперкальциемия вызывала уменшение реабсорбции по целому канальцу.

(4) Гиперкальциемия вызывала сильную реабсорбцию фосфата в проксимальном отделе канальца и в петле Генле.

(5) Увеличение уровня кальция в сыворотке крови не изменяло реабсорбцию фосфата в дистальном отделе канальца.

Предполагается, что регуляция реабсорбции кальция вдоль всего нефрона осуществляется одним и тем же механизмом. Полученные результаты позволяют предполагать, что реабсорбция фосфата в проксимальном канальце и в петле Генле является функцией внутриклеточной концентрации кальция, которая изменяется параллельно с изменением уровня кальция в сыворотке крови.

ЭФФЕКТЫ АЦИДОЗА И ИНФУЗИИ НОРАДРЕНАЛИНА НА ПРИЕМ ^{14}C- НОРАДРЕНАЛИНА МИОКАРДОМ КРЫС

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Ранние эксперименты обнаружили, что концентрация норадреналина в миокарде повышалась при ацидозе. Настоящие данные указывают, что прием меченого норадреналина меньше при ацидозе. Повышение концентрации норадреналина с одной стороны, и понижение его приема, с другой стороны, привели к предположению, что гипернорадреналинемия, появляющаяся при ацидозе, играет важную роль в этих противоположных наблюдениях. Было найдено, что инфузия немеченого норадреналина (1,5 мкг/мин/20 мин) значительно подавляла прием ^{14}C-норадреналина тканями желудочков и предсердий. Пониженный прием норадреналина в течение ацидоза можно объяснить тем, что связывающие места были заняты эндогенным норадреналином, освобожденным из экстракардиальных источников.

ДЕЙСТВИЕ ХЛОРОКВИНА ФОСФАТА НА ИЗОЛИРОВАННУЮ НЕБЕРЕМЕННУЮ И БЕРЕМЕННУЮ МАТКУ РАЗЛИЧНЫХ ВИДОВ ЖИВОТНЫХ

А. АБДЕЛ-АЗИЗ и Н. БАКРИ

Обнаружено межвидовое различие в действии хлороквина на изолированные матки беременных и небеременных крыс, кроликов и морских свинок. Этот препарат оказывал на матку крысы или тормозящее или возбуждающее действие в зависимости от того, была матка беременной или нет, а также в зависимости от типа использованной жидкости. Хлороквин оказывал стимулирующее действие на беременную матку крыс и морских свинок в растворах Де Жалон или Рингер-Лока. Стимулирующее действие хлороквина было зависимым от Ca^{++}, антагонистический эффект оказывали тетракаин и 2,4-динитрофенол; атропин, лизергид и феноксибензамин не оказывали заметного влияния.

АНАЛИЗ ОКСОТРЕМОРИНОВОЙ ГИПЕРГЛИКЕМИИ У КРЫС

Д. В. ФАН, Л. ДЬЁРДЬ и Й. МАРТОН

Систематическое и интрацеребровентрикулярное применение оксотреморина вызывает гипергликемию у бодрствующих крыс. Метилатропин тормозит действие интраперитонеально введенного оксотреморина, но этот эффект исчезает после предварительной интрацеребровентрикулярной инъекции холиномиметиков. Гипергликемия, вызываемая интраперитонеальным введением оксотреморина тормозилась после адреналэктомии и уменьшалась после гипофизэктомии. Предварительное введение животным аминоглютетимида, лекарства, тормозящего синтез стероидов, снижало гипергликемическое действие оксотреморина у тех животных, которые имели очень низкий уровень кортикостерона в крови.

Гипергликемия является периферической реакцией по своему происхождению, но имеется и центральное симпатическое звено, которое участвует в ее развитии. Для проявления гипергликемии также необходимо участие гипофизарно–адренокортиковой системы.

ДЕЙСТВИЕ ОКСОТРЕМОРИНА НА ПОВЕДЕНИЕ И ЭЭГ У КРЫС, КОТОРЫМ ПРЕДВАРИТЕЛЬНО ВВОДИЛИ РЕЗЕРПИН

Д. В. ФАН, А. БИТЕ и Л. ДЬЁРДЬ

Высокая доза оксотреморина иногда вызывает судорожные появления у крыс. После предварительной дачи резерпина и метилатропина, однако, развивались хронические припадки; в то же самое время могли наблюдаться изменения ЭЭГ, состоящие в величинах активность 6—7 ц/сек, длительностью 1—3 сек, которые появлялись с 15—20 сек интервалами. Подобные паттерны наблюдали у крыс, которым предварительно вводили резерпин, после применения физостигмина.

ЭЭГ активность, вызванная оксотреморином, тормозится атропином и пиримидоном; мекамиламин не изменяет ее.

ДВА ТИПА ИЗМЕНЕНИЙ ГИПОТАЛАМИЧЕСКОЙ САМОСТИМУЛЯЦИИ У КОШЕК ВЫЗВАННЫХ ВВЕДЕНИЕМ НОРАДРЕНАЛИНА

Л. АНДЬЯН, А. ДАРБИНЯН и Л. ШОЙМОШИ

На четырех кошках было установлено, что внутримышечная инъекция адреналина может различным образом изменять протекание реакции самостимуляции в зависимости от дозы. Малая доза норадреналина сильно облегчает реакцию нажатия на педаль, в то время, как большая доза подавляла эту реакцию. Изменения артериального давления крови являются отличным показателем влияния норадреналина на реакцию самостимуляции.

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Physiologia

DISC-ELECTROPHORETIC STUDY OF SERUM PROTEINS IN MATERNAL AND CORD BLOOD AND AMNIOTIC FLUID

By

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The serum proteins of 25 normal women were studied by disc-electrophoresis at the time of delivery and eight weeks post partum. Proteins of the amniotic fluid and of the umbilical sera (both arterious and venous) were also analyzed. The quantitative as well as qualitative changes found in the maternal sera were not present in any of the cord blood sera or in the amniotic fluid or in the sera of the same women eight weeks post partum. The quantitative changes thought to be characteristic of pregnancy consisted of an increase in the fast and slow postalbumins and transferrin. Two protein fractions (pregnancy protein$_1$ and pregnancy protein$_2$) in the alpha-2-globulin region not present in any of the other specimens were also identified in the maternal sera in about half of the cases.

The proteins of maternal and umbilical cord sera and of the amniotic fluid have intensely been studied in recent years by various methods (SMITHIES 1959; BREZINSKI et al. 1961; AFONSO and DE ALVAREZ 1964; ROBINSON et al. 1966; BAYER 1966; FISCHBACHER and QUINLIVAN 1970).

It was found that the changes in maternal serum are characterized by a quantitative increase of some alpha-1-globulins and by the appearance of two alpha-2-globulins not present in non-pregnant sera, whereas total serum protein concentration was decreased. The protein composition of the sera of the umbilical artery and vein and of the amniotic fluid differed from that of the mother quantitatively as well as qualitatively.

Evaluating the results obtained in the course of these investigations, we have concluded that the pregnancy-specific maternal protein changes could be interpreted more accurately if analysis of the maternal serum was combined with that of the cord sera and of the amniotic fluid and if examination of the maternal serum was repeated two months after delivery.

Methods

Blood was taken from the cubital vein of 25 healthy women at the end of the first stage of normal labour, and from the umbilical vein and one of the umbilical vein and one of the umbilical arteries immediately after birth. At the beginning of labour, amniotic fluid was also obtained by vaginal amniocentesis in each case. The samples were centrifuged and the amniotic fluid samples were filtered. All of the specimens were kept in a refrigerator at 5 °C for 36 hours. Total protein concentration was determined by refractometry in the serum while by the biuret method in the amniotic fluid.

From each sample an amount containing 250 μg of protein was subjected to polyacrylamide gel electrophoresis as described by DAVIS (1964). Each assay was carried out in duplicate, adding a few drops of a haemoglobin stock solution to one of each pair before the run. Electrophoresis was carried out at 5 mA for 75 minutes using a tris-glycine buffer pH 8.6. The untreated sample was stained with amido-black, while the sample mixed with haemoglobin was stained with benzidine for the identification of haptoglobins. The protein fractions were determined immunologically with monospecific antisera (transferrin, coeruloplasmin-antiserum, etc.). The pherograms were evaluated by means of an ERI 65 type densitometer.

The same procedure was used with the sera obtained from the same women eight weeks post partum. As a control, the sera of 25 healthy, non-pregnant women were also analyzed; in these cases the blood was drawn at the early proliferative phase.

Results

Mean total protein concentration in the maternal sera at term was 6.20 $\pm$ 0.40 g per 100 ml; in the umbilical artery, 5.37 $\pm$ 0.38 g and in the umbilical vein, 5.40 $\pm$ 0.45 g per 100 ml. Eight weeks post partum it increased to 6.76 $\pm$ 0.25 g per 100 ml, while in the non-pregnant controls it was 7.56 $\pm$ $\pm$ 0.30 g. Mean protein concentrations in the amniotic fluid at term 0.25 $\pm$ $\pm$ 0.09 g (Table I).

Table I

Protein concentrations in the samples studied

	No. of patients	Mean concentration of total protein, g per 100 ml $\pm$ S.D.
Non-pregnant controls	25	7.56$\pm$0.30
Maternal serum at birth	25	6.20$\pm$0.40
Maternal serum 8 weeks post partum	25	6.76$\pm$0.25
Amniotic fluid at term	25	0.25$\pm$0.09
Arterial cord serum	25	5.37$\pm$0,38
Venous cord serum	25	5.40$\pm$0.45

In the maternal sera at the time of delivery an increase in the fast (CBG, TBG) and slow postalbumins and transferrin was observed as compared with the non-pregnant sera or those obtained eight weeks post partum. The fast postalbumin and transferrin fractions were significantly higher in the

sera of the parturients than two months later ($p < 0.05$). The difference in the amounts of postalbumin was not significant ($p < 0.1$).

In the umbilical sera, haptoglobin was absent in every case and in the alpha-2 region fewer fractions were seen that in the same region of the control or maternal sera. The quantitative changes seen in the maternal sera were absent from the umbilical sera. The only difference between the arterial and venous cord sera was an increase in IgG in the venous sample.

The protein composition of the amniotic fluid was similar as that of the cord sera, but in some cases haptoglobin type 1-1 was also found.

In addition to these quantitative changes very distinct qualitative ones were also detected in the maternal sera. At term there were two well-separated pregnancy protein fractions between transferrin and S-alpha-2-macroglobulin. One of them found in 8 cases was near to transferrin, while the other, identified in 12 cases was localized near to S-alpha-2-macroglobulin. These fractions disappeared by eight weeks post partum and were never present in the cord and control sera or in the amniotic fluid (Fig. 1).

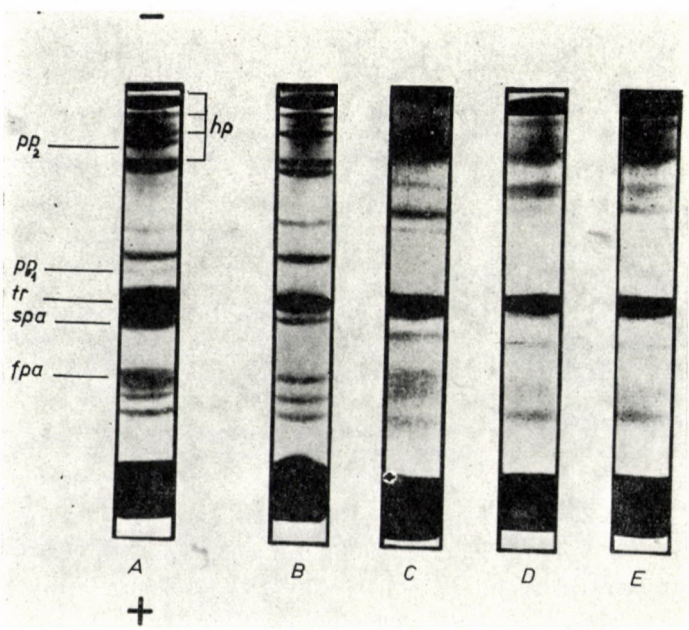

Fig. 1. A: Disc-electrophoretogram of the serum of a healthy woman. The blood was taken at the end of the first stage of labour. Note the increase of the fast and slow postalbumins and of transferrin with the appearance of two globulin (pp_1 and pp_2) fractions indicated by arrows. B: Serum of the same women eight weeks post partum. C: Pherogram of the amniotic fluid. D: Pherogram of blood serum of one of the umbilical arteries. E: Pherogram of the blood-serum of the umbilical vein. Abbreviations: fpa: fast postalbumins; spa: slow post-albumins; tr: transferrin; hp: haptoglobulins; pp_1: pregnancy protein$_1$; pp_2: pregnancy protein$_2$

Discussion

A quantitative increase in some serum protein fractions in pregnant women has already been observed (SLAUNWHITE and SANDBERG 1959; DOWLING et al. 1965; MAN and WHITEHEAD 1968; THAN et al. 1970). The increase in the fast postalbumins (CBG, TBG) was explained by the increased oestrogen level and the modified thyroxine metabolism, while the increase in the slow postalbumins was attributed to the elevated coeruloplasmin level, and for the increase in transferrin, the enhanced iron transport was held responsible.

The lower protein concentration in the cord sera, the absence of haptoglobins and of the other changes in maternal sera suggests that foetal protein production is independent of that of the mother, though the selective transplacental passage of some maternal protein fractions (albumin, IgG) has been definitively proven (DANCIS et al. 1961; HAWORTH et al. 1965). The maternal origin of amniotic fluid proteins seems very probable (MCKAY et al. 1958; ABBAS and TOVEY 1960; USATEGUI-GOMEZ and MORGAN 1966a, b, 1967). The amount and nature of maternal proteins crossing the foetal membranes depend on the selective activity of these barriers. The role of the placenta (WIRTSCHAFTER and WILLIAMS 1957) and of the foetus (BREZINSKI et al. 1961) in the synthesis of some of the proteins of the amniotic fluid cannot, however, be excluded. In our investigations the protein composition of the amniotic fluid resembled that of the cord sera, but the presence of 1-1 type haptoglobin in some cases has to be considered a direct proof of the membranes being selectively penetrable for some maternal proteins.

SMITHIES (1959), AFONSO and DEALVAREZ (1964), DAVIS (1964) and others described the existence of a pregnancy-associated alpha-2-globulin. In addition to this HIRSCHFELD and SÖDERBERG (1960), WILKEN (1963), BAYER (1966), BUNDSCHUH (1967) and HOFMANN et al. (1970, 1972) demonstrated a pregnancy specific beta-1-globulin.

In our material the alpha-2-beta-1-globulin near to transferrin in the maternal serum seems to be identical with the pregnancy protein demonstrated by disk-electrophoresis by DAVIS (1964) (pregnancy protein$_1$). The other fraction, near to S-alfa-2-macroglobulin in our series, was observed also by SMITHIES (1959) and AFONSO and DEALVAREZ (1964) in starch-gel and by HOFMANN et al. (1972) in disk-electrophoresis (pregnancy protein$_2$). The role of these fractions is not clear. They are not identical with any of the known protein hormones and no steroid is bound to any of them (WILKEN 1963; BAYER 1966). They differ from oxytocinase and phosphatase enzymes (AFONSO and DEALVAREZ 1964; ROBINSON et al. 1966) and are present in about one third or half of pregnant women. They were found in cases of trophoblastic tumour and after long term oestrogen treatment in non-pregnant women as

well (AFONSO and DEALVAREZ 1964; ROBINSON et al. 1966; BUNDSCHUH 1967; KADACH 1970).

Our observations indicate that these fractions are not crossing the placental or amniotic membranes in detectable amounts and disappear eight weeks post partum. This means that they are fairly specific of pregnancy. It cannot, however, be excluded that these fractions are regular components of the normal serum and only their concentration is below the sensitivity of the usual methods; during pregnancy their level may increase and thus they may become detectable.

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DER EINFLUSS VON PROSTAGLANDIN E_1 AUF DIE ZUSAMMENSETZUNG DER FREIEN FETTSÄUREN IM BLUTPLASMA BEIM KANINCHEN

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Die Untersuchungen der Wirkung des exogenen Prostaglandins E_1 auf das Spektrum der freien Fettsäuren im Blutplasma von Kaninchen wurde untersucht. Die ungesättigten Fettsäuren wiesen eine deutliche Erhöhung auf, insbesondere die Linolsäure und weniger die Öl- und Arachidonsäure. Der Anteil der gesättigten Fettsäuren verminderte sich mit Ausnahme der Stearinsäure. Die gewonnenen Ergebnisse wurden mit den metabolischen Funktionen des PGE_1 erklärt und mit anderen Beobachtungen verglichen.

Die Mannigfaltigkeit der physiologischen Effekte der Prostaglandine (PG), die nach einer Vielzahl experimenteller Beobachtungen nachgewiesen werden konnten, wobei widersprüchliche Wirkungen nicht selten auftreten, erfordert sowohl zielgerichtete Untersuchungen als auch Gegenüberstellungen und Synthese der gewonnenen analytischen Daten.

Die PG der Gruppe E üben verschiedene, oft sehr starke Einflüsse auf die metabolischen Prozesse der Gewebe und Organe aus. Diese Wirkungen auf die Nahrungs- und Wasseraufnahme des adipösen Gewebes (BAILE et al. 1973) und auf die lipolytischen und glykämischen Prozesse stehen mit der physiologischen Aufgabe der PG als Mediatoren eines Feedback-Mechanismus zur Freisetzung der adrenergischen Transmitter in Verbindung (HEDQUIST 1972; SAMUELSSON und WENNMALM 1971). Die renale Freisetzung von PG bei Nervenstimulation (DURHAM und ZIMMERMANN 1970) sowie die Erhöhung des Noradrenalinsiegels bei Inhibition der PG-Synthese deuten an, daß diese Mechanismen den funktionellen Zustand der biologischen Systeme kontrollieren.

Während die Biokonversion der polyenen Fettsäuren in PG sowie die günstige Beeinflussung ihrer Biosynthese durch die Infusion essentieller Fettsäuren oder die Stimulierung der Phospholipase A nachgewiesen werden konnten (BERGSTRÖM et al. 1964; VAN DORP et al. 1964), fand man, daß eine Infusion von PG in kleiner Dosis die Folgen eines Defizits an essentiellen Fettsäuren nicht kompensiert (KUPIECKY et al. 1968). Wir stellten uns daher die Aufgabe, den Effekt des applizierten PGE_1 auf die Zusammensetzung der freien Fettsäuren (FFS) im Blutplasma im Tierexperiment zu untersuchen.

Methode

Als Versuchsmaterial dienten 25 einjährige mischrassige Kaninchen beiderlei Geschlechts mit einem Durchschnittsgewicht von 4 kg, die unter Standardnahrung in Einzelkäfigen gehalten wurden. Bei 15 Tieren wurde PGE_1 (Upjohn Co., Kalamazoo, Michigan) in die V. marginalis eines Ohres injiziert (Versuchsgruppe), und zwar am 1. Tag 200 μg, am 2.—4. Tag 100 μg. In der viertägigen Versuchsperiode nahmen die Tiere weniger Nahrung auf; ihr Gewicht verringerte sich um ca. 200 g. 10 Kaninchen dienten als Kontrollgruppe; sie erhielten Injektionen von physiologischen Kochsalzlösung, Bei diesen Tieren ergaben sich keine Nahrungs- und Gewichtsabweichungen.

Nach 12stündiger Nahrungskarenz wurde den Tieren mittels Herzpunktion bei konstanten Versuchsbedingungen Blut entnommen. Die Extraktion der Lipide aus dem Plasma erfolgte mit dem Chloroform–Methanol–Verfahren, die Fraktionierung der Lipide durch Dünnschichtchromatographie mit Kieselgel G/Merck (MANGOLD 1962). Für die Gaschromatographie wurden die FFS mit Methanol/Salzsäure verestert. Die Zusammensetzung der FFS bestimmten wir gaschromatographisch (Gasofragt TP 500) mit Flammenionisationsdetektor unter folgenden Bedingungen: Säulenfüllung 10% EGSS-X (Applied Science Lab. Inc.) auf Gaschrom P, 80–100 mesh; Säulenlänge 200 cm $\times$ 4 mm; Trägergas: Stickstoff 2,5 l/Std; Temperatur: Säule 170 °C, Injektionspunkt 240 °C, Detektor 220 °C; Papiervorschub 300 bzw. 600 mm/Std; Empfindlichkeit $5 \cdot 10^{-9} - 5 \cdot 10^{-10}$ A. Aus dem mit einem Integrator ausgewerteten Peakflächen wurden die Anteile der einzelnen Fettsäuren errechnet. Zur statistischen Auswertung der Resultate wurden Korrelationsprüfung und t-Test herangezogen.

Tabelle I

Mittelwerte der FFS mit Standardabweichungen bei mit physiologischer Kochsalzlösung behandelter Kontrollgruppe und mit Prostaglandin E_1 behandelter Versuchsgruppe

C-Zahl	Kontrollgruppe		Versuchsgruppe		
	Gesättigte Fettsäuren in %				
C_{12}	0,5	$\pm$0,2	0,4	$\pm$0,2	
C_{14}	1,5	$\pm$0,3	0,8	$\pm$0,3	
C_{16}	42,8	$\pm$3,9	22,2	$\pm$2,4	($p < 0,05$)
C_{18}	4,4	$\pm$0,4	9,6	$\pm$1,3	($p < 0,05$)
C_{20}	2,6	$\pm$0,5	0,8	$\pm$0,3	
	$\Sigma = 51,8$	$\pm$4,5	33,8	$\pm$3,5	($p < 0,05$)
	Ungesättigte Fettsäuren in %				
$C_{14:1}$	0,2		0,3		
$C_{16:1}$	5,4	$\pm$0,4	1,3	$\pm$0,2	($p < 0,05$)
$C_{18:1}$	24,8	$\pm$2,2	30,4	$\pm$2,8	($p < 0,05$)
$C_{18:2}$	7,5	$\pm$0,8	23,7	$\pm$2,2	($p < 0,01$)
$C_{18:3}$	5,0	$\pm$0,4	1,5	$\pm$0,2	($p < 0,05$)
$C_{20:4}$	5,3	$\pm$0,4	9,0	$\pm$0,8	($p < 0,05$)
	$\Sigma = 48,2$	$\pm$4,5	66,2	$\pm$6,0	($p < 0,05$)

Ergebnisse

Das PGE_1 rief bei den Kaninchen deutliche Veränderungen in der Zusammensetzung der FFS im Plasma im Vergleich zu den Kontrolltieren hervor. Tabelle I demonstriert die Ergebnisse der einzelnen FFS bei beiden Untersuchungsgruppen. Die Veränderungen in der Versuchsgruppe mit PGE_1 sind folgenderweise charakterisiert: 1. Die Gesamtmenge der ungesättigten FFS ist erhöht. 2. Der Anteil der gesättigten FFS vermindert sich entsprechend mit Ausnahme der Stearinsäure (C_{18}), die sogar ansteigt. 3. Die ausgeprägteste Erhöhung zeigt die Linolsäure ($C_{18:2}$). 4. Die Arachidonsäure ($C_{20:4}$) ist deutlich angereichert. 5. Die Ölsäure ($C_{18:1}$) zeigt ebenfalls erhöhte Werte. 6. Palmitolein-($C_{16:1}$) und Linolensäure ($C_{18:3}$) vermindern sich relativ.

Diskussion

Die i.v. Applikation von PGE_1 erhöht die PG-Konzentration im Blutplasma, und seine lokale Wirkung im Gewebe besitzt eine teilweise Analogie zu der erhöhten PG-Konzentration im Blut nach vermehrter Produktion in der Niere bei Nervenstimulation. Die stimulierten physiologischen Funktionen gehen, wie bekannt, mit der Freisetzung energiereicher Verbindungen einher. Der Anstieg der Menge der ungesättigten Fettsäuren steht mit dem metabolischen Effekt einer erhöhten PG-Konzentration im Blutkreislauf in Verbindung. Zur Erläuterung unserer Ergebnisse können einige physiologische Mechanismen herangezogen werden. PG inhibiert die Aktivität des sympathischen Nervensystems und kann einige physiologische Systeme beeinflussen (Horton 1973). Das injizierte PG interferiert durch einen Feedback-Mechanismus mit den Effekten der Katecholamine inklusive ihrer Wirkung auf die Lipolyse und Utilisation der FFS. Eine Erhöhung der Menge der FFS, besonders der ungesättigten FFS, kann als Folge der Verwirklichung der Kontrollfunktion über metabolische Prozesse entstehen. Es ist bekannt, daß das PG die ketogene Wirkung des Noradrenalins abschwächt und die Glykolyse beschleunigt (Lemberg et al. 1971). In Prozessen der Lipolyse bilden sich endogene PG, die den Gehalt des zyklischen AMP senken und auf diesem Wege die Lipolyse beeinflussen (Iliano und Cuatrecasas 1971; Micheli 1970). Durch Verabreichung von PGE_1, die die Konzentration von PG im Blut ansteigen läßt, können die physiologischen PG-Bedürfnisse gedeckt werden, wodurch ein einsparender Effekt in der endogenen Synthese entsteht, der die Vermehrung der zirkulierenden polyenen Fettsäuren ermöglicht. Eine Inhibition der PG-Synthese erfolgt durch Öl-, Linolen- und besonders durch Linolsäure, da sie um die PG-Synthese mit der Arachidonsäure konkurrieren (Pace-Asciak und Wolfe 1968). Die vielseitige Wirkung des PGE_1 auf die Lipolyse sowie die Tatsache der Stimulierung der Adenylzyklase durch PG (Kimberg et al. 1971;

PEERY et al. 1971) zeigen, daß das PG die Mobilisierung der Fette aus den Depots nicht immer verhindern kann. Dabei kann sogar eine erhöhte FFS-Konzentration im Plasma beobachtet werden (CARLSON et al. 1970). Die Stimulierung des Kohlenhydrat-Metabolismus nach PG-Verabreichung mit verdoppelten glykämischen Werten (LEMBERG et al. 1971; ONAGA und SOLOMON 1970) führt zur Modifizierung der Fettsäureaufnahme in den Zellen. Die festgestellte Vermehrung der Steroidhormone im Plasma durch PG kann auch mit der lipolytischen Wirkung im Zusammenhang stehen (TAI et al. 1970). Die Erhöhung der ungesättigten Fettsäuren, insbesondere der Linolsäure, erweist sich wahrscheinlich als ungenügend, die Folgen ihres chronischen Defizits zu beseitigen im Hinblick auf die beschränkte Anwendung hoher Dosen für längere Zeit und auf die durch PG gehemmte Zufuhr von polyenen Fettsäuren. Außerdem werden die polyenen Fettsäuren vielseitig kontrolliert und für verschiedene Körperfunktionen benötigt. Die ermittelte Erhöhung der polyenen Fettsäuren erscheint unzureichend, um die angestiegene Nachfrage decken zu können. PGE_1 beeinflusst die Zusammensetzung des FFS Spiegels, dessen Veränderungen mit bestimmten physiologischen Funktionen dieser Substanzen in Verbindung stehen.

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EFFECT OF TENOTOMY ON THE LIPID COMPOSITION OF MUSCLES WITH DIFFERENT BIOLOGICAL FUNCTIONS

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The lipid composition of the tonic soleus muscle, the intermediate-type (containing tonic and tetanic fibres) gastrocnemius muscle and the tetanic vastus lateralis muscle of the rabbit was examined one and two weeks after tenotomy. One week after tenotomy the amount of triglycerides and phospholipids increased only in the tonic muscle. This increase was more pronounced after two weeks. At this time the amount of the above-mentioned lipids increased in the intermediate-type muscle, too. On the other hand, in the tetanic muscle the lipid composition did not change significantly even two weeks after tenotomy.

Regarding the phospholipids, qualitative changes could also be observed in the tonic muscle two weeks after tenotomy: the fatty aldehyde content of choline phosphatides and non-choline phosphatides increased. The observed changes of phospholipids in tonic muscle following tenotomy could be prevented by simultaneously performed denervation.

Owing to the biological diversity of mammalian skeletal muscles, generalizations concerning their responses to denervation, immobilization and metabolic alterations, are questionable. According to our previous results, the lipid composition of muscles with different biological functions, the so-called tonic and tetanic muscles, differ from each other (HEINER and DOMONKOS 1970). On the other hand, following denervation (HEINER et al. 1970, 1971a) and in dexamethasone-induced myopathy (HEINER et al. 1971b), the amount and the qualitative composition of phospholips change in a characteristic manner in tonic and tetanic muscles. It seemed therefore of some interest to investigate the lipid composition of muscles following tenotomy, another type of muscle damage.

Methods

Adult rabbits kept on a normal diet were used. The animals were anaesthetized with sodium pentobarbital. In the first group of animals the tendon of the right soleus, in the second group the tendon of the gastrocnemius and in the third one the tendon of the white portion of the vastus lateralis muscle were transected and one or two weeks later the animals were killed. Muscle specimens were obtained from the soleus (tonic), vastus lateralis (tetanic) and gastrocnemius (intermediate, containing both tonic and tetanic fibres) muscles. The contralateral muscles served as controls. After removing the connective tissue, the muscles

were homogenized and extracted with chloroform–methanol (2 : 1 v/v) at room temperature. Separation of lipids was performed in the same manner as in earlier studies (HEINER and DOMONKOS 1970). Determination of the muscle cell phase was based upon the estimation of non-collagenous protein according to LILIENTHAL et al. (1950). After treating the homogenized muscle with 0.05 N NaOH, the eluted protein was determined according to HILLER et al. (1948), fatty aldehydes by the method of WARNER and LANDS (1961). Phosphorus was determined according to FISKE and SUBBAROW (1925), after H_2SO_4 treatment, neutral glycerides according to CARLSON (1963).

Results

Table I shows the effect of tenotomy on the cell protein content of tonic, intermediate and tetanic muscles. One week after tenotomy the amount of non-collagenous protein decreased significantly only in the tonic muscle. This decrease was more pronounced after two weeks. Then the amount of non-collagenous protein decreased in the intermediate muscle too. In the tetanic muscle, the amount of non-collagenous protein was unchanged two weeks after tenotomy.

Table I

Effect of tenotomy on the amount of non-collagenous protein in various muscles (mg protein per g muscle wet weight)

	Contralateral muscle	Tenotomized muscle
Tonic muscle		
1 week	157.0±13 (10)*	116.0±23 (10)
2 weeks	161.0±23 (6)+	96.0±29 (6)+
Intermediate muscle		
1 week	172.0±14 (9)	156.0±15 (9)
2 weeks	169.0± 8 (7)	131.0±15 (7)+
Tetanic muscle		
2 weeks	177.0±15 (7)	176.0±19 (7)

* Mean ± S.D. Number of experiments in brackets
+ $p < 0.01$

Table II shows the neutral lipids content of tenotomized muscles. Two weeks after tenotomy the most striking changes occurred in tonic muscle, where both the content of triglycerides and neutral plasmalogens (triglycerides containing fatty aldehydes) increased markedly. In the intermediate muscle the neutral lipids showed less pronounced changes. In the tetanic muscle the amount of neutral lipids did not change significantly by two weeks after tenotomy. One week after tenotomy changes in the amount of neutral lipids were observed only in the tonic muscle. By this time, both the triglyceride and

Table II

Effect of tenotomy on the amount of neutral lipids in various muscles (μ mol per g non-collagenous protein)

	Tonic muscle	Intermediate muscle	Tetanic muscle
Triglycerides (expressed in glycerol)			
1 week			
C	153± 81 (10)*	12.9± 3.2 (9)	—
T	297±164 (10)	15.8± 4.5 (9)	—
2 weeks			
C	124± 38 (6)	13.6± 3.1 (7)	7.35±1.31 (6)
T	631±289 (6)+	36.0±10.3 (7)+	10.80±1.70 (6)
"Neutral plasmalogen" (expressed in palmitic aldehyde)			
1 week			
C	0.95±0.25 (10)	0.30±0.16 (9)	—
T	1.66±0.60 (10)+	0.33±0.18 (9)	—
2 weeks			
T	0.43±0.09 (6)	0.24±0.15 (7)	0.33±0.09 (6)
T	1.79±0.63 (6)+	0.45±0.24 (7)	0.37±0.06 (6)

C: contralateral muscle
T: tenotomized muscle
* Mean ± S.D. Number of experiments in brackets
± $p < 0.01$

neutral plasmalogen content increased. This increase was more significant in neutral plasmalogen.

Table III shows the effect of tenotomy on the amount of lipid-P and fatty aldehyde in the choline-phosphatide fraction of the muscles. In the tonic muscle, the amount of lipid-P and fatty aldehyde was increased one week after tenotomy. The increase was more pronounced two weeks after the operation. In the intermediate muscle one week after tenotomy the amount of lipid-P and fatty aldehyde were not altered significantly. At two weeks, a pronounced increase was, however, observed first of all in the amount of lipid-P. The fatty aldehyde content of choline phospholipids increased slightly. In the tetanic muscle, the amount of choline phosphatides did not change significantly even at two weeks after tenotomy.

Table IV illustrates the lipid-P and fatty aldehyde content in the non-choline phosphatide fraction of the muscles following tenotomy. In the tonic

Table III

Effect of tenotomy on the amount of lipid phosphorus and fatty aldehyde in choline phosphatides in various muscles (per g non-collagenous protein)

	Tonic muscle	Intermediate muscle	Tetanic muscle
Lipid phosphorus, microatom			
1 week			
C	30 0± 3.1 (10)*	26.4± 2.7 (9)	—
T	62.4±17.6 (10)	31.6± 3.8 (9)	—
2 weeks			
C	32.7± 3.2 (6)	31.3± 3.2 (7)	33.8±5.6 (6)
T	86.9±27.1 (6)	52.7±10.3 (7)+	39.9±4.8 (6)
Fatty aldehydes, micromole			
1 week			
C	1.54±0.30 (10)	1.71±0.74 (9)	—
T	2.96±0.93 (10)+	1.92±0.73 (9)	—
2 weeks			
C	2.70±1.08 (6)	3.07±1.25 (7)	0.94±0.20 (6)
T	10.70±3.00 (6)+	6.43±2.93 (7)	1.21±0.24 (6)

C: contralateral muscle
T: tenotomized muscle
* Mean ± S.D. Number of experiments in brackets
+ $p < 0.01$

muscle after both one and two weeks the amount of lipid-P and fatty aldehyde increased significantly. In the intermediate muscle there was no change after one week, while after two weeks the amount of both lipid-P and fatty aldehyde increased markedly. In the tetanic muscle neither the amount of lipid-P, nor that of fatty aldehyde had changed at two weeks after tenotomy.

Discussion

Only KOSTIC et al. (1965) investigated the effect of tenotomy on the lipid composition of muscles. According to their results, after transection of the tendon of the m. triceps surae, the amount of phospholipids increased in the muscle. However, muscles different in biological function were not investigated.

Our results showed that after tenotomy first of all the lipid composition of tonic muscle changes; one week after the operation the amounts of neutral

Table IV

Effect of tenotomy on the amount of lipid phosphorus and fatty aldehydes in non-choline phosphatides on various muscles (per g non-collagenous protein)

	Tonic muscle	Intermediate muscle	Tetanic muscle
Lipid phosphorus, microatom			
1 week			
C	28.0± 2.5 (10)*	20.0±3.9 (9)	—
T	54.0±16.1 (10)+	22.8±3.5 (9)	—
2 weeks			
C	21.9± 1.8 (6)	20.6±5.4 (7)	20.7±3.1 (6)
T	51.8±13.0 (6)+	33.2±3.3 (7)+	21.1±3.2 (6)
Fatty aldehyde, micromole			
1 week			
C	6.43±0.72 (10)	4.75±0.67 (9)	—
T	13.60±3.10 (10)+	5.45±0.95 (9)	—
2 weeks			
C	7.90±1.34 (6)	6.30±0.42 (7)	5.46±0.82 (6)
T	21.80±5.90 (6)+	12.10±3.40 (7)+	5.80±0.85 (6)

C: contralateral muscle
T: tenotomized muscle
* Mean ± S.D. Number of experiments in brackets
+ $p < 0.01$

lipids and phospholipids were increased. The increase was more pronounced after two weeks. The gastrocnemius is an intermediate-type muscle, in which tetanic fibres are predominant (HENNEMAN and OLSON 1965). The amount of lipids in this muscle increase significantly only two weeks after tenotomy and the increase was less than the pronounced one observed in the tetanic muscle. While in the tetanic muscle even two weeks after tenotomy there was no change in the composition of lipids, in the m. gastrocnemius containing both tonic and tetanic fibres the observed changes were probably due only to changes in tonic fibres.

The changes observed in the tonic muscle two weeks after tenotomy were not limited to an increase in the amount of phospholipids. Their composition displayed also qualitative changes. The rate of increase in the amount of phospholipids and that of the fatty aldehydes in phosphatides was not the same as in the choline or non-choline phosphatides of the tonic muscle two weeks after tenotomy.

As Table V shows, the ratio of fatty aldehyde to lipid phosphorus increased. As compared to the increase in choline-phosphatides, this increase was more pronounced in the non-choline phosphatides. The increase in the ratios suggests that under the effect of tenotomy in tonic muscle the synthesis of phospholipids containing vinyl ether bond (plasmalogens) is greater than that of phospholipids with diacyl ester bond. Similar qualitative changes could not be observed in intermediate-type and tetanic muscles. It was stated previously (HEINER et al. 1970) that at 3—6 weeks following denervation, the amount of fatty aldehyde is increased in phospholipids both in tonic and tetanic muscles. On the other hand, in dexamethasone myopathy, the content of plasmalogens decreases mainly in the tetanic muscle (HEINER et al. 1971b). Our recent findings, as well as our previous results suggest that these various

Table V

Effect of tenotomy on fatty aldehyde contents of phospholipids in various muscles (per cent [A/P·100])

	Tonic muscle	Intermediate muscle	Tetanic muscle
Coline phosphatides			
1 week			
C	5.2±1.2	6.5±2.7	—
T	5.1±1.7	6.3±2.1	—
2 weeks			
C	8.2±2.9	10.0±4.2	2.8±0.7
T	12.4±1.3+	11.9±4.0	3.6±0.9
Non-choline phosphatides			
1 week			
C	23.2±3.4	24.2±2.9	—
T	25.9±4.9	24.0±2.7	—
2 weeks			
C	36.0±3.0	32.5± 8.9	26.4±2.5
T	41.9±1.9+	36.5±10.8	27.8±2.8

$$A/P \cdot 100 = 100 \cdot \frac{\mu \text{ moles fatty aldehyde/g non-collagenous protein}}{\mu \text{ atoms lipid phosphorus/g non-collagenous protein}}$$

Values computed from data of Tables III and IV
C: contralateral muscle
T: tenotomized muscle
+ $p < 0.05$

experimental muscle alterations are influencing the plasmalogen content differently in the different types of muscle.

Concerning the results, the question arises why only the lipid composition of the tonic muscle changes so markedly and rapidly. The fact that tenotomy affects the structural integrity of the slow (tonic) muscle more than that of the fast (tetanic) one was shown by ECCLES (1944). The histological changes found in the tenotomized soleus are much more severe than those occurring after division of the motor nerve (GUTMANN and ZELENÁ 1959) and their onset is more rapid. Tenotomy, which decreases the activity of tonic muscle, converts it into a tetanic-type muscle; the activity of tenotomized tetanic muscle is not affected, neither are its mechanical characteristics (VRBOVÁ 1962, 1963). Recently, TOMANEK and COOPER (1972) have investigated the ultrastructural changes occurring in tenotomized muscles. According to their results, after tenotomy the soleus undergoes a more rapid and marked degeneration than the tetanic fibres do in the vastus lateralis muscle. A localized degenerative process occurs in most soleus fibres within five days following tenotomy. On the other hand, degenerative foci in the tenotomized rat gastrocnemius are infrequent and limited (WALKER et al. 1965) or not evident (LISSÁK et al. 1963).

Severe degeneration following tenotomy in the soleus muscle can be prevented by denervation or spinal cord section. Thus, degenerative changes in the soleus following tenotomy might be due to impulses of supraspinal origin (MCMINN and VRBOVÁ 1964).

Table VI

Common effect of tenotomy and denervation on the amount of lipid-phosphorus and fatty aldehyde in phosphatides of soleus muscle (per g non-collagenous protein)

	Choline phosphatides	Non-choline phosphatides
Lipid phosphorus microatom		
C	23.6±4.9 (9)*	24.4±2.4 (9)
T + D	39.2±7.6 (9)+	28.0±8.7 (8)
Fatty aldehyde micromole		
C	2.60±1.04 (9)	6.30±1.44 (9)
T + D	3.30±1.30 (9)	8.00±2.11 (8)

C: contralateral muscle
T + D: 2 weeks after tenotomy and denervation
* Mean ± S.D. Number of experiments in brackets
+ $p < 0.05$

The role of the nervous system in the degeneration of the tenotomized soleus muscle have been confirmed by our results, too. If soleus tenotomy and sciatic nerve division were performed together, the amount of phospholipids increased less than after tenotomy alone (Table VI). Two weeks after denervation and tenotomy, there was no significant change in the amount of phospholipid of the soleus muscle. The slight increase was similar to that observed after two weeks of denervation (HEINER et al. 1970).

The amount of non-collagenous protein did not decrease significantly; its value was 128.0 $\pm$ 27 mg/g muscle, while on the contralateral side, 159.0 $\pm$ $\pm$ 14 mg/g muscle. These results also indicate that tenotomy together with denervation "prevents" the effects of tenotomy by itself.

Changes of phosphatides and mainly of plasmalogens of skeletal muscles with different biological functions in various experimentally induced pathological states suggests the important role of phospholipids, first of the plasmalogens, in muscle function.

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EFFECT OF ELEVATED RENAL VENOUS PRESSURE ON INTRARENAL HAEMODYNAMICS

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Experiments were carried out in non-diuretic dogs under pentobarbital anaesthesia to investigate the effect of increased renal venous pressure on intrarenal circulation. Venous pressure was elevated to 30 mm Hg by partial venous occlusion on the left side. The right kidney served as control. Total renal blood flow (TRBF) was measured directly by collecting the venous outflow. Renal, cortical and medullary blood flows were estimated by tissue extraction of Rb (Rb-RBF). TRBF remained unchanged, while Rb-RBF fell in proportion to the decreased arterial–venous pressure difference and medullary blood flow decreased in excess of the cortical flow. Such a discrepancy between TRBF and Rb-RBF was not found in the controls. GFR, urine flow and sodium excretion were also reduced. A reduction of blood flow as determined by a shift of blood from nutritional towards non-nutritional capillaries. Calculation of vascular resistance suggested that the redistribution of blood flow could be localized mainly to the postglomerular segment of the iuxtamedullary circulation.

Elevation of renal venous pressure may exert different effects of urine flow and sodium excretion (BLAKE et al. 1949; HALL and SELKURT 1951; WINTON 1931) depending upon the previous electrolyte and water intake. WATHEN and SELKURT (1969) found that urine flow and sodium excretion increased in face of unchanged GFR in the hydropenic animal but decreased together with GFR in extracellular hypervolaemia. Recent observations on the relationship between the intrarenal distribution of RBF and GFR and the rate of water and sodium excretion (EARLY and FRIEDLER 1965; HOLLENBERG et al. 1970; HORSTER and THURAU 1968) may provide a clue to explaining the above difference. The purpose of the present experiments was to investigate the effect of elevated renal venous pressure on the intrarenal distribution of blood flow applying Sapirstein's isotope fractionation method.

Methods

Experiments were performed on 11 mongrel dogs of both sexes, weighing 13 to 20 kg, in non-diuretic conditions under sodium pentobarbital anaesthesia. Food was withdrawn 24 hours prior to the experiments but water intake was not restricted.

The left renal hilum was exposed through a flank incision, and the renal vein was stripped and cannulated. The left renal and left external jugular veins were connected with a polyethylene tube. Coagulation of blood was inhibited by 5 mg/kg of heparin. Both ureters were cannulated supravesically. Arterial blood pressure was measured in the left femoral artery by a mercury manometer, renal venous pressure in the left renal vein by a saline manom-

eter through a T extension of the connecting tube. Arterial blood samples were withdrawn from the right femoral artery, and the left femoral vein was used for infusions.

After a priming dose, PAH and creatinine were infused intravenously at 1 ml/min constant rate to maintain the plasma concentrations somewhat below 2 mg per 100 ml and around 20 mg per 100 ml, respectively. The experiment was started after 45 to 60 min had elapsed.

Urine was collected in 3 periods of 10 min each. At the mid-point of each period, arterial and venous blood samples were withdrawn and renal venous outflow was measured through the T extension of the connecting tube. After the control periods, the connecting tube was constricted till renal venous pressure had increased to 30 mm Hg. After stabilization of the elevated venous pressure, further 3 collection periods of 10 min each were made, blood samples were withdrawn and venous outflow was measured as above. After the last period, 50 μCi of ^{86}RbCl were injected into the left external jugular vein through the connecting tube; immediately thereafter, arterial blood was collected continuously in 2-sec fractions, and in the 60th sec the animal was killed by an intravenous injection of saturated KCl solution. Prior to and 50 sec after the injection of ^{86}RbCl, renal venous outflow was determined. The kidneys were removed promptly, then decapsulated, weighed, and tissue aliquots weighing 600–1000 mg were excised from the cortex, outer and inner medulla; the kidney remnant was again weighed.

PAH and creatinine concentrations in plasma and urine were determined by a method reported previously (HÁRSING et al. 1964); clearance and extraction ratio of PAH and creatinine were calculated as usually. Activity of ^{86}Rb in the arterial blood samples, renal tissue aliquots and in the kidney remnant was measured by a well-type crystal scintillation counter. Sodium concentration was determined by a Zeiss flame photometer.

Renal blood flow was measured directly and calculated from the clearance and extraction ratio of PAH as well as from the tissue and arterial concentration of ^{86}Rb on the basis of the formula

$$\frac{Q}{\int_0^T Ca\, dt},$$

where Q means the concentration of ^{86}Rb in the renal, cortical and medullary tissue, Ca the ^{86}Rb concentration in arterial blood and T the time of the first circulation (HÁRSING, et al. 1967). Mean concentration of ^{86}Rb in the medulla was calculated from the ^{86}Rb content of the outer and inner medulla with consideration of the relative weight of the tissues.

The following formulae were used for calculation of vascular resistances:

$$\text{total renal vascular resistance} = \frac{PA - RVP}{TRBF};$$

$$\text{renal prevenous resistance} = \frac{PA - IRVP}{TRBF};$$

$$\text{renal preglomerular (arteriolar) resistance} = \frac{PA - PG}{TRBF};$$

$$\text{renal postglomerular (arteriolar + capillary) resistance} = \frac{PG - IRVP}{TRBF};$$

$$\text{cortical prevenous vascular resistance} = \frac{PA - IRVP}{Rb\text{-}CBF};$$

$$\text{medullary prevenous vascular resistance} = \frac{PA - IRVP}{Rb\text{-}MBF},$$

where PA means arterial, RVP renal venous, IRVP intrarenal venous, PG glomerular capillary pressure, TRBF renal blood flow measured directly, Rb-CBF renal cortical and Rb-MBF renal medullary blood flow estimated by the Rb technique. IRVP was not determined, but considered to be 20 mm Hg in the control (ABE et al. 1970; BÁLINT et al. 1971; NAVAR 1970; THURAU and HENNE 1964), and 30 mm Hg in the experimental periods (GOTTSCHALK and

MYLLE 1956). PG was calculated from the formula $PG = \frac{GFR}{k} + COP + IRVP$, where k stands for the glomerular filtration coefficient and COP for plasma colloid osmotic pressure (ABE et al. 1970; BÁLINT et al. 1971; NAVAR 1970). The value of 0.0196 ml/min/mm Hg/g was used for k (BÁLINT et al. 1971), and 25 mm Hg for COP. Conclusions were based on relative changes in the resistance of the different vascular segments and were not influenced by eventual changes of the values for IRVP, k and COP, chosen arbitrarily on the basis of experiments performed by others.

Statistical evaluation of the results was undertaken by variance analysis and by Student's *t*-test using paired samples.

Results

Table I shows the mean values obtained in the right and left kidneys in the control periods (Period 1) and in the right kidney at normal venous pressure when left renal venous pressure was elevated (Period 2). The good agreement between the figures allows the assumption that the values for renal, cortical and medullary blood flows in the right kidney estimated by the Rb technique in Period 2 can be applied to the left kidney as well.

Table I

Values for right and left kidneys at normal renal venous pressure

	Period 1		Period 2
	right	left	right
Renal blood flow, ml/min/g kidney	5.05±0.42*	4.80±0.38+ 4.65±0.42++	5.05±0.40+ 4.90±0.42+++
Glomerular filtration rate, ml/min/g kidney	0.74±0.07	0.72±0.05	0.77±0.05
Urine flow, μl/min/g kidney	12.70±2.20	10.50±1.90	13.00±3.80
Sodium excretion, μEq/min/g kidney	2.10±0.47	1.67±0.17	1.74±0.52

* S.E.
\+ calculated from the clearance and extraction ratio of PAH
\++ measured directly
\+++ estimated by the Rb method

Table II represents the means and their standard errors obtained in the 3 control and 3 experimental periods with elevated renal venous pressure. The values for the individual periods were the same within reasonable limits and were therefore not indicated separately. Renal blood flow measured directly remained unchanged, while it fell to 83% of the control when estimated by the Rb method. Cortical and medullary blood flows were reduced to 88

Table II

Response of renal function to the elevation of renal venous pressure

	Control	Elevated renal venous pressure
Blood pressure, mm Hg	120.8±5.1*	121.0±5.2
Renal venous pressure, mm Hg	4.0±0.6+	30.0+
Renal blood flow (measured directly), ml/min/g kidney	4.65±0.42	4.95±0.42
Renal blood flow (measured by Rb technique), ml/min/g kidney	4.90±0.42+	4.09±0.40+
Cortical blood flow, ml/min/g cortex	5.35±0.44+	4.70±0.35+
Medullary blood flow, ml/min/g medulla	3.88±0.42+	2.64±0.31+
Glomerular filtration rate, ml/min/g kidney	0.72±0.05+	0.57±0.06+
Urine flow, μl/min/g kidney	10.5±1.9+	5.1±1.3+
Sodium excretion, μEq/min/g kidney	1.67±0.17+	0.58±0.16+

* S.E.
+ $p < 0.05$

and 68% of control values, respectively. There was a decrease of GFR, urine flow and sodium excretion as well.

Values for vascular resistances are summarized in Table III. Total renal as well as renal prevenous resistance decreased during elevated renal venous pressure. Reduction of the prevenous vascular resistance was due mainly to the decreased postglomerular resistance. Since renal blood flow estimated by the Rb technique was significantly lower than that measured directly, cortical and medullary vascular resistances calculated from flow values obtained by the Rb method were denoted as apparent resistances. There was no change in the cortical but a considerable increase in the medullary apparent resistance.

Discussion

Reduction of renal vascular resistance during elevated renal venous pressure is generally considered to be the result of renal autoregulation, i.e. vasodilation in the preglomerular arterioles (THURAU 1964; THURAU and HENNE 1964). Calculations for vascular resistance in the present experiments show that the decrease occurred in the postglomerular segment. The absence

Table III

Vascular resistances during elevated renal venous pressure

Vascular resistance, mm Hg · min · ml^{-1}/g	Control	Elevated renal venous pressure
Renal total	27.7±2.5*+	20.9±1.9+
Renal prevenous	24.0±2.8+	20.9±1.9+
Renal preglomerular	7.9±0.2	7.6±0.2
Renal postglomerular arteriolar and capillary	16.1±1.9+	13.3±1.3+
Cortical prevenous++	20.3±3.9	21.1±2.4
Medullary prevenous++	29.2±3.9	43.4±8.5

* S.E.
+ $p < 0.05$
++ apparent resistance (see in text)

of an autoregulatory response may have been the result of the comparatively low preglomerular arteriolar resistance in the control period (Table III).

The different effect of elevated renal venous pressure on directly measured renal blood flow and that estimated by the Rb method allowed to advance a tentative hypothesis for explaining the reduction of postglomerular resistance, urine flow and sodium excretion. Extraction of a substance with diffusion-limited transcapillary exchange is considerably influenced by the ratio of capillary diffusional capacity, i.e., capillary permeability and surface product (PS), and the blood flow (Q) (Crone 1963; Renkin 1968; Yudilevich et al. 1968). During the elevated renal venous pressure, PS rather increased as a result of the distension of capillaries and venules, while total renal blood flow remained unchanged. Therefore, as a possible explanation for the reduced Rb extraction, a redistribution of blood flow in the non-homogeneous intrarenal capillary bed could be taken into consideration. The nearly complete renal extraction of Rb under normal conditions indicates that practically the total amount of blood flows through capillaries having an optimum PS/flow ratio (nutritional capillaries). Diminished extraction of Rb during elevated renal venous pressure may be interpreted by assuming that a fraction of blood perfuses capillaries of lower resistance and of smaller diffusional surface, i.e. short capillaries in which conditions do not favour the extraction of Rb (non-nutritional capillaries). The opening of such short capillaries arranged in parallel during elevated renal venous pressure could have been responsible for the reduction of postglomerular vascular resistance even if the resistance of the vascular pathways perfused under control conditions had remained constant.

A decrease in the arterial–venous pressure difference as a consequence of elevated renal venous pressure with no change in the pre- and postglomerular vascular resistance would result in a proportional fall of blood flows measured directly and estimated by the Rb method. However, if the postglomerular resistance decreased at the same time as a result of the opening of non-nutritional capillaries, while the resistance of the originally patent vascular pathways remained constant, blood flow measured directly and by the Rb technique will deviate from each other; the former does not fall but increases and the latter decreases more than would be expected from the diminished arterial–venous pressure difference (Fig. 1). Cortical blood flow fell from 5.35 to 4.70 ml/min/g which corresponded well to the decrement of arterial–venous pressure differences, and apparent cortical resistance (calculated from flow values obtained by the Rb method) remained essentially unchanged. Medullary blood flow, however, diminished more markedly, from 3.88 to 2.64 ml/min/g, and the apparent medullary resistance increased. This suggests that the redistribution of blood flow between nutritional and non-nutritional capillaries could

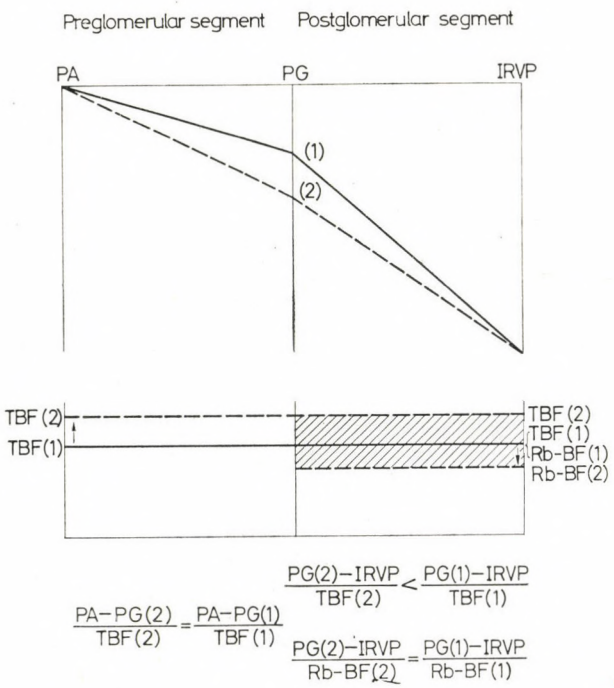

Fig. 1. Pressure gradient and blood flow measured directly (TBF) and by the Rb method (Rb-BF) in a renal vascular area when postglomerular resistance is reduced by opening of non-nutritional vascular chanels. Shaded area represents the difference between blood flow determined directly and estimated by the Rb method. PA: arterial pressure; PG: glomerular capillary pressure; IRVP: intrarenal venous pressure

be localized to the medullary circulation. Medullary Rb blood flow calculated from Tables II and III, assuming that there was no change in the pre- and postglomerular resistance of the originally functioning vascular channels, is in agreement with the value found in the experiments, considering that some Rb excapes from the blood while flowing through the glomeruli and non-nutritional capillaries.*

This conclusion seems to be supported by the results of MIYAZAKI and MCNAY (1971) who concluded from the distribution of microspheres that during elevated renal venous pressure a smaller fraction of renal blood flow perfuses the superficial and a larger one the deeper (iuxtamedullary) lyers of the cortex. Morphological evidence also favours this conception. Blood flowing through the long branches of the vasa recta could be partially shunted by shorter capillaries connecting them in the more outer layers of the medulla. Such differences in the structural arrangement of the capillaries do not exist in the cortex.

If this conclusion is correct, during elevated renal venous pressure there must be no change in the cortical pre- and postglomerular vascular resistance, consequently in the pressure and filtration rate in the cortical glomeruli.

However, a decrease of the postglomerular resistance in the medulla with unchanged preglomerular resistance results in a fall of pressure and filtration rate in the juxtamedullary glomeruli diminishing total GFR, urine flow and sodium excretion. It seems worth mentioning that our results are consistent with the findings of WATHEN and SELKURT (1969) in saline-loaded dogs, further that the comparatively high urine flow, sodium excretion and medullary blood flow also indicated an extracellular hypervolaemia in the present experiments.

The response of the renal circulation to elevated renal venous pressure in hydropenia or in any other condition associated with a sufficiently high preglomerular resistance and a good autoregulatory capacity may be different from the above mechanism. Preglomerular vasodilation increases pressure and filtration rate in the cortical glomeruli, bluting or even annihilating the effects of decreased medullary postglomerular resistance on the pressure and filtration rate in the iuxtamedullary glomeruli. The net result of this may be constant or augmented total GFR, with increased urine flow and sodium excretion.

It has been suggested (LEWY and WINDHAGER 1968; MARTINO and EARLEY 1967) that hydrostatic pressure in the peritubular capillaries is a

$$* \text{ Rb-MBF} = \frac{(\text{PA} - \text{IRVP}) - \text{RA}(\text{TRBF} - \text{Rb-RBF})}{\text{RA} + \text{RE}},$$

where RA and RE stand for control values of medullary pre- and postglomerular resistance. Medullary preglomerular resistance (RA) was considered to be equal to renal preglomerular resistance (TRBF — Rb-RBF) was calculated for 1 g of medulla.

factor determining tubular sodium transport. Our results do not exclude the possibility of different changes of the filtration rate in the cortical and juxtamedullary glomeruli, exerting a secondary, modifying effect on the primary tubular mechanism.

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THE EFFECT OF HYPOXIA ON THERMOREGULATORY HEAT PRODUCTION AND BODY TEMPERATURE IN THE NEW-BORN AND YOUNG GUINEA PIG

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The response of colonic temperature (T_c) and heat production (VO_2) to exposure to 12% O_2 and 8% O_2 was studied in guinea pigs aged less that 48 hours, 3 to 6 days, 7 to 9 days, 10 to 20 days, and 30 to 60 days at an ambient temperature (T_a) of 20 °C. For comparison, corresponding data for adult guinea pigs were included. Exposure to 12% O_2 failed to reduce mean VO_2 in all age-groups. After termination of exposure, mean VO_2 increased significantly from the age of 7 days. The mean of the changes during exposure to 12% O_2 recorded in individual observations (ΔO_2) showed a statistically significant increase in heat production in the groups aged to 3 to 6 and 7 to 9 days. Distribution of ΔO_2 in response to 12% O_2 of the animals aged less than 10 days differed significantly from that of the older age-groups. In contrast, T_c fell in all age-groups significantly in response to 12% O_2, the fall being significantly smaller in the 3 to 6 and 7 to 9 day-old than in either the younger or the older animals. The post-exposure increase in T_c was highly significant in all age-groups. The distribution of ΔT_c in the age-groups of 3 to 6 and 7 to 9 days differed significantly from that in the groups aged less than 48 hours, 10 to 20, and 30 to 60 days, whereas there was no difference in the distribution of ΔT_c-s between the three last mentioned groups. In response to exposure to 8% O_2, both VO_2 and T_c fell highly significantly. After termination of exposure, VO_2 increased above the pre-exposure level and concomitantly T_c increased rapidly. ΔO_2 in response to 8% O_2 was significantly smaller in the two youngest groups than in the older new-born groups. The distribution of ΔO_2 in the three combined younger groups differed significantly from that of the combined groups aged 10 to 20 and 30 to 60 days. ΔT_c in response to 8% O_2 was greatest, and almost equal, at the ages of less than 48 hours and of 10 to 20 days, both differing significantly from the other age-groups. In the 15 min subsequent to termination of exposure, ΔT_c was highly significantly greater in the youngest group than in any of the others. ΔT_c-s in the other new-born groups were identical inspite of the preceding fall during hypoxia and the level of T_c at termination of hypoxia having differed significantly between these groups. In contrast to the rat and rabbit, in which species the neonatal changes are characterized by a gradual approach to the responses of the adult animal, in the guinea pig the ΔT_c of animals aged less than 48 hours resembled that of the 10 to 20-day-old much more closely than that of the 3 to 6 and 7 to 9-day-old. The responses to cold and to hypoxia of the thermoregulatory system differed in the neonatal period: whereas the fall in T_c in response to cold was by far the smallest at the age of less than 48 hours, the fall in response to hypoxia was significantly greater in this age-group than in the 3 to 6 and 7 to 9-day-old animals.

Since LINTZEL's (1931) original communication it has abundantly been confirmed that at ambient temperatures (T_a) below the thermoneutral zone hypoxic hypoxia elicits a fall in heat production and deep body temperature in most, if not all, mammalian species. This has been shown for the human neonate (CROSS et al. 1955; BRODIE et al. 1957), the new-born dog (MOORE 1956a), rabbit (ADAMSONS 1959; BLATTEIS 1964; VÁRNAI et al. 1971a), kitten

(MOORE 1956b, 1959; HILL 1959; MOORE and UNDERWOOD 1963), monkey (DAWES et al. 1960), rat (MOUREK 1959; TAYLOR 1960; VÁRNAI et al. 1970a), and mouse (CASSIN 1963).

In contrast to the abundance of observations concerned with the thermoregulatory response of new-born guinea pigs to changes in ambient temperature, to catecholamines, etc. (DAWES and MESTYÁN 1963; BRÜCK and WÜNNENBERG 1965a, b, c, 1966a, b; ZEISBERGER and BRÜCK 1967; ZEISBERGER et al. 1967; ADAMSONS et al. 1969; FARKAS and DONHOFFER 1974), data concerning the effect of hypoxia on thermoregulatory responses in the new-born guinea pig are not available. Even observations on adult guinea pigs are sparse and, in part, contradictory (FARKAS and DONHOFFER 1973). An investigation of the thermoregulatory response of new-born guinea pigs to hypoxia appeared to be of interest, since a) the guinea pig is born at an advanced stage of maturity and has been considered to maintain a fairly stable core temperature; b) more severe hypoxia is required in the adult guinea pig for eliciting a similar response of heat production as in the young rabbit and the adult rat; and c) thermoregulatory responses undergo changes in the neonatal period (BRÜCK and WÜNNENBERG 1965c, 1966a; VÁRNAI and DONHOFFER 1970; VÁRNAI et al. 1970a, b, c, 1971a, b; FARKAS et al.

Methods

Newly born guinea pigs of both sexes, born and reared in the Institute's animal house were used. The observations were made throughout 1971, 1972, and the first half of 1973. For the sake of comparison, corresponding data on adult guinea pigs (FARKAS and DONHOFFER 1973) were included.

Oxygen consumption was measured by a modified Noyons-Kipp diaferometer using an air-flow of 0.7 to 1.4 litre/min (for the adult animals 2.0 litre/min had been used). Colonic temperature was recorded by copper–constantan thermocouples introduced to a depth of 5 to 10 cm according to the size of the animal. The strictly standardized thermal conditions and other details of the experimental setup have been described earlier (DONHOFFER et al. 1958; SZEGVÁRI et al. 1961). After introducing the animals into a metabolic chamber of appropriate size, this was submerged into a water-bath providing an ambient temperature (T_a) of 35 °C for the two youngest groups, and either 35 °C or 30 °C for the 7 to 9 and 10 to 20-day-old groups. Animals aged 30 to 60 days were always placed into the water-bath providing T_a 30 °C, as had been the adults. Measurements were started 30 to 45 min later; another 30 min thereafter animals exposed initially to 35 °C were transferred to T_a 30 °C, and from there after 30 min to T_a 20 °C, as were those of the older animals not exposed initially to T_a 35 °C. Thirty min after transfer to T_a 20 °C, 12% O_2 in N_2 was supplied to the animals for 30 min, after which room air was restored again; subsequently the animals were exposed for 30 min to 8% O_2 in N_2 after which room air was restored and measurements were continued for a further 30 min. In some instances, exposure to 12% O_2 has been omitted in animals aged less than 48 hours.

Figures for VO_2 represent, when not stated otherwise, averages of readings taken every minute during the last 10—15 min of each 30-min period. In the post-hypoxic periods, VO_2 represents averages obtained from readings during the 10th to 15th and the 20th to 30th min. Data for T_c represent the levels attained at the end of the pre-hypoxic and hypoxic periods, and those recorded at the 15th and 30th min after the termination of hypoxia. The recording galvanometers were, however, read every minute. Student's *t*-test and the Chi square test with Yates' modification for small numbers were used for statistical evaluation. Body surface was calculated according to the formula $10 \cdot kg^{0.67}$.

Results

Exposure to 12% O_2

Heat production. Table I summarizes the effect of breathing 12% O_2 at T_a 20 °C: on the average, no significant reduction in heat production (VO_2) was observed in the course of exposure in any of the age-groups. On the contrary, statistically significant increases were recorded in guinea pigs aged 3 to 6 and 7 to 9 days. The mean of the changes in oxygen consumption in individual observations (ΔO_2) approached statistical significance in the youngest age-group as well (Table II). Following the termination of exposure, VO_2 showed no change in the two youngest groups, whereas in the older age-groups VO_2 exceeded the level recorded during exposure in the first 15 min after the termination of hypoxia. In the younger age-groups the same level of VO_2 was maintained during the second 15 min after the termination of exposure, whereas in the group aged 30 to 60 days VO_2 fell in the second 15 min below the level reached during the first 15 min after exposure, as it did in the adults. The decrease in the 30 to 60-day-old group approached, and in the adults it reached, statistical significance.

Part B of Table I contains the statistical analysis of mean VO_2 between age-groups. Up to the age of 60 days, mean VO_2 differed significantly from that of adult animals before, during, and after the termination of exposure to 12% O_2. Mean VO_2 of the groups aged 3 to 6 and 7 to 9 days differed also significantly from that of the 30 to 60-day-old animals before as well as during the first 15 min after the termination of exposure.

Part C of Table I shows that during the 10th to 15th min after the termination of exposure — with the exception of the group aged less than 48 hours — VO_2 significantly exceeded that before exposure, and even between the 20th to 30th min after the termination of hypoxia, VO_2 was significantly higher than before exposure in the groups aged 3 to 6, 7 to 9, and 10 to 20 days.

Table II contains the means of the individual changes in heat production (ΔO_2) during exposure to 12% O_2 and ΔO_2 between hypoxia and the 10th to 15th, and the 20th to 30th min, respectively, after the termination of hypoxia (Part A), and the statistical analysis of differences between age-groups (Part B). In the three younger age-groups, the increase in heat production during hypoxia either attained, or approached, statistical significance, whereas no significant change was recorded in the older groups. In contrast, after the termination of hypoxia, no further change was observed in heat production in the two younger age-groups, whereas in all of the older groups significant increases above the hypoxic level were observed during the 10th to 15th min after the termination of exposure, and significantly, or almost significantly higher levels were maintained even during the 20th to 30th min after the restoration of room air. Part B of Table II confirms statistically

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Table I

Oxygen consumption (VO_2) of guinea pigs before, during, and after termination of exposure to 12% O_2 at T_a 20 °C. In brackets, number of observations (Part A). Statistical evaluation of the differences between age-groups (Part B), and between VO_2 before and after termination of exposure to 12% O_2 (Part C)

Part A

	VO_2 ml/dm² · min (M ± S.E.)											
Age	Before exposure to 12% O_2		p		During exposure to 12% O_2		p		During the 10th to 15th min after exposure		p	During the 20th to 30th min after exposure
< 48 hrs	1.95±0.11 (15)	→	> 0.5	←	2.03±0.09 (16)	→	> 0.6	←	2.08±0.09 (14)	→	> 0.6	← 2.13±0.07 (15)
3 to 6 days	1.77±0.06 (23)	→	< 0.01	←	2.01±0.04 (23)	→	> 0.7	←	2.03±0.05 (23)	→	> 0.9	← 2.03±0.04 (22)
7 to 9 days	1.83±0.04 (20)	→	< 0.05	←	1.95±0.04 (20)	→	< 0.05	←	2.09±0.05 (21)	→	> 0.8	← 2.08±0.04 (20)
10 to 20 days	1.92±0.06 (25)	→	> 0.6	←	1.95±0.04 (24)	→	< 0.01	←	2.14±0.05 (26)	→	> 0.4	← 2.09±0.04 (26)
30 to 60 days	2.01±0.07 (17)	+	> 0.9	←	2.01±0.05 (17)	→	< 0.01	←	2.31±0.07 (20)	→	0.1 > p > 0.05	← 2.15±0.05 (17)
Adults	1.62±0.05 (15)	→	> 0.6	←	1.59±0.05 (15)	→	< 0.001	←	1.83±0.04 (15)	→	< 0.05	← 1.71±0.04 (15)

Part B

Age-groups	Before 12% O_2 p	During 12% O_2 p	10th to 15th min after 12% O_2 p	20th to 30th min after 12% O_2 p
< 48 hrs— 3 to 6 days	> 0.1	> 0.8	> 0.6	> 0.2
< 48 hrs— 7 to 9 days	> 0.2	> 0.4	> 0.9	> 0.5
< 48 hrs—10 to 20 days	> 0.8	> 0.4	> 0.5	> 0.6
< 48 hrs—30 to 60 days	> 0.6	> 0.8	0.1> p >0.05	> 0.7
< 48 hrs—Adults	< 0.02	< 0.001	< 0.02	< 0.001
3 to 6 days— 7 to 9 days	> 0.2	> 0.2	> 0.4	> 0.3
3 to 6 days—10 to 20 days	0.1> p >0.05	> 0.2	> 0.1	> 0.2
3 to 6 days—30 to 60 days	< 0.02	> 0.9	< 0.01	0.1> p >0.05
3 to 6 days—Adults	0.1> p >0.05	< 0.001	< 0.01	< 0.001
7 to 9 days—10 to 20 days	> 0.2	> 0.9	> 0.4	> 0.8
7 to 9 days—30 to 60 days	< 0.05	> 0.3	< 0.02	> 0.2
7 to 9 days—Adults	< 0.01	< 0.001	< 0.001	< 0.001
10 to 20 days—30 to 60 days	> 0.3	> 0.3	0.1> p >0.05	> 0.3
10 to 20 days—Adults	< 0.001	< 0.001	< 0.001	< 0.001
30 to 60 days—Adults	< 0.001	< 0.001	< 0.001	< 0.001

Part C

Age	VO_2 before exposure to 12% O_2 and VO_2 from 10th to 15th min after termination of exposure p	VO_2 before exposure to 12% O_2 and VO_2 from 20th to 30th min after termination of exposure p
< 48 hrs	> 0.3	> 0.1
3 to 6 days	< 0.01	< 0.01
7 to 9 days	< 0.001	< 0.001
10 to 20 days	< 0.01	< 0.05
30 to 60 days	< 0.01	> 0.1
Adults	< 0.01	> 0.1

the differences between the responses to hypoxia of the 3 to 6-day-old and the other animals, as well as the differences between the changes in heat production 10 to 15 min after the termination of hypoxia between the two youngest groups, on the one hand, and those of the older animals, on the other hand.

The last column in Table II shows the changes in VO_2 (ΔO_2) between the 10th to 15th and the 20th to 30th min after termination of exposure.

Table II

Changes in oxygen consumption (ΔO_2) during exposure to 12% O_2, between VO_2 during and VO_2 in the 10th to 15th, and the 20th to 30th min after termination of exposure, and ΔO_2 between the 10th to 15th min and the 20th to 30th min after termination of exposure. In brackets number of observations (Part A). Statistical evaluation of the differences between age groups (Part B)

Part A

ΔO_2 ml/dm^2 · min (M ± S.E.)

Age	During exposure to 12% O_2	Between VO_2 during, and VO_2 in the 10th to 15th min after termination of exposure to 12% O_2	Between VO_2 during, and VO_2 in the 20th to 30th min after termination of exposure to 12% O_2	Between the 10th to 15th min and the 20th to 30th min after termination of exposure to 12% O_2
< 48 hrs	+0.10±0.05 0.1 > p > 0.05 (15)	+0.05±0.05 > 0.3 (14)	+0.08±0.06 > 0.2 (16)	+0.03±0.04 > 0.4 (14)
3 to 6 days	+0.24±0.05 < 0.001 (23)	+0.02±0.03 > 0.5 (22)	±0.00±0.03 > 0.9 (23)	+0.01±0.04 > 0.7 (23)
7 to 9 days	+0.12±0.05 < 0.05 (20)	+0.14±0.04 < 0.01 (20)	+0.14±0.03 < 0.001 (20)	±0.00±0.02 > 0.9 (20)
10 to 20 days	+0.02±0.07 > 0.7 (25)	+0.20±0.04 < 0.005 (24)	+0.14±0.07 0.1 > p > 0.05 (24)	−0.06±0.03 0.1 > p > 0.05 (24)
30 to 60 days	±0.00±0.06 > 0.9 (17)	+0.31±0.07 < 0.01 (18)	+0.14±0.07 0.1 > p > 0.05 (17)	−0.12±0.03 < 0.001 (19)
Adults	−0.02±0.08 > 0.8 (15)	+0.24±0.06 < 0.01 (15)	+0.12±0.05 < 0.05 (15)	−0.12±0.04 < 0.01 (15)

Part B

Age-groups	During exposure p	10th to 15th min after exposure p	20th to 30th min after exposure p	From the 10th to 15th min after termination of exposure to the 20th to 30th min p
< 48 hrs— 3 to 6 days	0.1 > p > 0.05	> 0.6	> 0.2	> 0.7
< 48 hrs— 7 to 9 days	> 0.8	0.1 > p > 0.05	> 0.3	> 0.5
< 48 hrs—10 to 20 days	> 0.3	< 0.01	> 0.5	0.1 > p > 0.05
< 48 hrs—30 to 60 days	> 0.2	< 0.01	> 0.5	< 0.01
< 48 hrs—Adults	> 0.2	< 0.01	> 0.6	< 0.01
3 to 6 days— 7 to 9 days	0.1 > p > 0.05	< 0.01	< 0.01	> 0.8
3 to 6 days—10 to 20 days	< 0.02	< 0.001	0.1 > p > 0.05	> 0.1
3 to 6 days—30 to 60 days	< 0.01	< 0.001	0.1 > p > 0.05	< 0.02
3 to 6 days—Adults	< 0.01	< 0.001	< 0.05	< 0.02
7 to 9 days—10 to 20 days	> 0.2	> 0.1	> 0.9	> 0.1
7 to 9 days—30 to 60 days	> 0.1	> 0.1	> 0.9	< 0.01
7 to 9 days—Adults	> 0.1	0.1 > p > 0.05	> 0.7	< 0.01
10 to 20 days—30 to 60 days	> 0.8	> 0.5	> 0.9	> 0.1
10 to 20 days—Adults	> 0.7	> 0.7	> 0.8	> 0.1
30 to 60 days—Adults	> 0.8	> 0.7	> 0.8	> 0.9

Table III

Distribution of changes in oxygen consumption (ΔO_2) in response to exposure to 12% O_2 at Ta 20 °C (Part A). Chi² square test comparing increases exceeding +0.10 ml $O_2/dm^2 \cdot min$ with decreases exceeding −0.10 ml $O_2/dm^2 \cdot min$. In brackets, enumeration data (Part B).

Part A

		ΔO_2 ml/dm² · min						
Age	n	> −0.50	−0.50 to −0.30	−0.30 to −0.10	±0.10	+0.10 to +0.30	+0.30 to +0.50	> +0.50
< 48 hrs	16	—	—	2 (12.5%)	6 (37.5%)	7 (43.8%)	1 (6.2%)	—
3 to 6 days	23	—	—	3 (13%)	3 (13%)	9 (39%)	5 (22%)	3 (13%)
7 to 9 days	20	—	—	3 (15%)	9 (45%)	5 (25%)	1 (5%)	2 (10%)
10 to 20 days	25	1 (4%)	4 (16%)	3 (12%)	9 (36%)	2 (8%)	4 (16%)	2 (8%)
30 to 60 days	19	—	1 (5%)	2 (11%)	8 (42%)	5 (26%)	2 (11%)	1 (5%)
Adults	15	1 (7%)	2 (13%)	2 (13%)	5 (34%)	3 (20%)	2 (13%)	—

Part B

Age-groups			Chi²	p
< 10 days (8 ; 33——41)	vs.	older (16 ; 21——37)	4.341	< 0.05
< 10 days (8 ; 33——41)	vs.	10 to 20 + 30 to 60 days (11 ; 16——27)	2.749	0.1 > p > 0.05
< 10 days (8 ; 33——41)	vs.	Adults (5 ; 5——10)	2.645	> 0.1
10 to 20 + 30 to 60 days (11 ; 16——27)	vs.	Adults (5 ; 5——10)	0.050	> 0.8

Whereas no change was observed in the three younger age-groups, an almost significant decrease occurred in the 10 to 20-day-old and a highly significant one in the 30 to 60-day-old and the adult animals. Averages tend to obscure the variety of individual responses, therefore, the distribution of ΔO_2 in response to exposure to 12% O_2 was also analyzed (Table III). Evidently, guinea pigs aged up to 9 days tend to respond to 12% O_2 considerably less frequently with a significant fall in heat production and more frequently with an increase in heat production then older animals do.

Colonic temperature. Table IV demonstrates colonic temperatures (T_c) before, during and after the termination of exposure to 12% O_2. Table V presents the changes in colonic temperature (ΔT_c) calculated on the basis of differences in individual observations. Part A of Table IV shows that T_c tended to be higher until the age of 6 days before, during, and after the termination of exposure than in the 7 to 9 and 10 to 20-day-old animals, whereas T_c of the 30 to 60-day-old and T_c of the adult animals were similar to those recorded up to the age of 6 days.

Part B of table IV contains the statistical analysis of the differences between age-groups. The three younger age-groups differed highly significantly from the 10 to 20-day-old, with the exception of the 7 to 9-day-old, in which before exposure statistical significance was only approached. At the termination of, as well as 15 and 30 min after exposure, T_c of the 7 to 9-day-old differed, however, highly significantly from T_c of the 10 to 20-day-old animals as did those of the younger age-groups. T_c of the 10 to 20-day-old animals was highly significantly lower throughout than T_c of the 30 to 60-day-old animals and that of the adult ones. T_c in the two youngest groups did not differ significantly from that in the two oldest groups, whereas T_c in the 7 to 9-day-old was significantly lower than in the adults before, at, and 15 min after, the termination of exposure.

Table IV

Colonic temperature (T_c) of guinea pigs before, at termination, and 15 min and 30 min after termination of exposure to 12% O_2 at T_a 20 °C. In brackets, number of observations (Part A). Statistical evaluation of differences between age-groups (Part B)

Part A

Age	T_c °C (M ± S.E.) Before exposure to 12% O_2	p	At termination of exposure to 12% O_2	p	15 min after termination of exposure to 12% O_2	p	30 min after termination of exposure to 12% O_2
< 48 hrs	38.55±0.19 (15)	→ < 0.02 ←	37.78±0.27 (15)	→ > 0.2 ←	38.21±0.24 (14)	→ > 0.9 ←	38.24±0.24 (15)
3 to 6 days	38.19±0.13 (24)	→ 0.1 > p > 0.05 ←	37.83±0.15 (24)	→ > 0.2 ←	38.07±0.15 (24)	→ < 0.2 ←	38.29±0.13 (24)
7 to 9 days	37.94±0.14 (21)	→ < 0.05 ←	37.54±0.13 (21)	→ > 0.1 ←	37.81±0.12 (21)	→ > 0.2 ←	38.00±0.13 (21)
10 to 20 days	37.60±0.15 (25)	→ < 0.001 ←	36.85±0.12 (25)	→ > 0.1 ←	37.20±0.14 (25)	→ > 0.4 ←	37.30±0.15 (25)
30 to 60 days	38.25±0.14 (20)	→ < 0.001 ←	37.44±0.17 (20)	→ 0.1 > p > 0.05 ←	37.90±0.17 (20)	→ > 0.6 ←	38.00±0.16 (20)
Adults	38.46±0.13 (15)	→ < 0.05 ←	37.98±0.15 (15)	→ > 0.1 ←	38.28±0.17 (15)	→ > 0.6 ←	38.39±0.19 (15)

Part B

Age-groups	Before exposure to 12% O_2 p	At termination of exposure to 12% O_2 p	15 min after termination of exposure to 12% O_2 p	30 min after termination of exposure to 12% O_2 p
< 48 hrs— 3 to 6 days	$0.1 > p > 0.05$	> 0.8	> 0.6	> 0.8
< 48 hrs— 7 to 9 days	< 0.05	> 0.4	> 0.1	> 0.3
< 48 hrs—10 to 20 days	< 0.001	< 0.01	< 0.001	< 0.01
< 48 hrs—30 to 60 days	> 0.2	> 0.2	> 0.3	> 0.4
< 48 hrs—Adults	> 0.6	> 0.5	> 0.8	> 0.6
3 to 6 days— 7 to 9 days	> 0.2	> 0.1	> 0.1	> 0.1
3 to 6 days—10 to 20 days	< 0.01	< 0.001	< 0.001	< 0.001
3 to 6 days—30 to 60 days	> 0.7	$0.1 > p > 0.05$	> 0.4	> 0.1
3 to 6 days—Adults	> 0.1	> 0.4	> 0.3	> 0.6
7 to 9 days—10 to 20 days	$0.1 > p > 0.05$	< 0.001	< 0.01	< 0.001
7 to 9 days—30 to 60 days	> 0.1	> 0.6	> 0.6	> 0.9
7 to 9 days—Adults	< 0.02	< 0.05	< 0.05	> 0.1
10 to 20 days—30 to 60 days	< 0.01	< 0.01	< 0.01	< 0.01
10 to 20 days—Adults	< 0.001	< 0.001	< 0.001	< 0.001
30 to 60 days—Adults	> 0.2	< 0.05	> 0.1	> 0.1

A somewhat different picture emerged from the analysis of the means of the individual changes in colonic temperature (ΔT_c) presented in Table V. Whereas T_c, as shown in Table IV, tended to decrease up to the age of 20 days and to increase thereafter, ΔT_c-s were very similar in the course of exposure to 12% O_2 in animals aged less than 48 hours, 10 to 20 and 30 to 60 days, whereas in the 3 to 6, and 7 to 9-day-old groups ΔT_c was considerably smaller than in the youngest, or the 10 to 20 and 30 to 60-day-old groups and very similar to ΔT_c in adult animals (Part A). The differences between the ΔT_c-s of the 3 to 6 and 7 to 9-day-old animals on the one hand, and the 10 to 20 and the 30 to 60-day-old ones, on the other hand, were highly significant. In adult animals, ΔT_c in response to 12% O_2 was significantly smaller than in the 10 to 20, and the 30 to 60-day-old animals (Part B). In comparison, inter-age differences of ΔT_c after the termination of hypoxia were rather small. Fifteen min after the termination of exposure to 12% O_2, ΔT_c was significantly greater in the group aged 30 to 60 days than in the three youngest groups and in the adult animals. Thirty min after the termination of hypoxia, the difference in ΔT_c approached statistical significance only between the group aged less than 48 hours and the 30 to 60-day-old group.

Table V

Changes in body temperature (ΔT_c) during exposure to 12% O_2, and between T_c at termination of exposure and in the 15th and 30th min after termination of exposure, and ΔT_c between the 15th and the 30th min after termination of exposure. In brackets, number of observations (Part A). Statistical evaluation of the differences between the age-groups (Part B)

Part A

Age	During exposure to 12% O_2	Between termination of exposure and the 15th min thereafter	Between termination of exposure and the 30th min thereafter	Between the 15th and 30th min after termination of exposure
	ΔT_c °C (M ± S.E.)			
< 48 hrs	-0.70 ± 0.11 < 0.001 (14)	$+0.21 \pm 0.05$ < 0.01 (14)	$+0.39 \pm 0.06$ < 0.001 (15)	$+0.14 \pm 0.04$ < 0.01 (14)
3 to 6 days	-0.36 ± 0.05 < 0.001 (23)	$+0.28 \pm 0.04$ < 0.001 (23)	$+0.46 \pm 0.04$ < 0.001 (23)	$+0.18 \pm 0.03$ < 0.001 (23)
7 to 9 days	-0.40 ± 0.08 < 0.001 (21)	$+0.29 \pm 0.04$ < 0.001 (21)	$+0.48 \pm 0.06$ < 0.001 (21)	$+0.19 \pm 0.04$ < 0.001 (21)
10 to 20 days	-0.75 ± 0.07 < 0.001 (25)	$+0.35 \pm 0.05$ < 0.001 (25)	$+0.45 \pm 0.08$ < 0.001 (25)	$+0.09 \pm 0.05$ 0.1 > p > 0.05 (25)
30 to 60 days	-0.80 ± 0.07 < 0.001 (21)	$+0.48 \pm 0.06$ < 0.001 (19)	$+0.56 \pm 0.07$ < 0.001 (20)	$+0.09 \pm 0.03$ < 0.01 (20)
Adults	-0.48 ± 0.08 < 0.001 (15)	$+0.30 \pm 0.04$ < 0.001 (15)	$+0.41 \pm 0.07$ < 0.001 (15)	$+0.11 \pm 0.04$ < 0.01 (15)

Part B

Age-groups	ΔT_c during exposure to 12% O_2	ΔT_c between termination of 12% O_2 and the 15th min thereafter	ΔT_c between termination of 12% O_2 and the 30th min thereafter	ΔT_c between the 15th and 30th min after termination of exposure to 12% O_2
	p	p	p	p
< 48 hrs — 3 to 6 days	< 0.01	> 0.2	> 0.5	> 0.4
< 48 hrs — 7 to 9 days	0.1 > p > 0.05	> 0.2	> 0.2	> 0.3
< 48 hrs — 10 to 20 days	> 0.7	0.1 > p > 0.05	> 0.6	> 0.4
< 48 hrs — 30 to 60 days	> 0.4	< 0.05	0.1 > p > 0.05	> 0.3
< 48 hrs — Adults	> 0.1	> 0.1	> 0.8	> 0.5
3 to 6 days — 7 to 9 days	> 0.3	> 0.9	> 0.8	> 0.8
3 to 6 days — 10 to 20 days	< 0.001	> 0.2	> 0.8	> 0.1
3 to 6 days — 30 to 60 days	< 0.001	< 0.01	> 0.3	0.1 > p > 0.05
3 to 6 days — Adults	> 0.2	> 0.7	> 0.7	> 0.1
7 to 9 days — 10 to 20 days	< 0.01	> 0.2	> 0.6	> 0.1
7 to 9 days — 30 to 60 days	< 0.01	< 0.01	> 0.3	0.1 > p > 0.05
7 to 9 days — Adults	> 0.7	> 0.5	> 0.4	> 0.1
10 to 20 days — 30 to 60 days	> 0.6	> 0.1	> 0.2	> 0.9
10 to 20 days — Adults	< 0.02	> 0.4	> 0.8	> 0.7
30 to 60 days — Adults	< 0.01	< 0.02	> 0.1	> 0.6

Table VI

Distribution of changes in colonic temperature (ΔT_c) in response to exposure to 12% O_2 at T_c 20 °C (Part A). Chi square test taking ΔT_c of −0.60 °C as the dividing line. In brackets, enumeration data (Part B)

Part A

Age	n	ΔT_c °C								
		−1.40°	−1.40° to −1.20°	−1.20° to −1.00°	−1.00° to −0.80°	−0.80° to −0.60°	−0.60° to −0.40°	−0.40° to −0.20°	−0.20° to +0°	±0° to +0.20°
< 48 hrs	17	4 (23.4%)	2 (11.8%)	1 (5.9%)	2 (11.8%)	2 (11.8%)	3 (17.6%)	1 (5.9%)	2 (11.8%)	—
3 to 6 days	24	—	—	1 (4%)	—	2 (8%)	5 (21%)	10 (42%)	5 (21%)	1 (4%)
7 to 9 days	21	1 (4.8%)	—	1 (4.8%)	1 (4.8%)	1 (4.8%)	5 (24%)	8 (38%)	3 (14%)	1 (4.8%)
10 to 20 days	27	1 (3.5%)	2 (7.5%)	5 (18.5%)	5 (18.5%)	4 (15%)	5 (18.5%)	2 (7.5%)	3 (11%)	—
30 to 60 days	22	—	1 (4.5%)	6 (27%)	3 (14%)	6 (27%)	3 (14%)	1 (4.5%)	2 (9%)	—
Adults	15	—	—	1 (6.7%)	1 (6.7%)	3 (20%)	5 (33.2%)	1 (6.7%)	3 (20%)	1 (6.7%)

Part B

Age-groups		Chi²	p			Chi²	p
< 48 hrs (11 ; 6——17)	3 to 6 days (3 ; 21——24)	9.873	< 0 01	3 to 6 days (3 ; 21——24)	7 to 9 days (4 ; 17——21)	0.027	> 0.8
< 48 hrs (11 ; 6——17)	7 to 9 days (4 ; 17——21)	6.704	< 0.01	3 to 6 days (3 ; 21——24)	10 to 20 days (17 ; 10——27)	11.494	< 0.001
< 48 hrs (11 ; 6——17)	10 to 20 days (17 ; 10——27)	0.016	> 0.8	3 to 6 days (3 ; 21——24)	30 to 60 days (16 ; 6——22)	14.718	< 0.001
< 48 hrs (11 ; 6——17)	30 to 60 days (16 ; 6——22)	0.044	> 0.8	3 to 6 days (3 ; 21——24)	Adults (5 ; 10——15)	1.299	> 0.2
< 48 hrs (11 ; 6——17)	Adults (5 ; 10——15)	2.008	> 0.1				
7 to 9 days (4 ; 17——21)	10 to 20 days (17 ; 10——27)	7.598	< 0.01	10 to 20 days (17 ; 10——27)	30 to 60 days (16 ; 6——22)	0.684	> 0.4
7 to 9 days (4 ; 17——21)	30 to 60 days (16 ; 6——22)	10.509	< 0.01	10 to 20 days (17 ; 10——21)	Adults (5 ; 10——15)	2.392	> 0.1
7 to 9 days (4 ; 17——21)	Adults (5 ; 10——15)	0.298	> 0.5	30 to 60 days (16 ; 6——22)	Adults (5 ; 10——15)	4.111	< 0.05

Table VII

Oxygen consumption (VO_2) of guinea pigs before, during, and after termination of exposure to 8% O_2. Ambient temperature (T_a) 20 °C. In brackets, number of observations (Part A). Statistical evaluation of the differences between the means of different age-groups (Part B) and between VO_2 before and after termination of exposure to 8% O_2 (Part C)

Part A

	VO_2 ml/dm² · min (M ± S.E.)								
Age	Before exposure to 8% O_2	p	During exposure to 8% O_2	p	During the 10th to 15th min after exposure				During the 20th to 30th min after exposure
< 48 hrs	2.10±0.06 (32)	→ < 0.001 ←	1.57±0.06 (29)	→ < 0.001 ←	2.54±0.09 (28)	→	> 0.1	←	2.39±0.07 (32)
3 to 6 days	2.03±0.04 (22)	→ < 0.001 ←	1.59±0.04 (22)	→ < 0.001 ←	2.31±0.07 (22)	→	0.1 > p > 0.05	←	2.15±0.05 (22)
7 to 9 days	2.08±0.04 (20)	→ < 0.001 ←	1.43±0.06 (19)	→ < 0.001 ←	2.42±0.08 (20)	→	0.1 > p > 0.05	←	2.22±0.06 (21)
10 to 20 days	2.09±0.04 (26)	→ < 0.001 ←	1.36±0.04 (26)	→ < 0.001 ←	2.35±0.05 (25)	→	< 0.01	←	2.08±0.06 (26)
30 to 60 days	2.16±0.06 (18)	→ < 0.001 ←	1.50±0.06 (18)	→ < 0.001 ←	2.66±0.07 (19)	→	< 0.001	←	2.31±0.06 (18)
Adults	1.67±0.08 (18)	→ < 0.001 ←	1.28±0.05 (18)	→ < 0.001 ←	1.88±0.06 (22)	→	< 0.02	←	1.68±0.05 (18)

Part B

Age-groups	Before 8% O_2 p	During 8% O_2 p	10th to 15th min after 8% O_2 p	20th to 30th min after 8% O_2 p
< 48 hrs— 3 to 6 days	> 0.3	> 0.7	0.1 > p > 0.05	< 0.01
< 48 hrs— 7 to 9 days	> 0.7	> 0.1	> 0.3	0.1 > p > 0.05
< 48 hrs—10 to 20 days	> 0.8	< 0.01	0.1 > p > 0.05	< 0.01
< 48 hrs—30 to 60 days	> 0.4	> 0.4	> 0.3	> 0.2
< 48 hrs—Adults	< 0.001	< 0.001	< 0.001	< 0.001
3 to 6 days— 7 to 9 days	> 0.3	< 0.05	> 0.1	> 0.3
3 to 6 days—10 to 20 days	> 0.2	< 0.001	> 0.6	> 0.3
3 to 6 days—30 to 60 days	0.1 > p > 0.05	> 0.2	< 0.01	< 0.05
3 to 6 days—Adults	< 0.001	< 0 001	< 0.001	< 0.001
7 to 9 days—10 to 20 days	> 0.8	> 0.3	> 0.4	> 0.1
7 to 9 days—30 to 60 days	> 0.3	> 0.4	< 0.05	> 0.2
7 to 9 days—Adults	< 0.001	0.1 > p > 0.05	< 0.001	< 0.001
10 to 20 days—30 to 60 days	> 0.4	0.1 > p > 0.05	< 0.001	< 0.01
10 to 20 days—Adults	< 0.001	> 0.2	< 0.001	< 0.001
30 to 60 days—Adults	< 0.001	< 0.01	< 0.001	< 0.001

Part C

Age	VO_2 before exposure to 8% O_2 and VO_2 10th to 15th min after termination of exposure p	VO_2 before exposure to 8% O_2 and VO_2 from 20th to 30th min after termination of exposure p
< 48 hrs	< 0.001	< 0.01
3 to 6 days	< 0.02	0.1 > p > 0.05
7 to 9 days	< 0.001	0.1 > p > 0.05
10 to 20 days	< 0.001	> 0.8
30 to 60 days	< 0.001	0.1 > p > 0.05
Adults	< 0.02	> 0.8

Table VI demonstrates the distribution of ΔT_c in response to 12% O_2. Statistical analysis (Part B) agreed, on the whole, with the inter-group analysis of T_c (Table V, Part B). However, whereas in the latter the difference between the groups aged less than 48 hours and 3 to 6 days only approached significance, the difference in the distribution of ΔT_c between these groups proved to be highly significant.

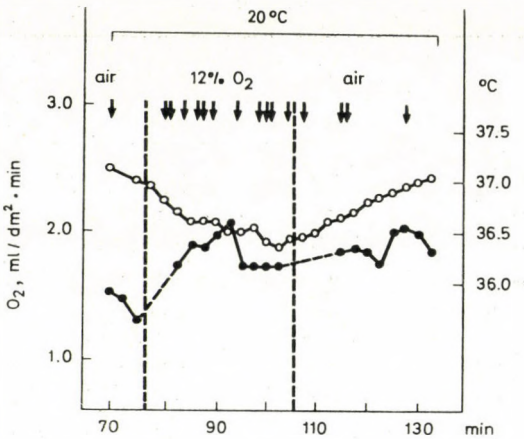

Fig. 1. Guinea pig aged 6 days, body weight 78 g. Oxygen consumption (•–•–•–•) and colonic temperature (○–○–○–○) before, during and after the termination of breathing 12% O_2

Table VIII

Changes in heat production (ΔO_2) during exposure to 8% O_2, and after termination of exposure, based on measurements during the 10th to 15th and the 20th to 30th min, and ΔO_2 between the 10th to 15th min and the 20th to 30th min. In brackets number of observations (Part A). Statistical evaluation of the differences between the different age-groups (Part B)

Part A

	ΔO_2 ml/dm · min (M ± S.E.)				
Age	During exposure to 8% O_2	Between VO_2 during, and VO_2 in the 10th to 15th min after termination of exposure to 8% O_2	p	Between VO_2 during, and VO_2 in the 20th to 30th min after termination of exposure to 8% O_2	Between the 10th to 15th min and the 20th to 30th min after exposure to 8% O_2
< 48 hrs	−0.53±0.05 < 0.001 (32)	+0.93±0.08 → < 0.001 (29)	> 0.2	← +0.82±0.06 < 0.001 (30)	−0.10±0.04 < 0.02 (29)
3 to 6 days	−0.44±0.03 < 0.001 (22)	+0.72±0.05 → < 0.001 (22)	< 0.05	← +0.56±0.05 < 0.001 (22)	−0.18±0.04 < 0.001 (22)
7 to 9 days	−0.65±0.06 < 0.001 (20)	+0.96±0.08 → < 0.001 (19)	0.1 > p > 0.05	← +0.77±0.05 < 0.001 (20)	−0.20±0.07 < 0.02 (19)
10 to 20 days	−0.72±0.06 < 0.001 (26)	+0.99±0.07 → < 0.001 (26)	< 0.01	← +0.72±0.06 < 0.001 (26)	−0.28±0.04 < 0.001 (25)
30 to 60 days	−0.70±0.09 < 0.001 (18)	+1.15±0.08 → < 0.001 (17)	< 0.05	← +0.84±0.08 < 0.001 (17)	−0.31±0.05 < 0.001 (17)
Adults	−0.38±0.05 < 0.001 (21)	+0.64±0.08 → < 0.001 (21)	< 0.01	← +0.38±0.05 < 0.001 (21)	−0.26±0.06 < 0.001 (21)

Part B

Age-groups	During exposure p	10th to 15th min after termination of exposure p	20th to 30th min after termination of exposure p	From the 10th to 15th min after termination of exposure to the 20th to 30th min p
< 48 hrs— 3 to 6 days	> 0.1	< 0.05	< 0.01	> 0.1
< 48 hrs— 7 to 9 days	> 0.1	> 0.7	> 0.7	> 0.2
< 48 hrs—10 to 20 days	< 0.02	> 0.5	> 0.2	< 0.01
< 48 hrs—30 to 60 days	> 0.1	> 0.1	> 0.6	< 0.001
< 48 hrs—Adults	< 0.05	< 0.02	< 0.001	< 0.05
3 to 6 days— 7 to 9 days	< 0.01	< 0.02	< 0.01	> 0.8
3 to 6 days—10 to 20 days	< 0.001	< 0.01	< 0.05	0.1 > p > 0.05
3 to 6 days—30 to 60 days	< 0.01	< 0.001	< 0.01	< 0.02
3 to 6 days— Adults	> 0.3	> 0.4	< 0.02	> 0.2
7 to 9 days—10 to 20 days	> 0.4	> 0.7	> 0.4	> 0.3
7 to 9 days—30 to 60 days	> 0.6	> 0.1	> 0.4	> 0.1
7 to 9 days—Adults	< 0.01	< 0.01	< 0.001	> 0.5
10 to 20 days—30 to 60 days	> 0.8	> 0.2	> 0.2	> 0.3
10 to 20 days—Adults	< 0.001	< 0.01	< 0.001	> 0.7
30 to 60 days—Adults	< 0.01	< 0.001	< 0.001	> 0.3

Tables I to VI demonstrated convincingly that exposure to 12% O_2 reduced T_c significantly even when heat production failed to decrease or even increased. The experiment recorded in Fig. 1 serves as an example.

Exposure to 8% O_2

Heat production. Table VII summarizes the data for VO_2 obtained by exposing the guinea pigs to 8% O_2. Exposure to 8% O_2 reduced VO_2 highly significantly in all age-groups. During the 10th to 15th min after termination of exposure VO_2 highly significantly exceeded not only the level recorded during exposure but, as shown by Part C, also the pre-hypoxic level. Twenty to 30 min after termination of exposure VO_2 was lower in all age-groups than during the 10th to 15th min after exposure. The differences were unequivocally significant in the three older groups and nearly so in the three younger ones. However, even during the 20th to 30th min after termination of exposure VO_2 exceeded, with the exception of the 10 to 20-day-old and the adult animals, the pre-exposure level, the difference being significant in the youngest group and approaching statistical significance in the others (Part C).

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Table IX

Distribution of changes in oxygen consumption (ΔO_2) in response to exposure to 8% O_2 at T_a 20 °C (Part A). Chi square test taking ΔO_2 of −0.55 ml/dm² · min as the dividing line. In brackets, enumeration data (Part B)

Part A

		Δ O_2 ml/dm² · min							
Age	n	−1.25 to −1.00	−1.00 to −0.85	−0.85 to −0.70	−0.70 to −0.55	−0.55 to −0.40	−0.40 to −0.25	−0.25 to −0.10	−0.10 to ±0.0
48 hrs	32	1 (3.1%)	4 (12.5%)	4 (12.5%)	4 (12.5%)	8 (25%)	7 (22%)	2 (6.2%)	2 (6.2%)
3 to 6 days	22	—	—	2 (9%)	3 (14%)	4 (18%)	11 (50%)	2 (9%)	—
7 to 9 days	20	4 (20%)	1 (5%)	—	5 (25%)	8 (40%)	2 (10%)	—	—
10 to 20 days	26	6 (23%)	1 (4%)	4 (15%)	7 (27%)	5 (19%)	3 (12%)	—	—
30 to 60 days	19	4 (22%)	3 (16%)	2 (10%)	3 (16%)	2 (10%)	3 (16%)	—	2 (10%)
Adults	18	—	1 (5.5%)	—	1 (5.5%)	8 (44.5%)	3 (17%)	4 (22%)	1 (5.5%)

Part B

Age-groups		Chi²	p			Chi²	p
< 48 hrs (13 ; 19——32)	3 to 6 days (5 ; 17——22)	1.199	> 0.2	3 to 6 days (5 ; 17——22)	7 to 9 days (10 ; 10——20)	2.395	> 0.1
< 48 hrs (13 ; 19——32)	7 to 9 days (10 ; 10——20)	0.161	> 0.5	3 to 6 days (5 ; 17——22)	10 to 20 days (18 ; 8——26)	8.407	< 0.01
< 48 hrs (13 ; 19——32)	10 to 20 days (18 ; 8——26)	3.631	0.1 > p > 0.05	3 to 6 days (5 ; 17——22)	30 to 60 days (12 ; 7——19)	5.238	< 0.05
< 48 hrs (13 ; 19——32)	30 to 60 days (12 ; 7——19)	1.114	> 0.2	3 to 6 days (5 ; 17——22)	Adults (2 ; 16——18)	0.342	> 0.5
< 48 hrs (13 ; 19——32)	Adults (2 ; 16——18)	3.476	0.1 > p > 0.05				
7 to 9 days (10 ; 10——20)	10 to 20 days (18 ; 8——26)	1.074	> 0.3	10 to 20 days (18 ; 8——26)	30 to 60 days (12 ; 7——19)	0.016	> 0.8
7 to 9 days (10 ; 10——20)	30 to 60 days (12 ; 7——19)	0.267	> 0.6	10 to 20 days (18 ; 8——26)	Adults (2 ; 16——18)	12.319	< 0.001
7 to 9 days (10 ; 10——20)	Adults (2 ; 16——18)	5.002	< 0.05	30 to 60 days (12 ; 7——19)	Adults (2 ; 16——18)	8.505	< 0.01

	Chi²	p
<10 days (28 ; 46——74) VS. 10 to 20 + 30 to 60 days (30 ; 15——45)	8.039	<0.01

Table X

Colonic temperature (T_c) of guinea pigs before, at termination, and 15 min and 30 min after termination, of exposure to 8% O_2 at T_a 20 °C. In brackets, number of observations (Part A). Statistical evaluation of differences between the different age-groups (Part B)

Part A

Age	Before exposure to 8% O_2	p	At termination of exposure to 8% O_2	p	15 min after termination of exposure to 8% O_2	p	30 min after termination of exposure to 8% O_2
< 48 hrs	38.65±0.13 (31)	→ < 0.001 ←	36.35±0.14 (31)	→ < 0.001 ←	37.96±0.19 (32)	→ < 0.05 ←	38.53±0.18 (31)
3 to 6 days	38.27±0.14 (22)	→ < 0.001 ←	36.59±0.13 (22)	→ < 0.001 ←	37.64±0.16 (22)	→ < 0.001 ←	38.41±0.13 (22)
7 to 9 days	38.00±0.13 (21)	→ < 0.001 ←	35.98±0.15 (21)	→ < 0.01 ←	36.90±0.22 (21)	→ < 0.001 ←	37.92±0.14 (21)
10 to 20 days	37.31±0.15 (25)	→ < 0.001 ←	35.02±0.17 (25)	→ < 0.01 ←	35.95±0.22 (26)	→ < 0.01 ←	36.82±0.23 (25)
30 to 60 days	38.08±0.15 (21)	→ < 0.001 ←	36.12±0.18 (21)	→ < 0.001 ←	37.23±0.20 (20)	→ > 0.1 ←	37.65±0.18 (21)
Adults	38.77±0.13 (22)	→ < 0.001 ←	37.61±0.16 (22)	→ < 0.05 ←	38.16±0.17 (23)	→ > 0.4 ←	38.45±0.30 (22)

Part B

Age-groups	Before exposure to 8% O_2 p	At termination of exposure to 8% O_2 p	15 min after termination of 8% O_2 p	30 min after termination of 8% O_2 p
< 48 hrs— 3 to 6 days	$0.1 > p > 0.05$	> 0.2	$0.1 > p > 0.05$	> 0.5
< 48 hrs— 7 to 9 days	< 0.001	$0.1 > p > 0.05$	< 0.001	< 0.02
< 48 hrs—10 to 20 days	< 0.001	< 0.001	< 0.01	< 0.001
< 48 hrs—30 to 60 days	< 0.01	> 0.1	< 0.02	< 0.01
< 48 hrs—Adults	> 0.5	< 0.01	> 0.4	> 0.8
3 to 6 days— 7 to 9 days	> 0.1	< 0.01	< 0.01	< 0.02
3 to 6 days—10 to 20 days	< 0.001	< 0.001	< 0.001	< 0.001
3 to 6 days—30 to 60 days	> 0.3	< 0.05	> 0.1	< 0.01
3 to 6 days—Adults	< 0.02	< 0.001	< 0.05	> 0.9
7 to 9 days—10 to 20 days	< 0.01	< 0.001	< 0.01	< 0.001
7 to 9 days—30 to 60 days	> 0.6	> 0.5	> 0.2	> 0.2
7 to 9 days—Adults	< 0.01	< 0.001	< 0.001	> 0.1
10 to 20 days—30 to 60 days	< 0.001	< 0.001	< 0.001	< 0.01
10 to 20 days—Adults	< 0.001	< 0.001	< 0.001	< 0.001
30 to 60 days—Adults	< 0.01	< 0.001	< 0.01	< 0.05

Part B of table VII contains the inter-group analysis of the data in Part A. Oxygen consumption of adult animals was lower throughout than that of any of the younger age-groups. During hypoxia the difference was not significant between the 10 to 20-day-old and the adults, and approached statistical significance only between the 7 to 9-day-old and the adults.

Table VIII demonstrates the changes in VO_2 (ΔO_2) during, and after termination of exposure to 8% O_2 based on the mean of differences in individual observations. The changes during, as well as after, termination of exposure were highly significant. The decrease in VO_2 between the 10th to 15th min and the 20 th to 30th min after termination of exposure which failed to reach statistical snigificance in the three youngest age-groups when mean VO_2 served as the basis of comparison, proved nevertheless significant when calculation was based on the mean of ΔO_2 of individual observations.

The most prominent differences between the various age-groups were that the reduction in heat production during exposure was smaller in the two youngest age-groups than in the groups aged 7 to 9, 10 to 20, and 30 to 60 days, and that the post-exposure increase in heat production was smaller in the 3 to 6-day-old than in any other group, although greater than in the adult animals. Statistical analysis of the differences between the age-groups

(Part B) showed that at the age of less than 48 hours ΔO_2 during exposure was significantly smaller than at the age of 10 to 20 days, and that in the 3 to 6-day-old ΔO_2 was significantly smaller than in the 7 to 9, the 10 to 20, and the 30 to 60-day-old animals. After the termination of exposure, ΔO_2 was significantly smaller in the 3 to 6-day-old than in any of the other young age-groups.

Table IX presents the distribution of changes in response to exposure to 8% O_2. The distribution of ΔO_2 of the 3 to 6-day-old differed significantly from that of the groups aged 10 to 20 days and 30 to 60 days, and the distribution in the combined three younger groups differed highly significantly from that in the combined groups aged 10 to 20 and 30 to 60 days. The difference in the distribution of ΔO_2 between the group aged less than 48 hours and of the adult animals approached statistical significance, while definitely no difference was observed between the age of 3 to 6 days and the adults. The 7 to 9, 10 to 20, and 30 to 60-day-old groups differed significantly in the distribution of ΔO_2 from that in the adult animals.

Body temperature. The data for T_c in Table X represent the counterpart to those for VO_2 in Table VII. Within one age-group, T_c before, at termination

Table XI

Changes in body temperature (ΔT_c) during exposure to 8% O_2, and between T_c at termination of exposure and the 15th and 30th min after termination of exposure, and ΔT_c between the 15th and 30th min after termination of exposure. In brackets, number of observations (Part A). Statistical evaluation of the differences between the age-groups (Part B)

Part A

Age	ΔT_c °C (M ± S.E.) During exposure to 8% O_2	Between termination of exposure and the 15th min thereafter	Between termination of exposure and the 30th min thereafter	Between the 15th and 30th min after termination of exposure
< 48 hrs	−2.30±0.13 (29)	+1.51±0.11 (30)	+2.17±0.13 (29)	+0.65±0.05 (30)
3 to 6 days	−1.68±0.12 (24)	+1.05±0.09 (22)	+1.82±0.12 (22)	+0.73±0.06 (22)
7 to 9 days	−2.01±0.11 (21)	+1.01±0.07 (21)	+1.89±0.10 (21)	+0.87±0.09 (21)
10 to 20 days	−2.25±0.15 (26)	+1.02±0.08 (24)	+1.83±0.15 (24)	+0.82±0.08 (24)
30 to 60 days	−1.85±0.09 (21)	+1.08±0.06 (20)	+1.56±0.07 (20)	+0.47±0.06 (20)
Adults	−1.16±0.08 (22)	+0.50±0.04 (21)	+0.84±0.06 (22)	+0.30±0.05 (21)

Part B

Age-groups	ΔT_c during exposure to 8% O_2 p	ΔT_c between termination of 8% O_2 and the 15th min thereafter p	ΔT_c between termination of 8% O_2 and the 30th min thereafter p	ΔT_c between the 15th and 30th min after termination of 8% O_2 p
< 48 hrs— 3 to 6 days	< 0.01	< 0.01	0.1 > p > 0.05	> 0.3
< 48 hrs— 7 to 9 days	< 0.05	< 0.001	0.1 > p > 0.05	< 0.05
< 48 hrs— 10 to 20 days	> 0.8	< 0.01	0.1 > p > 0.05	0.1 > p > 0.05
< 48 hrs— 30 to 60 days	< 0.01	< 0.01	< 0.001	< 0.05
< 48 hrs— Adults	< 0.001	< 0.001	< 0.001	< 0.001
3 to 6 days— 7 to 9 days	> 0.1	> 0.7	> 0.6	> 0.2
3 to 6 days— 10 to 20 days	< 0.01	> 0.8	> 0.9	> 0.3
3 to 6 days— 30 to 60 days	> 0.2	> 0.7	0.1 > p > 0.05	< 0.01
3 to 6 days— Adults	< 0.001	< 0.001	< 0.001	< 0.001
7 to 9 days— 10 to 20 days	< 0.05	> 0.9	> 0.7	> 0.6
7 to 9 days— 30 to 60 days	> 0.5	> 0.5	< 0.02	< 0.001
7 to 9 days— Adults	< 0.001	< 0.001	< 0.001	< 0.001
10 to 20 days— 30 to 60 days	< 0.05	> 0.5	> 0.1	< 0.01
10 to 20 days— Adults	< 0.001	< 0.001	< 0.001	< 0.001
30 to 60 days— Adults	< 0.001	< 0.001	< 0.001	< 0.05

of, exposure to 8% O_2, and at the 15th and 30th min thereafter, differed more markedly than in the corresponding observations with 12% O_2.

Accordingly, the differences were highly significant with the exception of the 30 to 60-day-old and the adult animals, in which T_c in the 15th and in the 30th min after termination of exposure failed to differ significantly, although the trend of T_c was similar to that in the other age-groups. Essentially the same applies to the differences between age-groups. As shown by Part B of Table X these were in many instances highly significant when the corresponding differences in the course of exposure to 12% O_2 (Table IV, Part B) were not, or only approached significance.

Table XI records the changes in T_c (ΔT_c) during and after termination of exposure to 8% O_2. Within each age-group all the changes were statistically highly significant ($p < 0.001$). In the course of exposure to 8% O_2, ΔT_c was greatest, and practically equal, at the ages of less than 48 hours and 10 to 20 days, whereas in all other age-groups ΔT_c was significantly smaller (Part B). The increase in T_c during the first 15 min after termination of exposure was highly significantly greater in the youngest age-group than in any of the others.

Table XII

Distribution of changes in colonic temperature (ΔT_c) in response to exposure to 8% O_2 (Part A). Chi square test taking ΔT_c of −2.0 °C as the dividing line. In brackets, enumeration data (Part B)

Part A

Age	n	ΔT_c °C						
		−3.5° to −3.0°	−3.0° to −2.5°	−2.5° to −2.0°	−2.0° to −1.5°	−1.5° to −1.0°	−1.0° to −0.5°	−0.5° to ±0°
< 48 hrs	33	4 (12%)	5 (15%)	16 (49%)	5 (15%)	2 (6%)	1 (3%)	—
3 to 6 days	22	—	3 (14%)	4 (18%)	6 (27%)	7 (32%)	2 (9%)	—
7 to 9 days	21	1 (5%)	3 (14%)	5 (24%)	9 (43%)	3 (14%)	—	—
10 to 20 days	26	3 (11%)	7 (27%)	7 (27%)	6 (23%)	2 (8)%	—	1 (4%)
30 to 60 days	21	—	1 (4%)	8 (38%)	6 (29%)	6 (29%)	—	—
Adults	22	—	—	—	5 (23%)	11 (50%)	6 (27%)	—

Part B

Age-groups		Chi²	p			Chi²	p
< 48 hrs (25 ; 8——33)	3 to 6 days (7 ; 15——22)	8.746	< 0.01	3 to 6 days (7 ; 15——22)	7 to 9 days (9 ; 12——21)	0.195	< 0.6
< 48 hrs (25 ; 8——33)	7 to 9 days (9 ; 12——21)	4.572	< 0.05	3 to 6 days (7 ; 15——22)	10 to 20 days (17 ; 9——26)	4.152	< 0.05
< 48 hrs (25 ; 8——33)	10 to 20 days (17 ; 9——26)	0.335	> 0.5	3 to 6 days (7 ; 15——22)	30 to 60 days (9 ; 12——21)	0.195	> 0.6
< 48 hrs (25 ; 8——33)	30 to 60 days (9 ; 12——21)	4.624	< 0.05	3 to 6 days (7 ; 15——22)	Adults (0 ; 22——22)	5.143	< 0.05
< 48 hrs (25 ; 8——33)	Adults (0 ; 22——22)	27.576	< 0.001				
7 to 9 days (9 ; 12——21)	10 to 20 days (17 ; 9——26)	1.533	> 0.2	10 to 20 days (17 ; 9——26)	30 to 60 days (9 ; 12——21)	1.535	> 0.2
7 to 9 days (9 ; 12——21)	30 to 60 days (9 ; 12——21)	0.000	> 0.9	10 to 20 days (17 ; 9——26)	Adults (0 ; 22——22)	19.549	< 0.001
7 to 9 days (9 ; 12——21)	Adults (0 ; 22——22)	9.453	< 0.01				
				30 to 60 days (9 ; 12——21)	Adults (0 ; 22——22)	9.453	< 0.01

Rather strikingly, ΔT_c in the course of the first 15 min after restoration of air was almost identical in the groups aged 3 to 6, 7 to 9, 10 to 20, and 30 to 60 days, and the same holds for the 30 min following the termination of exposure and, accordingly, for the second 15 min period in the case of the 3 to 6, 7 to 9, and 10 to 20-day-old groups. In the adult animals, ΔT_c was significantly smaller than in any one of the younger groups during, as well as after termination of exposure to 8% O_2.

Table XII demonstrates the distribution of ΔT_c in response to exposure to 8% O_2. The results of enumeration data analysis (Part B) correspond to those of the statistical analysis of ΔT_c in Table XI, Part B.

Discussion

The most striking feature of exposure to 12% O_2 was the discrepancy between the changes in heat production (ΔO_2) and the concomitant changes in colonic temperature (ΔT_c) as demonstrated most conspicuously by comparing Tables III and VI, and in the individual experiment shown in Fig. 1. This discrepancy, demonstrated previously for adult guinea pigs (FARKAS and DONHOFFER 1973), was even more impressive in the newly born. At the age of less than 48 hours, VO_2 increased in response to 12% O_2 by more than 0.1 ml/dm^2 · min in about 50%, at the age of 3 to 6 days in approximately 75% of the observations (Table III), whereas T_c concomitantly fell by more than -0.20 °C in 88% of the animals aged less than 48 hours, and in 75% of the 3 to 6-day-old ones. Essentially similar were the results for the older age-groups. Corresponding observations were made on new-born rabbits exposed at T_a 20 °C to 6% CO_2 (VÁRNAI et al. 1971b; DONHOFFER et al. 1973).

The interpretation of the fall in body temperature as a consequence of reduced heat production, as proposed even recently by BLATTEIS (1972) has, therefore, to be abandoned. The fact that T_c and VO_2 are falling concurrently when the animals are exposed to more severe hypoxia does not affect this conclusion. As has been pointed out earlier (FARKAS and DONHOFFER 1973), the lack of a decrease, or even the presence of an increase in heat production during hypoxia cannot be interpreted to indicate that thermoregulatory heat production was not impaired, since a similar fall in body temperature, e.g. during cold exposure, would have elicited a very marked increase in every instance.

Another point of interest is the fall in T_c in response to both 12% and 8% O_2. The group aged less than 48 hours responded to 12% O_2 with a fall in T_c twice that of the 3 to 6-day-old ($p < 0.01$), and exceeded almost significantly the ΔT_c of the 7 to 9-day-old animals ($0.1 < p < 0.05$). In the case of exposure to 8% O_2 the fall in body temperature (Table XI) was also signifi-

cantly greater in the youngest group than in either the 3 to 6-day-old ($p < 0.01$) or the 7 to 9-day-old group ($p < 0.05$).

This result was rather unexpected, since in response to transfer from T_a 30° to 20 °C, the fall in T_c was by far the smallest at the age of less than 48 hours as compared to the older new-born groups (FARKAS et al. 1974), which is consistent with the observation of BRÜCK and WÜNNENBERG (1965a) that the level of T_c at the age of 1 to 3 days exceeded in the cold the T_c of older guinea pigs up to the age of 21 days. It has to be concluded that the reponse of the central thermoregulatory system to the two stimuli, cold and hypoxia, changes during the neonatal period profoundly and in the opposite direction. Other factors, like pre-exposure levels of T_c and differences in body weight, can be excluded. Although the pre-exposure levels of T_c were lower at the age of 3 to 6 and 7 to 9 days than in the youngest group, T_c was even lower in the 10 to 20-day-old which responded to both 12% and 8% O_2 with a fall in T_c of a magnitude equal to that of animals aged less that 48 hours (Tables IV, V, X and XI). The similarity of ΔT_c in hypoxia at the age of less than 48 hours and at 10 to 20 days eliminates body weight as a decisive factor.

The changes in T_c during hypoxia, as well as after termination of hypoxia, deserve attention. In response to both 12% and 8% O_2, ΔT_c was significantly smaller at the age of 3 to 6 and 7 to 9 days than in the 10 to 20-day-old group, whereas the ΔT_c-s after termination of hypoxia were identical, and after exposure to 8% O_2, the same was the case in the 30 to 60-day-old animals (Tables V and XI). Post-hypoxic mean ΔT_c-s of the groups aged 3 to 6, 7 to 9, and 10 to 20 days appear, therefore, to be related neither to the mean T_c at the termination of hypoxia (Tables IV and X), nor to ΔT_c during hypoxia. The correlation was, however, significant between ΔT_c in response to 8% O_2 and ΔT_c after the termination of hypoxia within one and the same age-group (unpublished results).

Changes in the response of heat production and deep body temperature during the neonatal period to exposure to cold, hypoxia, and hypercapnia have been demonstrated in the rat (VÁRNAI and DONHOFFER 1970; VÁRNAI et al. 1970a, b), and the rabbit (VÁRNAI et al. 1970c, 1971a, b) and for exposure to cold, in the guinea pig (BRÜCK and WÜNNENBERG 1965a; FARKAS et al. 1974), and in some further mammalian species. The changes in thermoregulatory responses during the neonatal period in the guinea pig appear to be unique, and to differ profoundly from those of the other species. In the other species the changes are unidirectional, and approach gradually the responses characteristic for the adult animal. In contrast, the change in colonic temperature in response to cold resembles at the age of less than 48 hours more closely that of adult animals, and is much smaller than the decrease in T_c in anyof the older new-born groups (FARKAS et al. 1974). The

change in T_c in response to hypoxia was also bi-directional: the fall in T_c was significantly smaller at the age of 3 to 6 and 7 to 9 days, than either at the age of less than 48 hours or in the 10 to 20-day-old animals. Similarly, the fall in heat production in response to exposure to 8% O_2 was somewhat smaller in the 3 to 6-day-old than in the younger animals, and highly significantly smaller than in the groups aged 7 to 9, 10 to 20, and 30 to 60 days, and very close to that observed in adult animals (Table VIII).

The results obtained at the age of 30 to 60 days were of special interest. The fall in heat production in response to exposure to 8% O_2 was as great as that of the 7 to 9, and 10 to 20-day-old, and almost twice as great as that of the adult animals. The fall in body temperature during exposure to 8% O_2 was almost identical in the groups aged 3 to 6, 7 to 9, and 30 to 60 days, and equally highly significantly greater than in adult animals (Table XI). In response to exposure to 12% O_2, the fall in T_c was at the age of 30 to 60 days twice that recorded at the age of 3 to 6, and 7 to 9 days, and highly significantly greater than in the adults, whereas no significant difference could be demonstrated between the adults and the groups aged 3 to 6, and 7 to 9 days. This was the more remarkable, since in other aspects, e.g. body weight, 30 to 60-day-old animals are well advanced on the way to adulthood. These observations constitute further evidence for the conclusion arrived at earlier (FARKAS et al. 1974) that the changes in thermoregulatory responses during the neonatal period are due to changes in the working of the central cybernetic system governing the mechanisms of heat production and heat loss.

Limitations of space have precluded a discussion of all aspects of the data presented. Some of these have been pointed out in Results, others can be deduced from the Tables, and correlational analysis between different variables had to be reserved for a subsequent communication.

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EFFECT OF PHYSIOLOGICAL DOSES OF VASOPRESSIN AND OXYTOCIN ON AVOIDANCE AND EXPLORATORY BEHAVIOUR IN RATS

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The effect of small doses of vasopressin (30 mU/kg) and oxytocin (30 mU/kg) has been studied on active and passive avoidance behaviour and exploratory activity. Neither vasopressin nor oxytocin had any effect on acquisition of active avoidance behaviour, while extinction was delayed after vasopressin and facilitated after oxytocin treatment.

In passive shock avoidance response (PSAR), vasopressin treatment shortened the PSAR in repeated tests. Oxytocin was ineffective on PSAR. In open-field test, only the defecation rate was significantly lowered by vasopressin.

Oxytocin and vasopressin in physiological doses were shown to influence brain function and hypothalamic multi-unit activity in rats and rabbits (SCHWARZBERG 1968; SCHWARZBERG and UNGER 1970; UNGER and SCHWARZBERG 1970; SCHWARZBERG et al. 1973). Oxytocin inhibited while vasopressin facilitated neuronal multi-unit activity (SCHULZ et al. 1971, 1973).

In behavioural studies, vasopressin in high doses caused a delay in the extinction of active avoidance behaviour and of the passive avoidance reaction (DE WIED 1965, 1971, 1973; DE WIED and BOHUS 1966; BOHUS 1971).

The aim of the present experiments was to study the action of physiological doses of oxytocin and vasopressin on active and passive behaviour as well as an exploratory activity.

Methods

In the experiments, adult male R-Amsterdam albino rats were used, weighing 100—250 g. The animals were kept on a standard light–dark schedule (12 : 12), with the light period starting at 6.00 a.m. Food and water were allowed ad libitum.

Active avoidance. Active avoidance conditioning was performed in a platform jumping conditioning apparatus (TELEGDY et al. 1968; TELEGDY and RÓZSAHEGYI 1971). The conditional stimulus (CS) was the light of a 45 W electric bulb, while the unconditional stimulus (US) was an electric shock of 1.0 mA delivered through the grid floor of the apparatus on the paws of the rat. Ten conditioning trials were given daily in a fixed time schedule with 60 sec average intertrial intervals. The criterion of learning was 80% or more conditioned response (CAR) in three consecutive days. After reaching this criterion, extinction trials were run without US. The criterion of extinction was a 20% level of performance reached in three consecutive days.

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Passive avoidance. Passive avoidance behaviour was tested according to BOHUS et al. (1970) and KOVÁCS et al. (1974). The box consisted of two compartments, a light and a dark one connected by a hole. On the first day the untrained animals were placed in the light compartment for 180 sec and those entering the dark within 60 sec were selected for further studies. On the second day, the animals entering the dark compartment received an electric shock of 1.0 mA for 1 sec. On the following days the animals were tested for the time spent in the light compartment; this was expressed as an index of passive shock avoidance response (PSAR).

Exploratory activity. Exploratory activity was measured with the same animals as in the PSAR experiments. They were placed in an open-field box which consisted of 36 squares measuring 10×10 cm each, during the first three days immediately before PSAR testing. Activity was characterized by the total number of squares explored, the total number of rearings and groomings and the defecation boluses produced during 3 min sessions for three consecutive days.

Treatment. The animals received 30 mU/kg lysine-vasopressin (Sandoz, Basel) or 30 mU/kg synthetic oxytocin (Richter, Budapest) intraperitoneally in a volume of 0.5 ml 15—25 min prior testing. Control animals for vasopressin received the same volume of Ringer's solution and for oxytocin treatment the vehicle of oxytocin (Richter, Budapest). In the passive avoidance test, all drugs were given daily over a period of 6 days; in active avoidance conditioning, daily during acquisition and extinction.

Statistical analysis of the data was carried out by Mann and Whitney's U test. The probability level of 0.05 or less was accepted as a significant difference.

Results

Neither vasopressin nor oxytocin had any effect on acquisition of the active avoidance reflex. However, in the extinction trials, vasopressin caused a significantly higher rate of CARs than that observed in the control group ($p < 0.01$).

Oxytocin resulted in a lower rate of CARs ($p < 0.02$) than the vehicle treatment (Fig. 1).

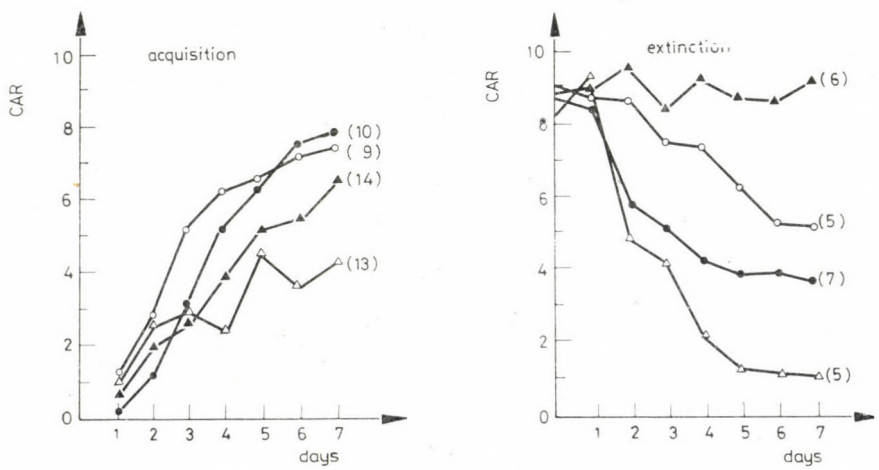

Fig. 1. Effect of vasopressin and oxytocin on acquisition and extinction of conditioned active avoidance reflex in rats. ▲—▲: vasopressin-treated group; △—△: Ringer's solution-treated group; ●—●: oxytocin-treated group; ○—○ vehicle for oxytocin-treated group. In brackets the number of animals used. CAR: conditioned avoidance response

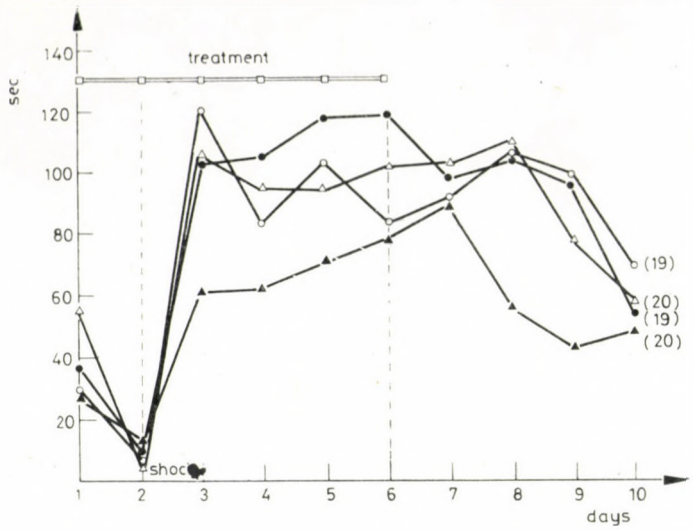

Fig. 2. Effect of vasopressin and oxytocin on the passive shock avoidance response in rats. Symbols as in Fig. 1

In passive avoidance behaviour, the PSAR of the vasopressin-treated rats was shortened in comparison to the control group ($p < 0.02$). Oxytocin showed a tendency to lengthen the PSAR, but the differences were not significant. The vasopressin-treated group showed a shortened PSAR after cessation of treatment, but in this period the differences were not significant. After cessation of oxytocin treatment there were no differences between the previously drug-treated animals and the vehicle-treated control animals (Fig. 2).

In open-field activity there were no differences in ambulation rate, grooming and rearing activity. The defecation rate in the vasopressin-treated group was significantly lower than in the controls ($p < 0.05$; Table I).

Table I

Effect of lysine-vasopressin and oxytocin on open-field activity

Treatment	No. of rats	Ambulation	Rearing	Grooming	Defecation
Ringer-controls	20	49.7±13*	9.5±2.0	12.9±2.1	2.9±0.2
Lysine-vasopressin	20	54.2±7	8.2±0.5	11.1±0.4	0.9±0.1**
Vehicle-controls	20	55.6±13	11.1±2.8	10.8±2.4	1.8±0.2
Oxytocin	20	56.5±13	8.4±2.4	10.6±2.5	1.8±0.2

* mean ± S.E.
** $p < 0.05$

Discussion

Physiological doses of vasopressin resulted in a similar delay of extinction of active avoidance behaviour as described earlier with higher doses of the hormone (DE WIED 1965, 1971, 1973; DE WIED and BOHUS 1966; BOHUS 1971; LANDE et al. 1971; BOHUS et al. 1972) and the PSAR was shortened. In passive avoidance, BOHUS (1971) observed an increased avoidance latency after pitressin administration. The discrepancy may have been due to differences in the preparations, application schedules, and the behavioural methods used.

Oxytocin treatment facilitated the extinciton of active avoidance reflex and showed a tendency to influence the PSAR in an opposite direction than did vasopressin. To our knowledge this is the first evidence that oxytocin and vasopressin should influence higher nervous function in an opposite manner.

The action of vasopressin and oxytocin is not mediated via the changed general motor activity. If, however, one accepts that the defecation rate is an index of emotionality, vasopressin might act at this level, too.

Although earlier no effect on animal behaviour could be observed after oxytocin treatment, some electrophysiological data (SCHULZ et al. 1971, 1973; SCHWARZBERG 1968; SCHWARZBERG and UNGER 1970; SCHWARZBERG et al. 1973; UNGER and SCHWARZBERG 1970) clearly indicate that vasopressin and oxytocin in low doses are influencing neuronal single and multi-unit activity and the EEG in an opposite direction in both rats and rabbits.

On the basis of the present data and those reported by the above authors it is tempting to suggest that posterior pituitary hormones like vasopressin and oxytocin have a modulating effect on brain function in mammals. Further investigations are, however, needed to clarify the details of this action.

Acknowledgement

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EFFECT OF MIDBRAIN RAPHE LESION ON DIURNAL AND STRESS-INDUCED CHANGES IN SEROTONIN CONTENT OF DISCRETE REGIONS OF THE LIMBIC SYSTEM AND IN ADRENAL FUNCTION IN THE RAT

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Serotonin level in the mesencephalon, hippocampus, septum, amygdala and hypothalamus, and plasma corticosterone content have been measured at 08, 16 and 24 hours in midbrain raphe-lesioned animals.

Following electrolytic lesion of the midbrain raphe nuclei, the diurnal variations of brain serotonin and plasma corticosterone level disappeared, the serotonin level decreased by 50—75% in each brain area investigated and the plasma corticosterone level increased about threefold of the normal morning level.

The stress response after ether and electric shock was facilitated in raphe-lesioned animals and return to the starting value was delayed. The hypothalamic serotonin level was lower than the control and did not change during stress.

The results suggest that the mesencephalic raphe nuclei are responsible for the serotonin rhythm of the limbic system which shows a negative correlation with pituitary–adrenal function.

A number of data show that the serotoninergic system in the brain originates mainly from the raphe nuclei. The pericarya of the serotonin-containing neurones are located mostly — if not exclusively — in the midbrain raphe nuclei (DAHLSTRÖM et al. 1965; FUXE 1965; ANDÉN et al. 1966; AGHAJANIAN et al. 1969). Electrolytic destruction of the midbrain raphe leads to a remarkable decrease in the serotonin content of the brain (HELLER and MOORE 1965; JOUVET et al. 1966; KOSTOWSKI et al. 1968; ROSECRANS and SHEARD 1969; KUHAR et al. 1971, 1972) and a decrease in the activity of tryptophan hydroxylase, the rate-limiting enzyme of serotonin synthesis (LOVENBERG et al. 1968) in the brain stem (POIRIER et al. 1969; KUHAR et al. 1971, 1972).

Earlier we have presented evidence of the inhibitory role of the serotoninergic system on hypothalamo–pituitary–adrenal function. Serotonin was able to inhibit the hypothalamic tissue-induced ACTH release in vitro (VERMES et al. 1972; TELEGDY and VERMES 1973). Intraventricular injection or implantation of serotonin into the baso-medial hypothalamus inhibited the stress response or the compensatory adrenal hypertrophy and hypersecretion (VERMES and TELEGDY 1972; VERMES et al. 1973).

In the present study the effect of midbrain raphe lesion on diurnal serotonin rhythm of the mesencephalon, hippocampus, septum, amygdala and hypothalamus, on the plasma corticosterone level, and on stress-induced changes in hypothalamic serotonin and plasma corticosterone level was investigated.

Methods

Adult male albino CFY rats weighing 185—235 g were used. The animals were kept in artificial light, with 12-hour light and 12-hour dark periods. The light period started at 6 a.m. The animals were fed a standard diet and water was given ad libitum.

The electric lesion was done under pentobarbital (50 mg/kg b.w.) anaesthesia with a stereotaxic apparatus using cathodal current (5 mA, 15 sec). The coordinates of the electrode set according to the atlas of BURES et al. (1967): AP (sagittal plane), 7; L (lateral plane), 0; F (frontal plane), 7 mm. In control (sham-operated) animals the electrode was introduced into the mesencephalon and than removed.

Localization of the lesion was verified in frozen brain sections of randomly selected animals.

The effect of lesion on the brain serotonin and plasma corticosterone levels was tested 7 days after the operation.

For the determination of brain serotonin content the animals were killed by decapitation, controls at 08, 12, 16, 20, 24 and 04 hours, raphe-lesioned animals at 08, 16 and 24 hours. The brain was rapidly removed and frozen, part of the dorsal hippocampus (80—100 mg), hypothalamus (20 mg), amygdala regions (15—20 mg), septal region (30—35 mg) and mesencephalon including the raphe region (40—50 mg) were dissected. The serotonin level in the different brain areas was measured by the method of SNYDER et al. (1965).

Corticosterone level in peripheral blood was determined from the trunk blood collected after decapitation, according to the method of ZENKER and BERNSTEIN (1958) as modified by PURVES and SIRETT (1965).

For stress, the animals were placed for 2 min in ether vapour or exposed to electric shock (alternating current 1.5 mA delivered to the pan for 5 sec every 15 sec during 2 min). The animals were tested 30 and 90 min after stress.

Statistical analysis was done by Student's *t*-test.

Results

A typical localization of the midbrain raphe lesion is shown in Fig. 1.

The animals showed a characteristic change in behaviour with increased spontaneous motor activity, aimless rotatory movements, and hypersensitivity to acoustic stimuli. The diurnal variations in the serotonin level in different regions of the limbic system are presented in Fig. 2.

The serotonin level in the mesencephalon, septum and amygdala was fairly constant between 08 and 16 hours. At the beginning of the dark period, a sharp decrease occurred in these regions, with the minimum at 20 hours. From that time on the level increased gradually, returning to the normal value at 04 hours in the mesencephalon and amygdala and at 24 hours in the septum. In the hippocampus and hypothalamus the serotonin level was fairly constant between 08 and 12 hours, followed by a gradual decrease till 20 hours. In the hypothalamus it gradually increased to the normal level at 08 hours

but in the hippocampus remained at this low level until 24 hours, then returned to the normal level at 04 hours.

The plasma corticosterone level showed an opposite trend. The minimum was at 08 hours and the maximum at 20 hours.

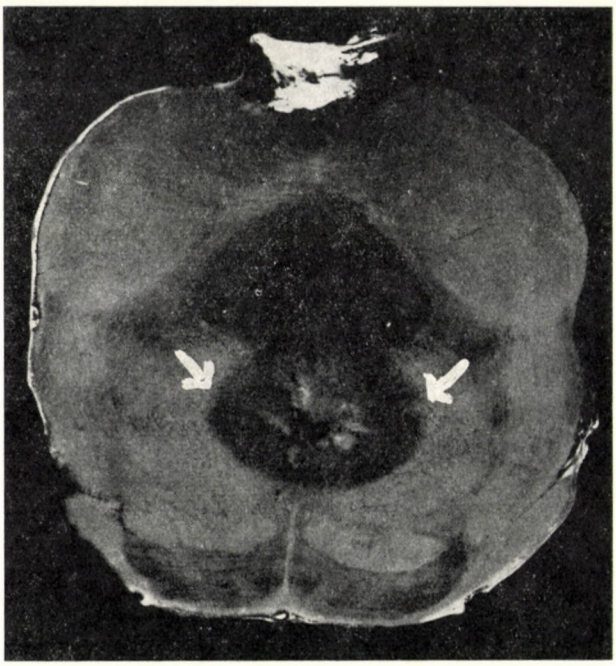

Fig. 1. Localization of electrolytic lesion of the midbrain raphe. The arrows indicate the lesion

On the seventh day following lesion of the midbrain raphe nuclei, in each brain region the serotonin level varied between 50—75% of the original morning level and was constant during the 24-hour period. The low level obtained following the raphe lesion was in the minimum range of the normal fluctuation. The plasma corticosterone content was high, threefold of the normal morning level, and remained constant in 24-hour period.

The stress, whether ether or electric shock, induced in shem-operated animals a sharp increase in the plasma corticosterone and a decrease in the hypothalamic serotonin level (Table I). In midbrain raphe-lesioned animals the stress response was facilitated, starting from a higher level and increased almost parallel with the normal reaction. The rate of return to the starting value was identical in the control and lesioned animals following ether stress but it was delayed after electric shock. The hypothalamic serotonin level was low in each case and did not change after exposure to stress (Table I).

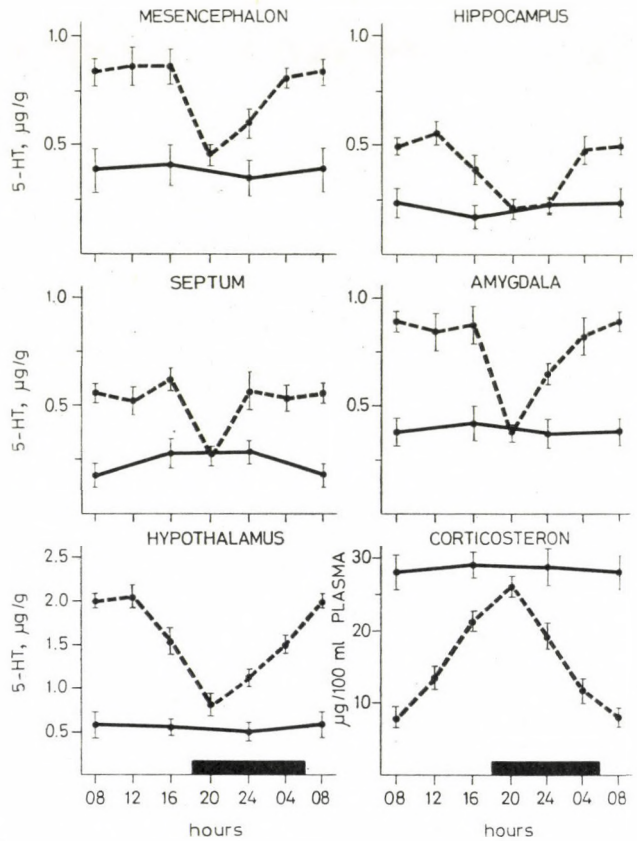

Fig. 2. **Diurnal variation in serotonin content of discrete regions of the brain, and in plasma corticosterone content of midbrain raphe-lesioned animals. Each vertical bar represents the mean ±S.E. of 14—58 rats. Dotted line: sham-operated; solid line: raphe-lesioned animals**

Discussion

The brain serotonin level shows a remarkable diurnal fluctuation. Most data agree in that the serotonin contant in the brain or in discrete areas of the brain decreases sharply at the beginning of the dark period. This diurnal rhythm is characteristic of the serotonin content of the whole brain of the mouse (Albrecht et al. 1956) and of the rat (Dixiu and Buckley 1967; Asano 1971; Okada 1971), of the telencephalon, diencephalon, and of the main part of the mesencephalon and rhombencephalon (Bobillier and Mouret 1971), of different cortical (Quay 1965) and subcortical areas (Quay 1968). Similar results have recently been reported by Héry et al. (1972) for the hypothalamus, cortex and brain stem. In the hippocampus and amygdala an opposite tendency has been found (Scapagnini et al. 1971). Our results

Table I

Effect of midbrain raphe lesion on electric shock- and ether stress-induced changes in plasma corticosterone level and hypothalamic serotonin content at 30 and 90 min following stress

Time (min)	Group	N	Plasma corticosterone		Hypothalamic serotonin	
			μg/ 100 ml	significance (p)	μg/g	significance (p)
0	1. Control	29	8.7±1.56*		1.94±0.07	
	2. Lesion	18	28.0±2.37	$p < 0.001$ vs. 1.	0.56±0.13	$p < 0.001$ vs. 1.
				Electric shock		
30	3. Control	12	40.0±2.15	$p < 0.001$ vs. 1.	0.67±0.16	$p < 0.001$ vs. 1.
	4. Lesion	14	61.7±3.20	$p < 0.001$ vs. 2.	0.38±0.08	NS vs. 2.
				$p < 0.01$ vs. 3.		$p < 0.05$ vs. 3.
				NS vs. 1.		$p < 0.05$ vs. 1.
90	5. Control	11	12.6±2.92	$p < 0.001$ vs. 3.	2.32±0.12	$p < 0\ 001$ vs. 3.
	6. Lesion	12	38.5±2.34	$p < 0.01$ vs. 2.	0.41±0.11	NS vs. 2.
				$p < 0.001$ vs. 4.		NS vs. 4.
				$p < 0.001$ vs. 5.		$p < 0.001$ vs. 5.
				Ether stress		
30	7. Control	16	41.2±1.90	$p < 0.001$ vs. 1.	0.94±0.11	$p < 0.001$ vs. 1.
	8. Lesion	14	62.3±4.12	$p < 0.001$ vs. 2.	0.35±0.09	NS vs. 2.
				$p < 0.01$ vs. 7.		$p < 0.01$ vs. 7.
				NS vs. 1.		$p < 0.05$ vs. 1.
90	9. Control	16	10.5±1.52	$p < 0.001$ vs. 7.	2.26±0.17	$p < 0.001$ vs. 7.
	10. Lesion	12	32.8±2.38	NS vs. 2.	0.42±0.12	NS vs. 2.
				$p < 0.001$ vs. 8.		NS vs. 8.
				$p < 0.001$ vs. 9.		$p < 0.001$ vs. 9.

* Values are means ± S.E.
NS: Not significant
N: Number of rats

showed that in each brain area (mesencephalon, hippocampus, septum, amygdala and hypothalamus) the serotonin level was lovest two hours after the beginning of the dark period.

The plasma corticosterone rhythm shows an opposite behaviour (Guillemin et al. 1959; Critchlow et al. 1963; Cheifetz et al. 1968; Rózsahegyi

et al. 1973), having the highest value at 20 hours. The same reversed correlation was observed by OKADA (1971) in adult rats and by RÓZSAHEGYI et al. (1973) in female rats. The present study has extended these data to show that practically all regions of the limbic system displayed a similar pattern. When the serotonin content was low, the plasma corticosterone was high, supporting our conception that the central nervous serotoninergic system exerts a tonic inhibition on the function of the hypothalamo–pituitary–adrenal axis.

Following the electrolytic lesion of the midbrain raphe nucleus, the brain serotonin level decreases (HELLER and MOORE 1965; JOUVET et al. 1966; KOSTOWSKY et al. 1968; ROSECRANS and SHEARD 1969; KUHAR et al. 1971, 1972). Our findings too demonstrated that after a raphe lesion the serotonin level decreased in each brain area investigated, the diurnal serotonin rhythm disappeared, and the basal plasma corticosterone level was elevated without showing a diurnal fluctuation.

In a different approach using 5,6-dihydroxytryptamine injection into the raphe nuclei, which causes a selective degeneration in the serotoninergic system, an elevated morning corticosterone level has been reported by FUXE et al. (1973).

In the raphe-lesioned animals, the response to stress was facilitated; it started from a higher than normal level and increased to a much higher value than in the controls. The present data supported the concept proposed earlier that serotonin exerts an inhibitory action on the hypothalamo–pituitary–adrenal system (VERMES and TELEGDY 1972; VERMES et al. 1972; TELEGDY and VERMES 1973; VERMES et al. 1973). It seems that in the limbic system serotoninergic transmission inhibits tonically the hypothalamo–pituitary–adrenal system. The diurnal variation of serotonin in the limbic system is inverse correlated to the plasma corticosterone level which would mean that when serotoninergic inhibition is low, the plasma corticosterone is high. This is in agreement with the data of KRIEGER and RIZZO (1969) who showed that the diurnal rhythm of the plasma corticosteroid disappeared after the inhibition of the serotonin receptors in the cat. The actual magnitude of the adrenal response to stress depends on the level of serotonin in the brain, especially in the hypothalamus.

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^{3}H-MELATONIN LEVEL IN CEREBROSPINAL FLUID AND CHOROID PLEXUS FOLLOWING INTRAVENOUS ADMINISTRATION OF THE LABELLED COMPOUND

By

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^{3}H-melatonin and ^{3}H-N-acetyl-serotonin (^{3}H-NAS) were injected intravenously into adult male rats. Radioactivity in cerebrospinal fluid (CSF), serum, the choroid plexus and the hypothalamus and mesencephalon was measured at different intervals ranging from 1 minute to 12 hours. CSF showed a high activity. The peak was reached 5 minutes after the injection of labelled melatonin, but even after 12 hours a considerable degree of activity was detected. The choroid plexus contained 3—12 times higher counts per 100 mg tissue than the rest of the brain. The high level of labelled melatonin in the choroid plexus was not due to the blood content of the organ, since it was not affected by perfusion of the brain with physiological saline. ^{3}H-NAS, like melatonin, was concentrated equally in the choroid plexus and the CSF. It is concluded that the choroid plexus takes up indole-amines from the circulation and secretes them into the CSF.

An important question raised by the Ciba Foundation's Symposium on the Pineal Gland (WOLSTENHOLME and KNIGHT 1971) was whether the pineal gland secreted its bioactive principles (e.g. melatonin) into the blood capillaries or into the cerebrospinal fluid (CSF). The possibility of a direct or indirect intraventricular route of pineal secretion was categorically denied by ARIENS-KAPPERS (1971). On the other hand, MARTINI and FIORETTI (1971) provided evidence that melatonin prolongs the sleep induced by barbiturates injected intraventricularly in the rat. Melatonin in the brain reaches a hundred times higher level if introduced directly into the CSF than by the intravenous route (ANTÓN-TAY and WURTMAN 1969; ANTÓN-TAY 1971). COLLU et al. (1971) reported that the intraventricular administration, but not the subcutaneous injection, of melatonin restricted spontaneous ovulation in normally cycling rats and concluded that the physiological route of melatonin secretion was through the CSF. Furthermore there is, at least in certain species, a close anatomical relationship between the pineal gland and the ventricular system of the brain (SHERIDAN et al. 1969).

Morphologists have to agree with the main argument of ARIENS-KAPPERS (1971): "... all light and electron microscopical data point to the extrusion of the pineal products into the general blood circulation." However,

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the pharmacological and physiological data presented by ANTÓN-TAY and WURTMAN (1969), MARTINI and FIORETTI (1971), and by COLLU et al. (1971) seem equally convincing. The question therefore is still far from being proven. Recently, REITER (1973) has quoted it as one of the most important but unsolved problems of pineal physiology. These points have made us to perform additional experiments in an attempt to provide more data to clarifying the problem.

Methods

Adult, intact, male albino rats (200—250 g body weight) under superficial ether anaesthesia were injected intravenously with 40 μCi of ^{3}H-melatonin (I.R.E., Belgium; specific activity, 460 mCi/mmole; the radioactive product was purified and its identity confirmed by thin-layer radiochromatography). Then 1, 2, 5, 10, 20, 30, 60, 120, 180, 360 and 720 minutes later CSF was withdrawn by puncture of the cisterna magna. Immediately after this procedure the abdominal aorta was exposed and the animals were killed by exanguination. Radioactivity in the CSF, serum, choroid plexus, the hypothalamus and the midbrain was measured. All tissues were dissolved in 1 ml of Soluene (Packard) and activity was determined by a Packard liquid scintillation counter, using the scintillation mixture, toluene : absolute ethanol 98 : 2 vol./vol. In 1000 ml of this solvent 4 g of PPO and 0.04 g of POPOP were dissolved. Each determination was made on a pool of 2 rats, and each experiment was repeated three times. Thus, the data summarized in Tables I and II represent mean values obtained in 6 rats. Radioactivity was calculated per 1 ml of CSF and of blood serum, and per 100 mg of the various fragments of wet brain tissue.

In the next experimental series rats injected with 60 μCi of ^{3}H-melatonin after withdrawal of CSF and blood were perfused with at least 100 ml of physiological saline per rat and the radioactivity of the brain and of the choroid plexus was counted in the perfused tissues.

In the final experiment, a labelled precursor of melatonin, ^{3}H-N-acetyl-serotonin (^{3}H-NAS) was administered. The dose of radioactivity was the same as for melatonin. The animals were sacrificed 12 hours after injection of the labelled compound. This experiment was repeated only twice, so data in Table III represent means for 4 rats.

Statistical significance was calculated by Student's t-test.

Results

Results for ^{3}H-melatonin are summarized in Table I. Labelled melatonin appeared in the CSF within 1 minute, the peak concentration was reached within 5 minutes. Then the activity decreased but a considerable amount was still detectable 12 hours after the injection.

In the serum, however, the activity continuously decreased from the first minute. After 12 hours, it was nearly the same in the blood as in the CSF.

Among the different areas of the brain studied, the choroid plexus showed the highest uptake; it was about 3—12 times higher than in other parts of the brain. This ratio subsequently tended to increase. The difference in activity between the choroid plexus, the hypothalamus and the mesencephalon was equally highly significant at every point of times.

In order to exclude the possibility that the high concentration of labelled melatonin found in the choroid plexus was due to the high blood content of this organ rather than to melatonin uptake by the choroid tissue, these experi-

Table I

^{3}H-melatonin levels in blood, CSF and different areas of the brain in the albino rat (dose of melatonin = 40 μCi/rat)

(CPM/ml of blood and CSF, and CPM/100 mg brain and choroid plexus)

Minutes after melatonin injection	Blood serum	CSF	Choroid* plexus	Hypo-thalamus	Mesence-phalon
1	43 230	9 950	6 306	2156	1879
2	29 838	10 698	5428	1735	1900
5	37 878	20 969	6606	1148	946
10	31 093	10 119	5202	1120	1140
20	22 123	8 800	3370	503	459
30	16 252	5 388	3419	470	481
60	8 046	3 801	1840	425	348
120	2 273	1 000	1985	324	165
180	2 497	1 636	999	210	242
360	1 624	890	2140	149	187
720	873	793	1781	206	207

* The statistical difference in uptake between choroid plexus, hypothalamus and mesencephalon was equally highly significant, $p < 0.001$

ments were repeated after perfusion of the brain with physiological saline until the whole brain and the choroid plexus had become completely pale. It is evident from Table II, that this caused no significant change either in the choroid plexus or in the rest of the brain. The slight increase of radio-

Table II

^{3}H-melatonin levels in choroid plexus, hypothalamus and mesencephalon, following removal of blood by perfusion of the brain with physiological saline (dose of melatonin = 60 μCi/rat)

(CPM/ml of blood and CSF, and CPM/100 mg brain and choroid plexus)

Minutes after melatonin injection	Blood serum	CSF	Choroid* plexus	Hypo-thalamus	Mesence-phalon
2	100 038	43 380	8210	3206	3318
10	75 756	31 056	4625	2089	2160
30	57 099	7 634	3196	630	530
60	57 471	3 770	1854	344	305
120	33 197	2 794	1835	321	293

* The statistical difference in uptake between choroid plexus, hypothalamus and mesencephalon was equally highly significant, $p < 0.001$

activity, especially in the blood, was due to the higher dose of melatonin injected.

In order to determine whether the choroid plexus and CSF take up exclusively melatonin or also other related indole-amines too, ^{3}H-NAS was injected instead of melatonin. This compound was concentrated in the CSF and in the choroid plexus nearly to the same extent as was melatonin, while hypothalamic and mesencephalic activity was not higher than the background (Table III).

Table III

^{3}H-N-acetyl-serotonin (NAS) levels in blood, CSF and different areas of the brain in the albino rat 12 hours after intravenous injection (dose of NAS = 40 μCi/rat)

(CPM/ml of blood and CSF, and CPM/100 mg brain and choroid plexus)

	Blood serum	CSF	Choroid* plexus	Hypo-thalamus	Mesence-phalon
	403	2747	587	155	190
	241	2217	620	180	154
	128	536	466	96	46
	—	663	509	93	40
Mean ± S.E.	257±79	1540±550	545±31	131±21	107±31

* The statistical difference in uptake between choroid plexus, hypothalamus and mesencephalon was equally highly significant, $p < 0.001$

Discussion

The results presented indicate that melatonin, NAS and probably other pineal indole-amines (Wurtman et al. 1968) pass from the blood stream into the CSF. The main difference between the results obtained with melatonin and NAS was observed in the serum, hypothalamus and mesencephalon. Twelve hours after ^{3}H-NAS administration, the number of counts was considerably lower than after labelled melatonin. The explanation of this finding is not clear; it is conceivable that NAS degradation in blood occurs faster than that of melatonin and its excretion is therefore also more rapid. This point, however, requires further experimental substantiation.

Antón-Tay and Wurtman (1969) and Cardinali et al. (1973) demonstrated that hypothalamus and midbrain are the cerebral areas which have the highest ^{3}H-melatonin concentration regardless to the route of administration (intravenous, intraventricular or intracisternal). In the present study, in which the level of melatonin in the choroid plexus was compared with that in the hypothalamus and midbrain, it was apparent that uptake by the choroid plexus was 3—12 times higher than by any of the mentioned

areas at all intervals after injection. This indicates that the choroid plexus concentrates intravenously administered indole-amines. It seems therefore very probable that this organ, which is responsible for the secretion of CSF (ROUGEMONT et al. 1960; MAREN 1967; DAVSON 1956), plays an active part in the transport of indole-amines from the blood into the CSF. The fact that the concentration of labelled melatonin did not decrease in the choroid plexus following perfusion of the brain, clearly demonstrated it was not only in the capillaries of the plexus that the compound was present. It appears that the epithelial cells, the secretory elements of the choroid plexus, are taking the indole-amines and secrete them into the CSF. Autoradiographic studies are in progress in our laboratory to verify the proposed site of binding of labelled melatonin.

Numerous data in the literature indicate that the choroid plexus takes up and concentrates different organic compounds from the blood plasma. SANDLER and WELCH (1967) reported a specific glucose-transport system of the choroid plexus which would "pump" glucose from the blood into the CSF. A previous autoradiographic study performed in our laboratory (MESS and KOLOUSEK 1962) has shown that on intravenous injection, ^{35}S-methionine reached much at higher concentrations in the choroid plexus than in other areas of the brain. Other compounds, less physiologic than sugars and amino acids, as e.g. Na-pertechnate-99 TC (WITCOFSKI et al. 1968), or benzoleamide (BRODER and OPPELT 1969), are also collected by the choroid plexus and secreted into the CSF.

An opposite direction of choroidal transport has, however, also been shown, i.e. the choroid plexus transports different organic compounds also from the CSF into the blood. CSÁKY and RIGOR (1964) found twenty times higher ^{14}C-glucose concentrations in the choroid plexus than in the incubation medium in an in vitro system. These authors therefore concluded to the presence of a "sugar pump mechanism" in the choroid plexus, which would transfer sugars from the CSF into the blood. Organic acid dyes are also transported by the choroid plexus into the CSF (RALL and SHELDON 1962).

Furthermore, there is evidence indicating the possibility of transport from the brain into the CSF. Biogenic amines such as dopamine (PORTIG et al. 1969), serotonin (GUILDBERG and YATES 1968), or norepinephrine SCHANDBERG et al. 1968) may pass from the brain into the CSF. CSERR (1971) assumed that the CSF would be a kind of transport medium between brain and blood, which brought nutritive compounds to, and removed metabolites from, the brain, both by an active transport through the choroid plexus.

On the basis of all these and the present results, it may be assumed that the pineal body secretes its bioactive principles into the capillaries, which are then drained into the large veins surrounding the organ (ARIENS-KAPPERS 1971). A possible venous blood flow from the pineal gland to the

choroidal tissue of the suprapineal recess and the roof of the third ventricle has been recently reported in the rat (QUAY 1973). The choroid plexus, then, functions as a "pump" secreting the indole-amines from the general circulation into the CSF. Since in our experiments the highest rate of ^{3}H-melatonin was found in the hypothalamus and the midbrain within 1 minute after intravenous injection, whereas the peak concentration in the choroid plexus and CSF occurred at 5 minutes (Table I), the opposite direction of melatonin transport cannot be excluded. However, as shown by several data in the literature (MESS and KOLOUSEK 1962; SANDLER and WELCH 1967; WITCOFSKI et al. 1968; BRODER and OPPELT 1969) and by the findings after intravenous administration of melatonin in the present experiment, the former direction of melatonin transport, i.e. blood → choroid plexus → CSF seems to be more probable. The real direction of melatonin transport between blood, CSF and brain tissue, as well as the physiological role of the pineal indole-amines in the CSF, need further elucidation.

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ACTIVITY OF THE CORPUS STRIATUM OF CATS DURING NATURAL SLEEP; A CORRELATION ANALYSIS STUDY

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Activity of the sensorimotor cortex (Mc), of the ventral posterolateral (VPL) and ventrolateral (VL) nuclei of the thalamus, of the caudate nucleus (Cau), of the globus pallidus (GP) and of the putamen (Pu) and the changes in their auto- and cross-correlograms were studied during natural sleep in nine cats prepared for chronic testing.

The frequency of the spindles in superficial and slow-wave sleep was 12 to 17 c/s in the sensorimotor cortex and in the two nuclei of the thalamus, 10 to 15 c/s in the globus pallidus, and 9 to 12 c/s in the caudate nucleus. A comparatively stable frequency and amplitude both in wakefulness and in slow-wave sleep characterized the putamen.

In all states a slow and a rapid component of significantly different relative power and coherence were detected in each of the investigated structures. Practically, only in the Mc did the slow wave of 2 to 3 c/s dominate. The changes in the activity of Mc and VPL were the same in the various states, while that of VL resembled both the first two and also that of the striatum. During REM sleep the rhytmic activities of VL, Cau, GP and Pu were identical.

The activities of VPL and VL are usually synchronous to that of the other structures; the phase relations are constant or change only slightly. Phase shifts appear almost always in the relation of GP, Cau and Pu and change in the same direction as sleep becomes deeper. The cross-coherence between the activity of various structures, particularly in relation to the striatum, is lower in SWS.

The possible functional importance of spindle activity, of slow waves and of the basal ganglia during sleep is being discussed.

Within the complex function of the corpus striatum the globus pallidus plays a role mainly in postural and reflectory motions and the putamen primarily in the regulation of the muscle tone, in close functional correlation with the cortex and the non-specific nuclei of the thalamus. The caudate nucleus has a paramount inhibitory or suppressor effect, its relationships are limited mainly or entirely to the centrum medianum, the cortex and the putamen (DENNY-BROWN 1962). Since these functions are present also in the passive changes of the level of wakefulness, we decided to study the frequency and time relations of the electrical manifestations of the cortico-striato-thalamo-cortical connections during sleep.

Method

Nine adult cats of both sexes were prepared for chronic tests. Bipolar nickel-chromium electrodes (diameter, 0.2 mm; uninsulated tip, 1 mm; intertip distance, 1 mm) were inserted under sodium pentobarbital anaesthesia into the ipsilateral ventrolateral (VL) and the ventral posterolateral (VPL) nuclei of the thalamus, into the caudate nucleus (Cau), the globus pallidus (GP) and the putamen (Pu). There was a 2 mm distance between the epidural electrodes (uninsulated disk surface, 1 mm²) placed above the sensorimotor cortex (Mc). The electrodes were placed in a localization defined by the stereotaxic map (JASPER and AJMONE-MARSAN 1954, 1961). Further disk electrodes were fixed on the supraorbital bone of frontal sinus for the recording of eye movements (EOG) and into the cervical muscle for the detection of changes in muscle tone. The outer end of the electrodes was soldered to a subminiature plug, which were fixed to the cranial bone by means of dental cement. The localization of the electrode tips was checked histologically at the end of the experiment.

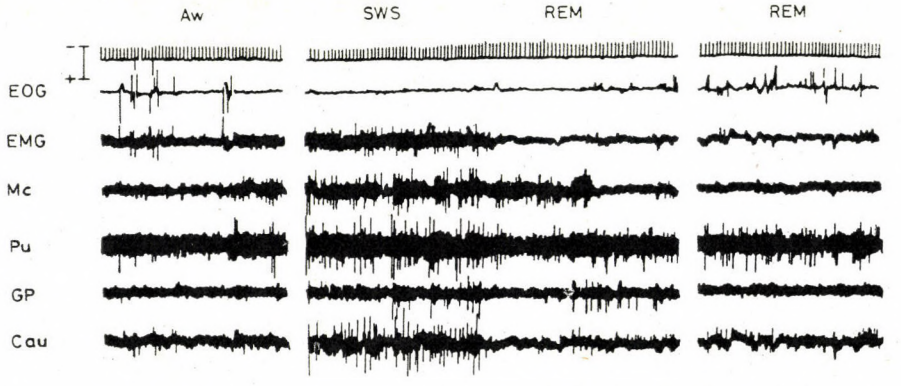

Fig. 1. Activity of cerebral structures at different levels of wakefulness. Upper channel: time in seconds. Calibration: 300 μV for EOG, 150 μV for EMG, 100 μV in the case of Mc, GP and Cau, and 50 μV in the case of Pu lead. Note in the SWS — REM transition the retardation of the activity characteristic of REM in Mc, as compared to its appearance in the other structures and at the same time the occasional spindling section in GP

The experiment began 4 to 5 weeks after the operation by which time the animals had completely recovered. The electric cerebral activity of the cat moving freely in a sound-insulated cage was recorded through an artefact-free cable by an 8-channel Elema-Schönander polygraph (Mingograph-8); its oscilloscope outputs were coupled to a 4-channel FM tape-recorder. The EEG was continuously recorded on paper, while an unambiguous period of at least 4 minutes duration of each successive stage of sleep was recorded on magnetic tape. After cyclic fluctuations during adaptation to the environment has ceased, the signals of 3 to 4 sleep periods of each cat were stored.

For correlation analysis a correlator plug-in (NE 299 Corall-B) of the NTA 512/A multi-channel analyzer was used. The integration time of the autocorrelograms was 3.5 min ($\Delta\tau = 6.4$ msec; $\tau = 0.82$ sec); that of the cross-correlograms, 2.5 min ($\Delta\tau = 2.56$ msec; $\tau = 0.33$ msec) in each time domain. The correlograms were written on a decimal printer and XY-recorder. The measurements were carried out on the basis of criteria determined according to former experiences (SARKADI et al. 1971).

The level of wakefulness was defined in agreement with data in the literature according to the following criteria (Fig. 1): 1) Awake (Aw): low voltage fast, irregular cortical activity. Rapid, high, irregular EMG. High, coarse waved EOG when the animal is moving or sitting with open eyes. 2) Drowsiness: beside the appearance of cortical sleep spindles there is no change in EMG. Eye movements, if any, are high and coarse waved when the animal is sitting with dropping or closed eyes. 3) Slow wave sleep (SWS): a cortical high amplitude activity of 2 to 6 c/s is mixed with one of 8 to 12 c/s. On the EMG there is hardly any change in voltage;

the curve is, however, more even and more rhythmical. There are practically no eye movements. The animal settles itself comfortable, but can be awaked easily. 4) Fast wave sleep (rapid eye movement, REM): cortically low voltage fast activity which is more regular than in waking state. There is a definite decrease of amplitude in the EMG and a slight frequency slow-down. The EOG shows a more or less continuous fluctuation of low voltage. The animal is lying relaxed with slack head and limbs and can be awaked with difficulty by repeated strong stimulation.

Results

In the majority of animals the most balanced and longest sleeps were recorded in the late afternoon or evening, when, as a rule, 3 to 5 cycles interrupted by a few brief wake intervals occurred during a period of 2 to 4 hours of continuous sleeping. The duration of one cycle was on an average 30 minutes within which SWS varied from 16 to 30 minutes. The REM stage lasted 10 to 17 minutes, but stages of 4 to 5 minutes were also recorded in agreement with PASSOUANT's data (1970). A total of 44 SWS and 39 REM stages were recorded and processed.

During drowsiness sections of spindles appeared in all structures. In some of the animals spindles of lower voltage appearing on one or two occasions could also be observed in the Aw and REM stages. The activity of Pu was characterized by a relatively stable frequency and amplitude with hardly any spindles. The frequency of the spindles varied between 12 and 17 c/s in Mc, VL and VPL, while in the GP it was between 10 and 15 c/s and in the Cau 9 to 12 c/s. Spindling in VL was not always accompanied by spindling in Mc, and the opposite case never occurred. In most instances spindling in VPL coincided with spindling in the other structures, first of all in Cau and GP, and also appeared sometimes during REM sleep, in this case always at a frequency of 15 c/s. While in the other structures the duration of the spindles varied between 8 and 12 sec, those in GP reached in one or two cats a maximum of 20 to 40 sec. In a single animal the spindles of Cau appeared in all stages, but only for a short period.

With the exception of Mc the activity of every structure was mixed with an activity of 4 to 6 c/s at all levels of wakefulness. In slow wave sleep this activity became more intensive in every structure with the exception of VL and appeared also in Mc. Fast wave sections lasting from 10 sec sometimes even to 90 sec were observed in all SWS stages, while slow wave groups appeared in REM on one or two occasions and these were localized in one of the cats to the GP alone. In another animal short alternations of fast and slow wave sections occurred without an unambiguous detectability of the level of weakfulness.

When passing from one sleep stage into another there was a simultaneous change in the activity of the structures. In five cats in some transitions from SWS to REM the VPL was ahead of Pu and the other structures, or Pu alone

or together with VPL was lagging behind by 10 to 20 sec in relation to the other structures. On one occasion Mc was behind Pu, GP and Cau nuclei by 10 sec (Fig. 1). Though these findings were not consistent, the development of the activity which characterizes fast wave sleep seemed to be delayed mainly in the Pu.

In cats the autocorrelograms are mainly of the noise type, with a low relative power of the rhythmic components (Fig. 2). In various stages of wakefulness the following changes of activity were observed in the structures

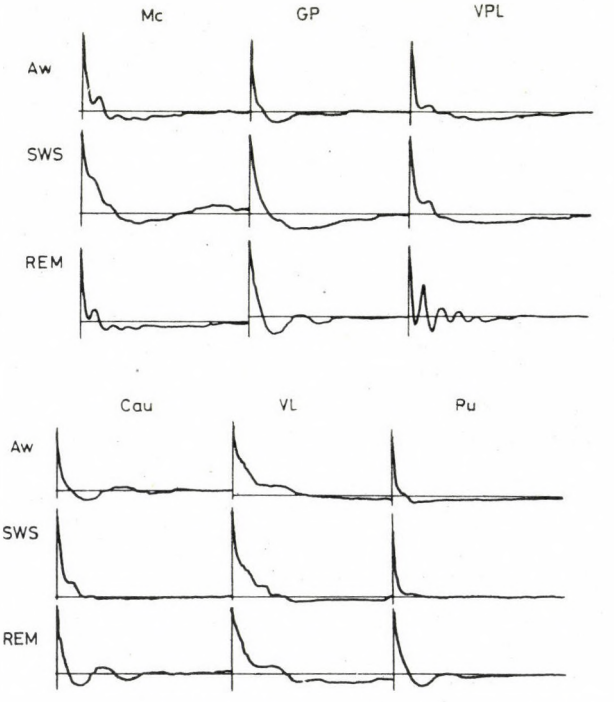

Fig. 2. Autocorrelograms of the various structures from Aw to the REM state. Integration time: 3.5 min. ($\Delta\tau = 6.4$ msec; $\tau = 0.82$ sec). For explanation, see text

under investigation. In Mc an activity of 12 to 14 c/s dominated in wakefulness. In slow wave sleep an activity of 15 to 18 c/s of lower power was predominant, superimposed on a rhythm of 2 to 3 c/s. In fast wave sleep the correlogram was similar to that of the waking state with a dominant frequency identical with the fast component of SWS. In VPL in wakefulness an activity of 11 to 14 c/s mixed with a very slow subharmonic component was predominant, in SWS the fast component was less marked than before, while in REM again the 15 to 20 c/s rhythm dominated. In VL the strongly noise-type activity

was preponderant in all stages, within which a 4 to 5 c/s rhythm was detected in the waking state. In SWS this rhythm was replaced by one of a frequency of 8 to 10 c/s and in REM by a more marked activity of 5 to 7 c/s.

The shape of correlograms of these three structures were almost identical in wakefulness and in fast wave sleep, though their dominant frequency was faster in REM. In SWS there was a striking increase in correlability of slow cortical activity with the simultaneous appearance of fast rhythmicity in the VL.

Changes in the correlograms of Cau were similar to those of VL but for a significantly more coherent activity. The dominant frequency in wakefulness

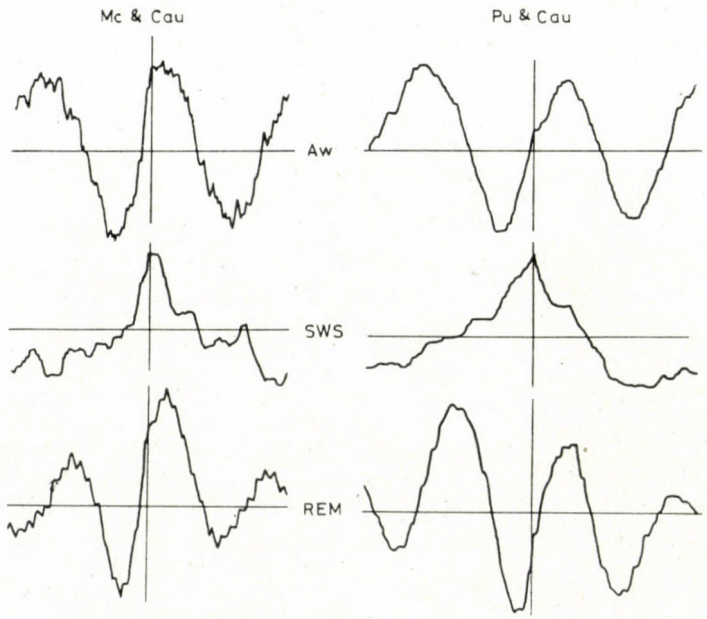

Fig. 3. Two cross-correlogram series showing the changes in the mutual relations of the activities of three structures. Note the disappearance of phase differences and the drop of coherence to a minimum in SWS. Integration time: 2.5 min ($\Delta\tau = 2.56$ msec; $\tau = 0.33$ sec)

was 3 to 5 c/s, in SWS in a noisy environment it was 10 to 14 c/s, and in REM 5 to 7 c/s. In GP in wakefulness there was a noise-type and mixed activity of 11 to 13 c/s, 5 to 7 c/s and 2 to 4 c/s frequencies. In SWS these were associated with a 0.5 to 1 c/s frequency while all these were replaced in REM by a fairly coherent 5 to 7 c/s activity. The activity of Pu was similar to that of GP, though with a more distinct fast component. In SWS a 2 to 3 c/s rhythmicity was observed. In REM a more coherent 4 to 7 c/s activity dominated.

The only difference between the correlograms of GP and Pu in wake-

fulness and in SWS was the more marked slow components in the GP during sleep. In REM the autocorrelograms of VL, Cau, GP and Pu were practically the same.

Figure 3 is an example of changes of the cross-correlograms. In 41 cases a phase difference was found, out of these 11 were observed in wakefulness, 9 in SWS and 21 in REM. The phase differences either ceased in SWS or were significantly smaller than in the waking state. In a single animal the activities were synchronous in all the investigated structures at all levels of wakefulness, while in another animal a phase shift was found between two structures in SWS, with synchronous activites in Aw and REM on the other hand.

The activity in VPL and VL was most often synchronous with that of the other structures, without change in the phase relations compared to the other structures or between themselves, or with a minimum and generally not unambiguous change. There was almost always a phase shift in the relations of the GP, Cau and Pu. Within these three structures — when investigating the phase shifts changing with the deepening of sleep in each cat — it was found that the degree of phase-lag of Cau increased (or the degree of its advance decreased) in relation to the other two structures, while its advance to Mc decreased. At the same time the phase difference between the activities of Pu and GP also increased.

In SWS the cross-coherence between the activities of the structures generally decreased. This was particularly pronounced in the relationships among Cau, GP, Pu and VPL. The least consistent was the change of cross-coherence in the various stages between Mc and the other structures.

On transition into REM, in animals in which the stage-specific activity appeared somewhat sooner or later, this deviation was not related to the direction of change of phase-shifts between these same structures.

Discussion

In cats during behavioural inhibition a characteristic cortical rhythm of 12 to 16 c/s frequency appears which is ascribed to active suppressive or inhibitory processes. During these periods, the animal is often fixed in some unusual tonic posture and after the cessation of the inhibitory stimulus, simultaneously with the appearance of the sleep spindles, it assumes a relaxed position (Grastyán et al. 1959; Donhoffer and Lissák 1962; Roth et al. 1967; Sarkadi and Tomka 1971). In the waking state of cats, during internal inhibition or behavioural immobility, the accompanying somatic and viscero-motor symptoms of the spindling rhythm appearing above the sensorimotor cortex will be in qualitative agreement with those observed during drowsiness

and slow wave sleep (CHASE and HARPER 1971). According to HOWE and STERMAN (1972), a rhythm corresponding to the waking phase appears also in the VPL and disappears on phasic motor activity, while the rhythms corresponding to the sleep spindles appear primarily in the VL and to a lesser degree in the centrum medianum in SWS. In this way the spindle rhythm reflects presumably an active inhibitory function which is directed at the suppression of the potential motion contents or motion possibility of a given situation or state. According to this would the morphology of the cortical spindles and the appearance of the spindles in the subcortical structures be variable. Our finding of a coincidence in time of the VPL spindles with spindles in the other structures, particularly in Cau and GP, would indicate that these spindles reflect the appearance — on specific pathways — of a suppression of muscle tone and potential motor intentions still vigorous in drowsiness and slow wave sleep. This might be related to the fact that there is a close overlap of spindle frequencies between Mc, VPL and VL, as well as between GP and Cau, and the overlap is the slightest between the first three and the last two structures.

It has been suggested that the spindle activity reflecting the inhibitory phasing function ("inhibitory phasing theory" of ANDERSEN and SEARS, 1964) might be in equilibrium with the activity of the reticular formation. ANDERSEN and ANDERSSON (1968) showed that the lateral thalamic nuclei and extensive areas of the cortex are capable of maintaining their spontaneous spindle forming capacity even without intact mesial line and intralaminar structures, while the removal of the lateral thalamic nuclei led to the disappearance of cortical spindles. The same authors (ANDERSEN et al. 1967, cit. ANDERSEN and ANDERSSON 1968) observed a definite decrease in the amplitude of spindles as a result of a moderate reduction of cortical temperature. This decrease in amplitude was not accompanied by a change in frequency. We have made the same observation when studying the effect of cortical cooling in chronic preparations (SARKADI et al. 1972) and came to the conclusion that in superficial sleep the reticular formation contributes indirectly to the appearance of cortical spindles. In slow wave sleep, particularly in EEG periods containing delta waves, bursts separated by quiet intervals longer than 200 msec occur in the cells of the thalamic reticular nucleus (MUKHAMETOV et al. 1970), while the cells of VL show group discharges deviating from their base rhythm just at the time of the appearance of spindles; and this is associated with a complete inhibition of the cerebellifugal and corticofugal synaptic transmission (STERIADE et al. 1969; STERIADE et al. 1971a, b), or a definite weakening of it (FILION et al. 1971).

Thus, slow wave sleep is a heterogeneous process in which every cerebral structure participates more or less pronouncedly and relatively actively or passively. This might explain the fact that in SWS — as compared to the Aw

and REM states — the noise-like nature of the activity of the investigated structures was more pronounced and the phase differences and coherences between these structures decreased considerably or disappeared. CALVET et al. (1973) distinguished a heterogeneous and a homogeneous form in the deepening of SWS, where the homogeneous form is characterized by negative slow waves indicating an inhibited state, which appear more and more often and over more and more extensive areas of the cortex. This inhibited state is presumably produced by an active phasing inhibition with an electrical manifestation of frequent spindling in superficial and occasional spindling in slow wave sleep.

Thus we might say that in cerebral electrical activity the transient phenomena are the equivalents of temporary control processes accompanied by active connections, while the average phenomena are characteristic of more or less continuous functional states. The REM state seems to be such a homogeneous state in which the own internal and the mutual coherence of the activities of the investigated structures is reestablished though in a quality which is different from that in Aw: the dominant activity of every structure is more rapid; the rhythmic activities of VL, Cau, GP and Pu are identical in this state only; the phase-shift of Cau to Mc has decreased and to the GP and Pu has increased.

The majority of efferent projections coming from the basal ganglia originate in the globus pallidus and end in the ventro-anterior and ventro-lateral nuclei of the thalamus from where they are projected in the direction of the motor cortex. The globus pallidus participates functionally not only in postural and motor reflexes, but also in the voluntar or instrumental motions of limbs (DELONG 1971). It may further be assumed that the direct role of the globus pallidus is decisive prior to the onset of movement (DELONG and EWARTS 1971). This might be the result of the synthesis of a large amount of information, since the injury of GP affects most seriously the performance of discriminative conditioned motor reactions (GAMBARIAN et al. 1970) and the restorability of avoidance conditioned reflexes (CHERKES et al. 1972), that is, processes which contain motor inhibition. The so called "arrest" behaviour consists dominantly of motor inhibition and in its development the caudate nucleus plays a decisive role (KLINGBERG and KUNZ 1972). According to our results, the rhythm of the putamen approches unanimously the activities of the other striatum nuclei only in REM sleep, when muscle tone reduced to a minimum. In this way it may be assumed that the slow rhythmic activity of corpus striatum during REM reflects a state of continuous motor inhibition extending also to the muscle tone. Such a state, however, can durably exist only independently of external stimuli and integrative functions. Since, according to our results the activity of and electrical relations between the investigated structures differ during REM slightly, but detectably from those

in wakefulness, it may be assumed that during REM the internal, intrinsic activity of the various structures is recorded, but without the integrative influences and functions present in the wakeful state.

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EFFECT OF CHOLINESTERASE INHIBITORS ON THE SUSCEPTIBILITY TO PENTETRAZOLE OF THE MOUSE

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Susceptibility of mice to pentetrazole is increased by physostigmine and paraoxone and decreased by neostigmine. Oxotremorine enhances the effect of pentetrazole in low doses and inhibits it in high doses.

The seizure-facilitatory action of physostigmine is inhibited by the anticholinergic drugs atropine and mecamylamine as well as by the amine oxidase inhibitors nialamide and tranylcypromine.

In the development of epileptic seizures a role has been ascribed to the cholinergic neurotransmission processes of the central nervous system. One of the compounds most often used for producing model epilepsy is pentetrazole. This drug decreases the cerebral acetylcholine level in the rat (GIARMAN and PEPEU 1962; SLATER 1971) and the guinea pig (BEANI et al. 1969) but does not influence it in the mouse (CONSOLO et al. 1972). This activity of pentetrazole is connected to its effect enhancing acetylcholine liberation (MITCHELL 1963; BELESLIN et al. 1965). An inhibitory action of the drug on acetylcholinesterase has also been described (MAHON and BRINK 1970). The acetylcholine level of the rat brain is decreased also by electroshock (RICHTER and CROSSLAND 1949), whereas the repeated application of pentetrazole increases the acetylcholinesterase activity (MODAK and STAVINOHA 1972).

There is some disagreement concerning the influence of cholinesterase inhibitors on seizure susceptibility. When injected into the cerebral ventricle, di-isopropyl fluorophosphate (DFP; FELDBERG and SHERWOOD 1954) and physostigmine (BELESLIN et al. 1973) elicit seizures in the cat. A similar effect has been observed after DFP and tetraethylpyrophosphate (TEPP) in the dog (EDERY 1962), as well as after the injection of DFP into the carotid artery in the rabbit (FRIEDMAN et al. 1949). WILLIAMS and RUSSEL (1941) have shown physostigmine to inhibit the petit mal-like EEG manifestations induced by hyperventilation in humans when applied in low doses but to enhance them in high dosage. Physostigmine inhibits the EEG manifestations evoked in this way also in the cat (DARROW et al. 1944), in which species it also inhibits

the appearance of penicillin-induced spike potentials (FUNDERBURK and CASE 1951). According to our earlier data, physostigmine injected intracerebrally causes tremor in the mouse; seizures, however, occur seldom and after large doses only. In sum, the data on the influence of cholinesterase inhibitors on seizure susceptibility are contradictory (see also MACHNE and UNNA 1963).

Only few authors have investigated how cholinesterase inhibitors would modify the susceptibility to convulsants. According to the data of HYDE et al. (1949), the effect of convulsants applied topically on the cerebral cortex of the cat is not influenced by intravenous DFP, and decreased by physostigmine and neostigmine. BAYLISS et al. (1957) showed that DFP did not influence the pentetrazole sensitivity of rats. In this same species, WÓRUM and PÓRSZÁSZ (1968) demonstrated physostigmine to increase the susceptibility to electroshock. In the mouse, the electroshock threshold is not influenced by physostigmine (CHEN et al. 1968).

Of the centrally acting cholinomimetics, pilocarpine elevates the electroshock threshold in the rat (WÓRUM and PÓRSZÁSZ 1968), and the same effect has been observed after large doses of oxotremorine in the mouse (CHEN et al. 1968).

In an attempt to obtain further data on the role played by cholinergic transmission in seizure activity, we have investigated how cholinesterase inhibitors influenced the susceptibility of the mouse to pentetrazole convulsions. After having proved the seizure-facilitatory effect of cholinesterase inhibitors, we studied the type of cholinergic receptor on which this effect is brought about and the influence of various anticholinergic compounds on the facilitation. Assuming an interaction to exist between the amines of the central nervous system the influence of amine oxidase inhibitors on the effect of cholinesterase inhibitors was also investigated.

Methods

The experiments were performed on Swiss mice of both sexes. The animals were placed in individual plastic cages 30—60 min prior to the experiments and treated in a randomized way.

Pentetrazole, in the form of a solution of 5 mg/ml, was injected into the tail vein of the animals, according to the method of ORLOFF et al. (1949). The dose causing tonic convulsions was expressed in terms of ml/10 g of body weight. The compounds investigated were injected subcutaneously (s.c.), intraperitoneally (i.p.) or intracerebrally (i.cer.) according to HALEY and MCCORMICK (1957).

The results were evaluated with two- and three-way analysis of variance (ANOVA).

The drugs used were as follows: atropin sulphate (Ph.Hg.VI.); mecamylamine (EGYT); neostigmine bromide (Stigmosan, Chinoin); nialamide (Nuredal, EGYT); oxotremorine oxalate (Institute of Experimental Medicine, Hungarian Academy of Sciences); liquid parafin (Ph.Hg. VI.); paraoxone (Chinorto, Chinoin); pentetrazole (Tetracor, Chinoin); physostigmine salicylate (Ph.Hg.VI.); tranylcypromine sulphate (Smith Kline and French). The doses mentioned refer to the corresponding salts.

Results

Susceptibility to pentetrazole seizures was not influenced by 0.03—0.2 mg/kg of physostigmine s.c., or if so, to a very slight extent only (Table I). Since even higher doses were not more effective but caused muscular fasciculation, it was assumed that the peripheral actions of the drug might interfere with its central effects. To eliminate this factor we injected the drug i.cer. and found that by this route of administration, it augmented the pentetrazole effect constantly and very considerably (Table I).

Table I

Influence of physostigmine on pentetrazole seizure threshold in the mouse. Convulsant doses of pentetrazole in ml/10 g
Mean ± S.E.

Compound and dose, mg/kg s.c., mg/animal i.cer.	Route of administration	Time of treatment	n	Dose of pentetrazole
NaCl	s.c.	−30 min	33	0.21±0.015
Physostigmine, 0.03	s.c.	−30 min	32	0.19±0.015
Physostigmine, 0.20	s.c.	−30 min	32	0.18±0.008
NaCl	i.cer.	−10 min	15	0.19±0.014
Physostigmine, 0.002	i.cer.	−10 min	15	0.14±0.005
Physostigmine, 0.005	i.cer.	−10 min	15	0.12±0.009

By the s.c. route only 0.2 mg/kg physostigmine was effective ($p \approx 0.05$)
By the i.cer. route both doses were effective ($p < 0.01$)

Our assumption concerning the peripheral effect of physostigmine was indirectly corroborated by the finding that neostigmine, a drug not penetrating into the central nervous system, instead of facilitating inhibited the convulsions induced by pentetrazole (Table II).

Table II

Influence of neostigmine on pentetrazol seizure threshold in the mouse. Convulsant doses of pentetrazole in ml/10 g
Mean ± S.E. Twelve mice in each group

Compound and dose, mg/kg s.c.	Time of treatment	Dose of pentetrazole
NaCl	−30 min	0.19±0.014
Neostigmine, 0.15	−30 min	0.18±0.006
Neostigmine, 0.20	−30 min	0.30±0.017

Neostigmine in a dose of 0.2 mg/kg was effective, $p < 0.01$

The influence of the anticholinergic agents atropine and mecamylamine on the seizure-potentiating effect of physostigmine is shown in Table III. The effect of i.cer. injected physostigmine was abolished by 2 mg/kg of atropine and decreased by 2 mg/kg of mecamylamine.

Table III

Influence of atropine and mecamylamine on the seizure-facilitatory effect of physostigmine in the mouse. Convulsant doses of pentetrazole in ml/10 g. Mean ± S.E. Physostigmine treatment: 0.005 mg/mouse, i.cer.

1st treatment, s.c., —60 min	2nd treatment, i.cer., —10 min	n	Dose of pentetrazole
NaCl	NaCl	10	0.17±0.011
NaCl	Physostigmine	10	0.11±0.010
Atropine, 2 mg/kg	NaCl	10	0.19±0.014
Atropine, 2 mg/kg	Physostigmine	10	0.18±0.014
NaCl	NaCl	20	0.21±0.010
NaCl	Physostigmine	20	0.12±0.010
Mecamylamine, 2 mg/kg	NaCl	20	0.20±0.010
Mecamylamine, 2 mg/kg	Physostigmine	20	0.17±0.010

Atropine abolished, mecamylamine inhibited but did not abolish, the effect of physostigmine

There was an interaction in both cases, $p < 0.01$

One mg/kg of paraoxone, given three hours prior to the administration of pentetrazole, was found to augment the seizure susceptibility of mice; this effect of paraoxone was counteracted by atropine (Table IV).

Diminution of the cerebral catecholamine and serotonin level facilitates (CHEN et al. 1954), and its augmentation inhibits (PROCOP et al. 1959), the

Table IV

Influence of atropine on the seizure-facilitatory effect of paraoxone in the mouse. Convulsant doses of pentetrazole in ml/10 g. Mean ± S.E. Twelve mice in each group

1st treatment, s.c., —180 min	2nd treatment, s.c., —120 min	Dose of pentetrazole
Paraffin	NaCl	0.19±0.019
Paraoxone, 1 mg/kg	NaCl	0.11±0.005
Paraffin	Atropine, 5 mg/kg	0.22±0.015
Paraoxone, 1 mg/kg	Atropine, 5 mg/kg	0.23±0.020

Interaction, $p < 0.01$. Paraoxone was effective, atropine inhibited its effect

appearance of seizures. Thus, we have investigated the influence of amine oxidase inhibitors elevating the cerebral amine level on the physostigmine-induced facilitation. As seen from Table V, the two amine oxidase inhibitors, tranylcypromine and nialamide, completely inhibited the seizure-potentiating activity of physostigmine in doses not influencing the susceptibility to pentetrazole.

Table V

Influence of tranylcypromine and nialamide on the seizure-facilitatory effect of physostigmine in the mouse. Convulsant doses of pentetrazole in ml/10 g. Mean ± S.E. Physostigmine treatment: 0.005 mg/mouse i.cer. Twelve mice in each group

1st treatment i.p.	Time of treatment	2nd treatment, i.cer., —20 min	Dose of pentetrazole
NaCl	—80 min	NaCl	0.18±0.012
NaCl	—80 min	Physostigmine	0.11±0.008
Tranylcypromine, 10 mg/kg	—80 min	NaCl	0.16±0.006
Tranylcypromine, 10 mg/kg	—80 min	Physostigmine	0.20±0.012
NaCl	—24 hours	NaCl	0.18±0.012
NaCl	—24 hours	Physostigmine	0.12±0.005
Nialamide, 100 mg/kg	—24 hours	NaCl	0.18±0.011
Nialamide, 100 mg/kg	—24 hours	Physostigmine	0.21±0.006

Physostigmine was effective in both series of experiments. Its effect was inhibited by tranylcypromine and nialamide, $p < 0.01$

Since atropine inhibits the seizure-facilitatory effect of cholinesterase inhibitors, the muscarine receptors of the central nervous system may be assumed to play a part in this effect. Thus, we investigated how oxotremorine, a drug of specific muscarinic activity and devoid of nicotine-like action (HASLETT 1963), would influence seizure susceptibility. The results of these experiments are shown in Table VI. As seen, low doses (0.01—0.02 mg/kg) of oxotremorine increased seizure susceptibility, medium doses were ineffective and high doses (0.1—0.6 mg/kg) inhibitory.

Discussion

According to our experiments, cholinesterase inhibitors increase the susceptibility of the mouse to pentetrazole convulsions by their central action. Neostigmine, a drug possessing exclusively peripheral activity, inhibits the effect of pentetrazole. A similar, i.e. peripheral, activity of physostigmine is assumed to diminish the drug's own facilitatory action of central

Table VI

Influence of oxotremorine on the pentetrazole seizure threshold in the mouse. Convulsant doses of pentetrazole in ml/10 g. Mean ± S.E. Five-day experiment, with 5 animals in each group on the first three days and with 7 animals on the 4th and 5th days

Day of experiment	Oxotremorine treatment, mg/kg, i.p., 30 min before pentetrazole							
	0.0	0.01	0.02	0.05	0.1	0.2	0.4	0.6
1	0.14 ±0.010			0.11 ±0.010	0.13 ±0.010	0.16 ±0.016	0.19** ±0.010	0.21** ±0.01
2	0.11 ±0.002			0.11 ±0.002	0.14** ±0.005	0.16** ±0.004	0.18** ±0.008	0.19** ±0.003
3	0.12 ±0.003			0.12 ±0.01	0.14 ±0.01	0.15* ±0.003	0.16** ±0.007	0.21** ±0.005
4	0.15 ±0.008	0.10** ±0.001	0.11** ±0.002	0.13 ±0.004				0.17* ±0.007
5	0.15 ±0.003	0.15 ±0.005	0.18** ±0.003	0.14 ±0.002				0.24** ±0.007

ANOVA-Dunnett test. The values significantly differing from controls not treated with oxotremorine (0.0 mg/kg): * < 0.05, ** $p < 0.01$

origin. This is why physostigmine is definitely effective after i. cer., but only slightly after s.c. application. It should be noted that physostigmine elicits marked tremor when injected i. cer; this occurs rarely after s.c. administration and is difficult to evaluate because of the muscular fasciculation (MOLNÁR et al. 1967).

The two groups of cholinomimetics produce different central nervous effects: nicotine causes convulsions whereas oxotremorine and arecoline evoke tremor. It has been assumed that two types of cholinergic receptors, i.e. muscarinic and nicotinic (M and N), are present in the central nervous system (PFEIFFER et al. 1967). The first type is inhibited by atropine, and the second type by mecamylamine.

The question arises of what type of receptor might be involved into the facilitatory action on pentetrazole convulsions of cholinesterase inhibitors which elevate the endogenous acetylcholine level in the brain. According to our results, mecamylamine decreases and atropine abolishes the effect of physostigmine. Accordingly, both N and M receptors must figure in the facilitation. In the knowledge of the convulsant action of nicotine, one does not have to add anything particular to its effect exerted on N-receptors. Accordingly, the nicotine-like activity of cholinesterase inhibitors and of endogenous acetylcholine plays a part in the facilitation. The role of muscarinic

receptors in the development of seizures is more debated. According to Cox and Potkonjak (1970), oxotremorine causes convulsions in the rat; in our experiments, this was seen to occur exclusively after reserpine pretreatment (Phan et al. 1974). Carbachol elicits convulsions after i.cer. injection in the mouse. The compound has a dual site of action (M and N); still these seizures are prevented by atropine (Decsi et al. 1963). Atropine inhibits the seizure-facilitatory action; thus we assume that central M receptors are also playing a part in this action of the cholinesterase inhibitors. This assumption is corroborated by the finding that low doses of oxotremorine, a drug of primarily M activity, diminish the convulsant dose of pentetrazole, just like the cholinesterase inhibitors do. Large doses of oxotremorine inhibit pentetrazole convulsions. These results are in accordance with the observation of Chen et al. (1968) who found low doses of oxotremorine (0.25 mg/kg as fumarate salt) to diminish, and high doses (2.0—4.0 mg/kg!) to elevate, the electroshock threshold. The mechanism of the anticonvulsant action of oxotremorine was not analyzed in the present experiments; this action may be a consequence of the ergotropic stimulatory action of the compound. This action is marked in the cat and manifest itself with a rage reaction and with signs of sympathetic excitation (György et al. 1971a). The effect is latent in the mouse: after repeated administrations the drug counteract the gradual decrease of spontaneous motility (György et al. 1971b); while the hypermotility-inducing effect of amphetamine is augmented by tremorine pretreatment in the mouse (unpublished data). Ergotropic excitation, on the other hand, is known to diminish seizure susceptibility (Procop et al. 1959).

As regards seizure facilitation, the monoamines and acetylcholine are probably of antagonistic activity in the central nervous system. (Administration of amine oxidase inhibitors counteracted the potentiating effect of physostigmine). Since amine oxidase inhibitors elevate the level of the catecholamines and of serotonin, it is an open question which of the amines might be figuring in the above antagonism. Experiments in this direction are now in progress in this laboratory.

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EFFECT OF DROPERIDOL ON THE ELECTRICAL ACTIVITY OF RABBIT ATRIAL CELLS

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Intracellular recordings of rabbit right atrial tissue were obtained under the influence of the neuroleptic agent droperidol. A shortening of action potential duration, dV/dt, occurred, the rate of discharge diminished and the heart stopped after long exposure to the drug. In latent pacemaker cells, the amplitude of diastolic depolarization and overshoot decreased. In contrast, true pacemaker cells increased in amplitude; their automatism was not affected by the drug.

It is concluded that high concentrations of or long exposure to, the drug cause exit blockade of impulses generated at the sinus node.

Droperidol is a neuroleptic drug. Its pharmacological properties have extensively been studied (JANSSEN et al. 1963, 1965a, b). Its cardiovascular effects include a slight lowering of blood pressure as a result of peripheral vasodilatation (SHAPER et al. 1963; TORUNCHA et al. 1972), a lack of influence on the mechanical properties (TORUNCHA et al. 1974), and an antiarrhythmic effect in ectopic ventricular rhythms (DAHLGREN 1970; LONG et al. 1967). Its action on heart cell membranes was studied by intracellular recordings by HAUSWIRTH (1968). This author observed a slowing of the rate of increase and a shortening of the duration of the action potentials with a prolonged functional refractory period, without any significant change in resting potential and membrane resistance. As an explanation, these effects were attributed to inhibition of the Na^+-transporting system of the cell membrane. KERN et al. (1971) confirmed these results by voltage-clamp measurements and they reported a progressive reduction of the amplitude of the action potential. This research was conducted in non-automatic tissue and, in both cases, prolonged exposure to the drug or high dosage resulted in complete and irreversible impulse arrest.

With the purpose of determining whether this effect of droperidol was due to a disturbance of impulse formation or to a conduction blockade, it was decided to study the action potentials of automatic atrial cells and their correlation with those encountered in non-specialized conducting tissue.

Methods

The right atrium from rabbits of both sexes killed by a blow on the neck was employed. The area limited by the atrial septum, the cristae terminalis, the base of the superior vena cava and the coronary sinus, were explored with floating micro-electrodes of less than 0.5 tip diameter and a resistance between 20 and 50 Megaohms. Tyrode solution of the following composition was used: NaCl, 124; KCl, 3.0; $CaCl_2$, 2.5; $NaHCO_3$, 15.0; $MgCl_2$, 1.2; NaH_2PO_4, 1.2; glucose, 5.5 mM. The solution was aereated with 95% O_2 and 5% CO_2, the temperature was maintained at 31—32 °C. Automatic cells of three types (VALLADARES and POLUNIN 1972), all with marked diastolic depolarization, and non-automatic cells were observed before and after addition of droperidol to the bathing solution. In some cases, it was possible to follow the changes caused by the drug in one cell throughout the experiment, in others an average observation of at least 10 cells was registered.

Droperidol (Janssen Pharmaceutica, Beerse, Belgium) was employed. The stock solution contained tartaric acid, of a pH between 3 and 4. Test solutions containing 10^{-5} droperidol were obtained by adding appropriate amounts of the stock solution to the tyrode solution in the bath.

Results

The influence of droperidol on the shape of the action potential of automatic cells after 10 and 30 minutes of exposure of the tissue to a concentration of 10^{-5} M of the drug is illustrated in Fig. 1. The amplitude of the action po-

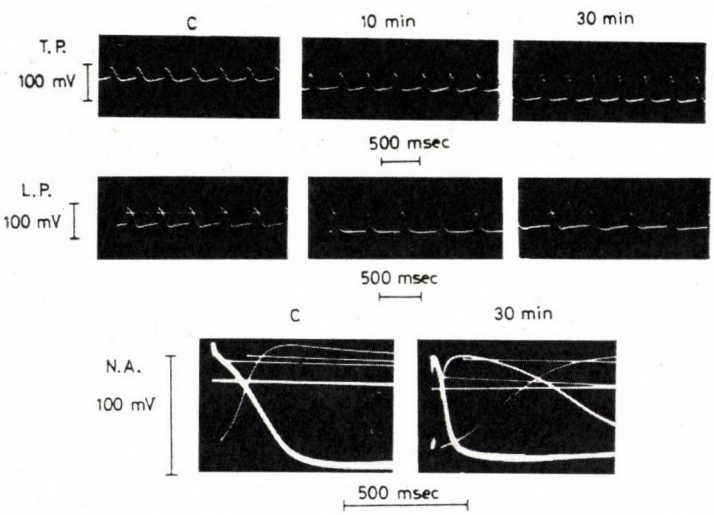

Fig. 1. Intracellular recordings of pacemaker (T.P.), latent pacemaker cells (L.P.), and non-automatic cells (N.A.), under the influence of 10^{-5} M droperidol. C: Control, before drug

tential increased in the cells in the central part of the sinus node, described by HOFFMAN and CRANEFIELD (1960) as true pacemaker cells, and as group I by VALLADARES and POLUNIN (1972). In contrast, the action potential of latent pacemakers or group II decreased in height. Non-automatic cells responded with a diminished action potential and with a marked delay of the

increase (see Fig. 1). Diastolic depolarization decreased, and in some cases was abolished only in latent pacemaker cells, while no significant change of this parameter was observed in true pacemaker cells. All the potentials were shortened in duration with the duration of exposure to the drug. The frequency of discharge also decreased with time, until a total arrest had occurred after 60 minutes in latent and non-automatic cells. In true pacemakers it was possible to register activation for more than 90 minutes.

Table I

Parameters of atrial fibre action potentials under the influence of droperidol

Cell type	n		R.P., mV	O.S., mV	D.D.A., mV	A.P.D., msec	R.R., V/sec
T.P.	26	C	53.0±1.4	7.0 ±0.21	16.0±0.5	266 ± 9.2	180 ± 6.4
		D	50.8±1.8	8.9*±0.18	15.6±0.3	174**± 3.5	172 ± 7.0
L.P.	40	C	84.0±1.7	22.2 ±1.1	9.2±0.2	226 ±11.4	221 ±11.2
		D	78.9±2.1	16.3*±1.2	7.0±0.21	108**± 7.2	132**± 8.0
N.A.	46	C	87.3±2.1	25.4±1.4	—	186 ± 9.0	328 ±12.7
		D	81.2±1.8	16.7*±1.1	—	101**± 7.1	183**± 8.1

R.P.: resting potential; O.S.: overshoot; D.D.A.: diastolic depolarization amplitude; A.P.D.: action potential duration; R.R.: rate of rise of phase O; T.P.: true pacemaker; cells; L.P.: latent pacemaker cells; N.A.: non-automatic cells; C: control values; D: after 10^{-5}M droperidol

* $p < 0.05$ from control value; ** $p < 0.01$ from control value

Results for 26 measurements on true pacemaker cells and 40 other types of automatic atrial cell, and 46 without automatism, can be seen on Table I. These observations were made after 20 minutes exposure to the drug at a concentration of 10^{-5} M.

Discussion

Prolonged exposure of non-automatic tissue to droperidol at high concentrations resulted in inexcitability preceded by a marked decrease in amplitude, duration and rate of increase of the action potential. Automatic latent pacemakers seemed to undergo similar changes and in addition tended to lose their diastolic depolarization. In contrast, the action potentials from true pacemaker cells gained in amplitude and did not alter their diastolic depolarization. Although no proof could be obtained that these changes had occurred simultaneously in all three types of cell because only one micro-electrode was

used, we still assumed it in view of the stability of the results and the randomization of measurements.

The relationship of the different functional structures of the heart is well-known (PAES DE CARVALHO et al. 1959; WAGNER et al. 1966; HOGAN and DAVIS 1968; STRAUSS and BIGGER 1972) and it is generally accepted that the sensibility of the response of myocardial cells differs and that the sinus node is the least labile of the structures involved (GELBAND et al. 1972; TEN EICK et al. 1971).

Thus, droperidol exerts a depressive effect on latent pacemakers and non-automatic atrial tissue, simultaneously relieving the true pacemaker cells from an inhibitory influence.

Considering that true pacemaker cells retain their automatism the neighbouring structures become inexcitable, the immediate cause of cardiac arrest would seem to be due to an exit blockade of the sinus node impulse.

It is concluded that droperidol causes exit blockade of impulses generated at the sinus node.

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NEW DATA ON THE MECHANISM OF THE DEPOLARIZING ACTION OF VERATRINE ON SKELETAL MUSCLE

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The mechanism of the depolarizing action of veratrine was investigated on the frog sartorius muscle.

1. Veratrine, applied after the removal of chloride ions from the extracellular space, caused the muscle to depolarize unchanged.

2. After removal of extracellular potassium, 0.1 mM of veratrine did not depolarize during the 2—3 hours of observation or, if so, only during a considerably longer period of time than in Ringer's solution containing 2.5 mM of potassium.

3. The characteristic depolarizing effect of 0.1 mM veratrine failed to come about in hypertonic solutions of different composition (normal Ringer + 150 mM NaCl; normal Ringer + 300 mM sucrose; normal Ringer + 300 mM glucose) in spite of persisting functioning of the spike-channel under these conditions.

4. A single electric stimulus applied in an appropriate phase of the latency period between veratrine application and the development of depolarization, was found to trigger the appearance of veratrine depolarization.

In previous experiments it has been shown that veratrine does not depolarize the muscle in a sodium-free (choline) Ringer. In fact, the muscle previously depolarized with veratrine in normal Ringer will be fully repolarized in choline-Ringer, in spite of the continued presence of veratrine. The depolarizing effect of veratrine is counteracted by tetrodotoxin (10^{-7} g/ml) in a reversible way (VARGA et al. 1972a). These observations and the data in the literature on neural structures show that the depolarizing effect of veratrine is a sodium-dependent process in the skeletal muscle. In accordance with this, we observed an increase of Na-influx and of $[Na]_i$ under the effect of veratrine (VARGA et al. 1972b). All this proves the role played by sodium ions in the depolarizing action of veratrine. On the other hand, the role of potassium and chloride ions is not quite clear.

The role of Cl^- transport was first raised by SHANES et al. (1953) who studied the characteristic changes of negative after-potentials upon the effect of veratrine. On the other hand, SPERELAKIS and PAPPANO (1969) found an unaltered depolarizing effect of veratrine on heart cells kept in chloride-free milieu (tissue culture) as compared to that observed in chloride-containing milieu. The role of potassium was first emphasized by BACQ (1939), and later SHANES (1952) showed that K-efflux from the nerve increased under the effect of veratrine. We too observed a transient increase in the rate coefficient

of K-efflux in the frog sartorius upon the effect of the drug. However, the potassium content calculated for the dry-substance of the muscle remained unchanged and it was only the concentration of potassium which diminished in proportion to the water uptake (VARGA et al. 1972b). On the basis the above data it seemed interesting to complete our previous investigations concerning veratrine depolarization with the experiments to be reported below.

Methods

The experiments were performed on the sartorius muscle of the frog *Rana esculenta*, between April, 1971, and May, 1973. Seasonal alterations were observed only a few winter frogs. These observations, perhaps useful in connection with the mechanism of the depolarizing effect of veratrine, will be published elsewhere. The muscle was prepared under the microscope, than kept in Ringer's solution for 30 min, and only those muscles were used in the experiments the fibres of which had remained intact. Intactness of the preparations was checked at the end of the experiment microscopically and on the basis of recovering of membrane potential as well as by controlling electrical excitability.

The experiments were performed on pairs of muscle. A great number of observation had revealed considerable individual deviations in latency and magnitude of the depolarization, the height of the amplitude of oscillation, its frequency, etc. For these reasons only results obtained on pairs of muscle can be compared reliably.

The sartorius muscle was stretched to 4/3 of its slack length and fixed in a separating chamber, originally constructed for separation of the pelvic part of the muscle, practically free of end-plates (PEZARD and MAY 1937), from its distal, so-called neural, part (GESZTELYI and KOVÁCS 1970). In addition, the separating chamber allows a continuous recording of the potential difference between the two parts of the muscle, separated with a rubber diaphragm. This latter not only separates the solutions on the two sides but, in an ideal case, also insulates electrically the surface of the muscle membrane pulled through it. Thus, in practice, it is the mean potential difference of the fibres of the two muscle parts which can be recorded from the incubation solutions (GESZTELYI 1973). The potential was measured by means of the amplifier of a Keithley 604 type electrometer. The output signal of the amplifier was recorded from a DISA Universal Indicator oscilloscope by means of a photorecorder. The slow (7.5 min/div) horizontal deflection corresponding to the beam of the oscilloscope was ensured by means of an external generator constructed for this purpose.

Since we explicitly wanted to study the effect of veratrine on the muscle membrane, the drug was always applied on the aneural part of the muscle in the separating chamber. The veratrine spikes of the aneural muscle part could pass through the rubber membrane from one compartment of the separating chamber to the other part of the muscle. To prevent this, the neural muscle part was incubated in Na-free (choline) Ringer. The solutions were changed at 10—15 min intervals on both the aneural and neural parts.

Intracellular potential measurements were carried out with microelectrodes of the Ling-Gerard type (LING and GERARD 1949). The tip potential of the electrodes was under —5 mV, its resistance between 5 and 10 Mohm (ADRIAN 1956). The membrane potential was measured with a Keithley 604 type electrometer. The membrane potential was determined on the basis of 5—10 measurements in each case.

Triggerability by an electric stimulus of the veratrine depolarization was studied by means of a DISA Multistim apparatus. As stimulating electrodes, platinum plates, 3.5×0.3 mm in size and placed parallel at a distance of 6 mm, were used. The amplitude of the impulse was 2.5-fold of the voltage corresponding to the stimulus threshold, the impulse width was 0.1 msec.

Composition of the solutions used is shown in Table I; their pH was 7.0 and their temperature between 20 and 23 °C.

A veratrine preparation made by Merck was used in the experiments. Its veratridine content, determined by a procedure prescribed by the factory was 33.3%. The other 2/3 of the alkaloid preparation was cevadine (VARGA et al. 1972a). The two alkaloids have an identical depolarizing effect on skeletal muscle (FALK 1961; VARGA et al. 1972a).

Choline chloride (Reanal), used for replacement of sodium, was recrystallized twice before administration. Tetrodotoxin (TTX, Sankyo) as well as d-tubocurarine (Sigma Chemical Co.) was recrystallized three times. All solutions were freshly prepared before use.

Results

Investigation of the depolarizing effect of veratrine in chloride-free milieu. In these experiments we studied whether the removal of $[Cl]_0$ would cause any significant change in the extent and time-course of the veratrine-induced depolarization. As known from the results of HODGKIN and HOROWICZ (1959), the muscle will be depolarized on an abrupt diminution of the chloride content of the Ringer's solution. This depolarization is a reversible one soon followed by repolarization even in chloride-free Ringer. Thus, when the membrane

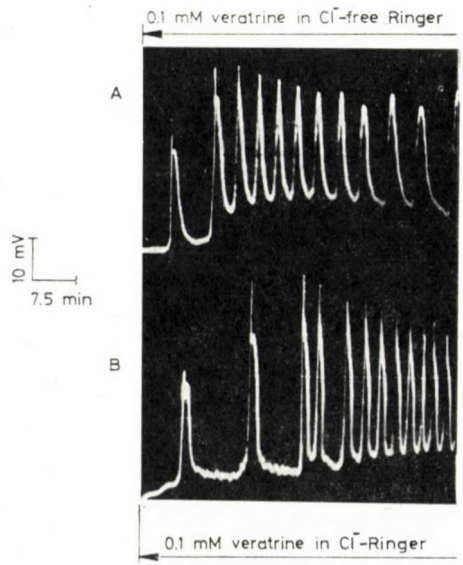

Fig. 1. Investigation of the depolarizing effect of veratrine in $[Cl^-]_0$-free milieu. Both members of the muscle pair were placed separately in a separating chamber. The aneural part of one muscle (A) was equilibrated in chloride-free (sulphate) Ringer (Table I, E), the neural part in choline-sulphate-Ringer (Table I, F) for 90 min before veratrine treatment. The aneural part of the control member (B) of the muscle pair was kept, similarly for 90 min, in a solution containing 80 mM Na, 1 mM Ca and 121 mM Cl (Table I, G), while the neural part in a solution of similar composition but containing choline instead of Na (Table I, H). Experiment reference: 37/46-III

potential had returned its original value, the sulphate-Ringer in the aneural part was replaced by a sulphate-Ringer containing 0.1 mM of veratrine. A typical experiment is shown in Fig. 1.

As seen from Fig. 1, the depolarizing effect of veratrine took place in a chloride-free milieu, too. There was no difference either in the time of appearance or the extent of depolarization in the average of 9 experiments.

The potential change seen in the "control", i.e. chloride-containing, Ringer differed from the characteristic pattern observed in normal Ringer

Table I

Composition of the solutions

Solutions	K^+	Cl^-	Na^+	Ca^{2+}	HPO_4^{2-}	$H_2PO_4^-$	SO_4^{2-}	Choline	TRIS buffer	Sucrose	Glucose
	mM										
A Normal Ringer	2.5	121.1	120	1.8	2.15	0.85					
B Na^+-free (choline) Ringer	2.5	123.6		1.8	1.08	0.43		120			
C K^+-free Ringer		121.1	122.5	1.8	2.15	0.85					
D K^+- and Na^+-free (choline) Ringer		123.6		1.8		*		120	5		
E Cl^--free (sulphate) Ringer	2.5		80	8	1.08	0.43	48			113	
F Cl^-- and Na^+-free Ringer	2.5			8	1.08	0.43	48	80		113	
G Ringer with reduced Na^+ and Ca^{2+} content	2.5	121.1	80	1.0	2.15	0.85		41.6			
H Na^+-free Ringer with reduced Ca^{2+} content	2.5	123.6		1.0	1.08	0.43		121.6			
I Hypertonic solution containing 270 mM NaCl	2.5	271.1	270	1.8	2.15	0.85					
J Hypertonic solution 270 mM choline	2.5	273.6		1.8	1.08	0.43		270			
K Hypertonic solution containing 300 mM sucrose	2.5	121.1	120	1.8	2.15	0.85				300	
L Na^+-free hypertonic solution containing 300 mM sucrose	2.5	123.6		1.8	1.08	0.43		120		300	
M Hypertonic solution containing 300 mM glucose	2.5	121.1	120	1.8	2.15	0.85					300
N Na^+-free hypertonic solution containing 300 mM glucose	2.5	123.6		1.8	1.08	0.43		120			300

* TRIS base was adjusted with ortho-phosphoric acid to pH 7.0 at 22 °C

under the effect of veratrine. Comparison of curve B in Fig. 1 with the control curves shown in Figs 2—6 revealed a striking difference in both the magnitude and character of the first wave of oscillation. This difference was probably due to the fact that the amount of ionized calcium was lower (1 mM, see HODGKIN and HOROWICZ 1959) in the sulphate-Ringer applied on muscle A (Table I, E) than in normal Ringer's solution. The same was true for the sodium

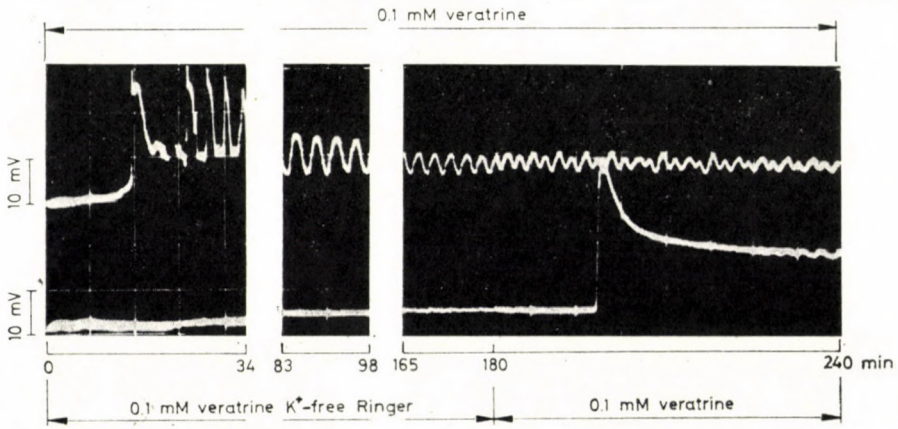

Fig. 2. Investigation of the depolarizing effect of veratrine in K-free milieu. One member of the muscle pair was equilibrated in K-free Ringer (aneural part: C; neural part: D, Table I) for 90 min (lower curve). The aneural part of the control muscle (upper curve) was kept in normal Ringer (Table I, A), the neural part in choline-Ringer (Table I, B), both for 90 min. To prevent the possible disturbing effect of potassium traces, the agar bridges were prepared with K-free, and not with normal Ringer solution in these experiments. Experiment reference: 37/3

content; we therefore applied 1 mM $CaCl_2$ and 80 mM Na also in the Ringer's solution of the "control" muscle (B).

Depolarizing effect of veratrine in K-free Ringer's solution. In some exploratory experiments, the aneural part of the muscle in the separating chamber was kept in K-free Ringer's solution (Table I, C) for 30—60 min before veratrine treatment. In two out of seven experiments no depolarization occurred under the effect of 0.1 mM veratrine within 80 and 120 min, respectively. In further five experiments a depolarization was though observed but this occurred after 58 $\pm$ 27.2 min on the average, in contrast to the average latency of 25.3 $\pm$ 10.4 min seen in the case of the other member of the muscle pair incubated in normal Ringer.

In view of the wide scattering of the results, in further experiments the duration of incubation in K-free Ringer was prolonged to 90 min. In addition, unlike in the exploratory experiments, the neural part serving as reference basis was also immersed into K-free Ringer. A characteristic record of an experiment performed in this way can be seen in Fig. 2.

As seen from Fig. 2, by the 14th min veratrine had induced a well-defined depolarization in the muscle kept in Ringer solution containing 2.5 mM potassium while a depolarization of only about 5 mV could be observed in the muscle kept in K-free Ringer for 90 min, even in the 180th min of incubation. The effect of K-free Ringer antagonizing veratrine depolarization was reversible; a depolarization equal in magnitude to the control developed 18 min after substitution of the K-free solution with veratrine-containing normal Ringer. In 11 similar experiments, veratrine depolarization developed in 21.5 ± 13.7 min in the control muscles, while in the muscles preincubated in K-free Ringer no depolarization was seen in any case during observations for 120—240 min (mean, 163 ± 28.3 min). Comparison of the two curves demonstrated in Fig. 2 reveals a considerable difference in the oscillation reaction of the control muscle and the muscle pretreated in K-free Ringer. The cause of this difference which was observed in every experiment, has not been investigated.

When evaluating the experiments, one will naturally be faced by the question of whether the development of veratrine depolarization was retarded and inhibited by the absence of potassium ions or by the hyperpolarization due to their absence. Therefore, we measured the membrane potential by means of intracellular microelectrodes in 430 superficial sartorius muscle fibres incubated in K-free Ringer solution for 60 min. Of the 430 fibres investigated, 336 were hyperpolarized with 23.9 ± 8.3 mV and 94 depolarized with 27.5 ± ± 13.9 mV on the average. Hyperpolarization, once it had developed, remained practically unchanged for 4—5 hours. This finding, and also our data on the ratio of hyperpolarized to depolarized fibres differ from the results of AKIYAMA and GRUNDFEST (1971). The discrepancy may have been due to the different experimental conditions. The authors mentioned prepared and isolated fibres from the semitendinosus muscle of *Rana pipiens* and used this specimen for following changes in resting potential caused by the K-free solution. In contrast, our measurements were performed on the superficial fibres of the sartorius muscle of *R. esculenta*. The fact, however, that the membrane potential even of the superficial fibres was of opposite direction and changed within wide limits means that the results for potential changes measured on the superficial fibres were *a priori* inadequate for answering the above question. Therefore, we used GESZTELYI's method (1973) and in seven further experiments followed the potential change of the aneural muscle part kept in K-free Ringer's solution with respect to the neural one. Under these conditions a hyperpolarization averaging 5.5 ± 2 mV was found which developed in about 60 min and lasted without change for hours.

Effect of hypertonicity on veratrine depolarization. The observations concerning the dependence of veratrine depolarization on sodium concentration are quite unequivocal (ULBRICHT 1969). In full accordance with this,

ULBRICHT and FLACKE (1965) as well as ULBRICHT (1969) found the after-potential increasing effect of veratrine to become even more marked when the sodium concentration of the Ringer solution was raised to the 2—3-fold. These authors applied the Na-rich solution for the short period of 15 sec, in order to avoid the side-effects of the hypertonic solution. It seemed therefore desirable to employ solutions of high sodium concentration in our experments. As known from the results of HODGKIN and HOROWICZ (1957),

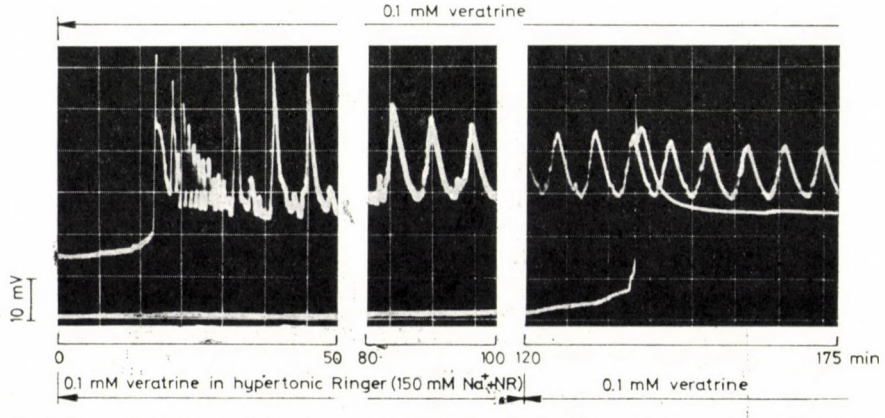

Fig. 3. Effect of hypertonicity on veratrine depolarization. One member (lower curve) of the muscle pair was equilibrated in hypertonic solution (aneural part: I; neural part: J; Table I) for 45 min before veratrine treatment. The aneural part of the control muscle (upper curve) was kept in normal Ringer (Table I, A), the neural part in choline-Ringer (Table I, B) for the same period. Experiment reference: 35/78-II

although an unchanged action potential can be demonstrated in skeletal muscle after treatment with hypertonic solutions, but the mechanical response fails to occur. It was therefore hoped that in muscle incubated in a sodium-rich solution the mechanical answer sometimes disturbing the recording will be absent and, on the other hand, that the depolarization will be more marked owing to the high sodium concentration.

In these experiments, 150 mM NaCl was added to normal Ringer's solution, and the aneural part of the muscle placed into the separating chamber was treated with it for 45 min. After this pretreatment, a hypertonic solution containing 0.1 mM veratrine was applied on the aneural part. A typical experiment is shown in Fig. 3.

As seen from Fig. 3, the otherwise characteristic potential change failed to develop under the effect of veratrine in the muscle incubated in hypertonic solution during the 120-min observation period. This inhibitory effect of the hypertonic solution was reversible since, when in the 120th min the muscle had been transferred into isotonic Ringer's solution similarly containing 0.1 mM of veratrine, the depolarizing effect reappeared in ten of experiments.

Further experiments were performed with a solution made hypertonic with 300 mM of sucrose, in order to decide whether it was really the hypertonicity and not the augmented sodium concentration which had antagonized the effect of veratrine.

As seen from Fig. 4, depolarization in the control muscle began with a characteristic oscillation wave by the 29th min of treatment with 0.1 mM of veratrine. No similar potential change was observed during the 140-min

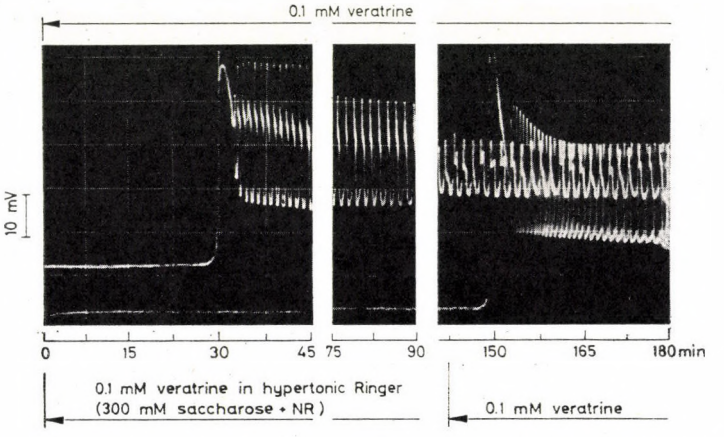

Fig. 4. **Investigation of the depolarizing effect of veratrine in hypertonic solution. One member (lower curve) of the muscle pair was equilibrated in hypertonic Ringer solution (aneural part: K; neural part: L, Table I) for 45 min before veratrine treatment. The aneural part of the control muscle (upper curve) was kept in normal Ringer (Table I, A), the neural part in choline-Ringer (Table I, B) for the same period of time. Experiment reference: 35/67-II**

observation period in the solution made hypertonic with sucrose. However, when the muscle was taken out from the hypertonic and put into an isotonic solution, a characteristic depolarization and oscillation developed after 8.5 min (seven experiments).

Similar results were obtained in six further experiments in which the Ringer's solution was made hypertonic with 300 mM of glucose. A typical record is shown in Fig. 5.

As seen from Fig. 5, the characteristic veratrine depolarization developed in both members of the muscle pair by the 8th and 13th min, respectively. By the 38th min of the experiment, the aneural part of one member of the muscle pair was transferred into a hypertonic solution (Table I, M) containing the same amount of veratrine. Oscillation immediately stopped and, though slowly, a repolarization took place. This process, too, proved reversible since returning the muscle into the veratrine-containing isotonic solution resulted in depolarization and oscillation by the 11th min.

Can the development of veratrine depolarization be triggered by electric stimulus? As stated in our first experiments, the neural muscle section was depolarized considerably sooner (5.7 ± 2.5 min in the average of 24 experiments) then the aneural part not containing neural elements (14 ± 7 min in the average of 167 experiments). This observation had already raised the possibility that the cause of the difference might be the spike developed on,

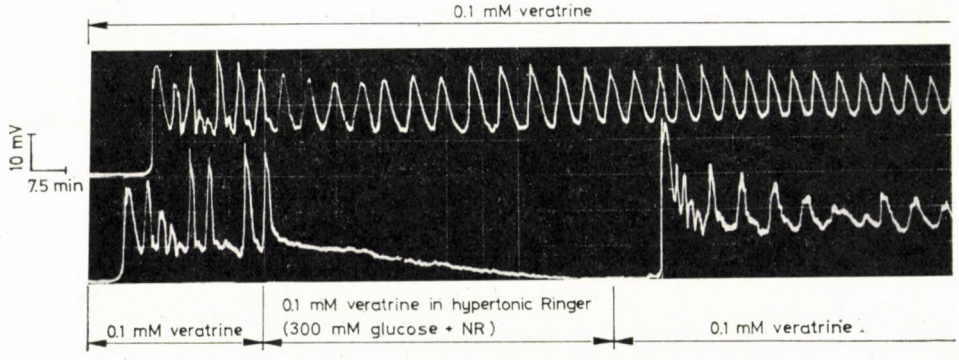

Fig. 5. Effect of hypertonicity on veratrine-induced depolarization. Both members of the muscle pair were in normal Ringer solution before veratrine treatment. Experiment reference: 34/116

and propagated from, the post-synaptic membrane under the effect of veratrine, which spike could possibly trigger the otherwise slowly developing veratrine depolarization. This assumption was substantiated by the observations of ULBRICHT and FLACKE (1965) as well as MEVES (1966).

In the course of these experiments, on one member of the muscle pair we determined the time necessary for the development of the depolarizing action of veratrine. Depolarization was considered to begin at the appearance of the first high depolarization wave. Then the other member of the muscle pair was treated in a similar way, with the difference that an electric impulse was given to the muscle through the external electrodes during the latency period between veratrine treatment and development of the expected depolarization.

One of such 15 experiments is shown in Fig. 6.

As shown by the lower curve in Fig. 6, no depolarization occurred in the control muscle up to the 29th min. Thereafter, a characteristic depolarization wave of nearly 30 mV appeared, followed by a characteristic oscillation. On the other member of the muscle pair depolarization and oscillation induced by the stimulus given in the 11th—12th min of veratrine treatment. Thus, this observation unequivocally showed that veratrine depolarization can in fact be triggered by an external stimulus. However, some external stimuli

applied in various phases of the latency period were ineffective. This usually occurred in cases when the external stimulus had been applied within the first quarter of the latency period of the control muscle. In such cases it was the sum of action potentials elicited by the external stimulus which could be recorded, in addition to the characteristically prolonged after-potential due to the presence of veratrine. This was followed by an interval of some length

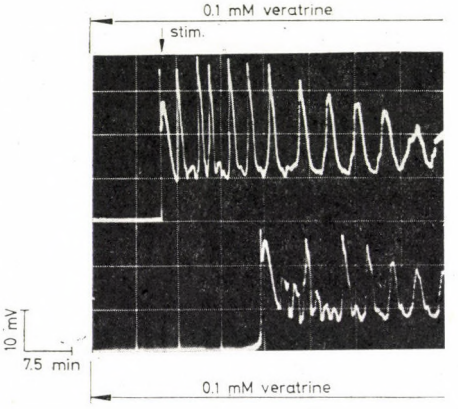

Fig. 6. Effect of electrical stimulus on the latency period of veratrine depolarization. For explanation, see text. Experiment reference: 33/99

and then by spontaneous depolarization and oscillation at about the same time as in the control muscle. Thus, the development of veratrine depolarization can be triggered by an external stimulus applied at the appropriate point of time.

Discussion

The results described above showed the muscle depolarizing effect of 0.1 mM of veratrine to be identical in chloride-free or normal Ringer's solution. Accordingly, no significance can be ascribed to chloride ions in the depolarizing effect of veratrine.

It was further demonstrated that on the effect of 0.1 mM veratrine no depolarization occurs in muscles incubated in K-free Ringer or, if so, then after a considerably longer period of time only. The question arises whether it is the presence of $[K]_0$ to which importance has to be ascribed in the mechanism of veratrine depolarization or is it the hyperpolarization due to potassium deficiency which antagonizes the veratrine effect? According to data in the literature, the hyperpolarized membrane exhibits a decreased sensitivity to veratrine, and what in more, the veratrine action already developed will cease upon the effect of a suitably strong anelectrotone (FLECKEN-

STEIN and RICHTER 1953; ULBRICHT 1969). It is, however, questionable whether the hyperpolarization measured by us was really sufficient for antagonizing the depolarizing effect of veratrine. In the lack of any direct evidence, we are reduced to assumptions for the time being.

The results described unequivocally prove that the depolarizing effect of veratrine on muscles treated with hypertonic solutions of different composition can be counteracted in a reversible way. This observation is especially interesting in the light of the findings of HODGKIN and HOROWICZ (1957) who proved that action potential can be generated on muscle fibres incubated in hypertonic solution. This was observed also under the conditions of our experiments. The question is whether the fact that action potential can be generated on the membrane and, at the same time, veratrine does not depolarize could be explained by the non-identity of the sodium-channel, involving in veratrine depolarization, with the spike-channel? In our opinion, another explanation is also possible. According to ULBRICHT's (1972a, b) convincing conclusions, no new channels will be open up made the effect of veratrine but the spike-channel will undergo certain modifications. To explain our observations on the basis of this theory, we may assume that a hypertonicity may render impossible the modification of the sodium-channel which modification would otherwise be brought about by the veratrine effect.

According to our findings, the depolarizing effect of veratrine is counteracted by any of the three different hypertonic solutions applied. This raises the question whether the changes in water content would not deserve special attention in future investigations into the mechanism of veratrine depolarization. In this respect, the pertaining literature fails to give directly utilizable information. We have already called attention to the fact that the water content of the muscle will be augmented upon the effect of veratrine treatment (VARGA et al. 1972b). In the present experiments, 0.1 mM veratrine in isotonic Ringer's solution caused a $16.1 \pm 4.8\%$ increase in the wet weight of the muscle during 60 min (mean of 40 experiments). On the other hand, the wet weight diminished by 22—25% in various hypertonic solutions during the 90-min pretreatment (eight pairs of muscle each). No swelling under the effect of veratrine was observed in such a milieu. Thus, it seems on the basis of these data that veratrine will depolarize the muscle in the case when the wet weight of the muscle increases, i.e. in the case of a water uptake. Whether this water uptake is causally associated with the mechanism of depolarization, or is just an accompanying phenomenon, cannot yet be answered.

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ACTION OF POLYMETHYLENE-BIS-TRIMETHYLAMMONIUM COMPOUNDS AND THEIR AFFINITY TO SKELETAL MUSCLE CHOLINORECEPTORS

By

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1. The effects of polymethylene-bis-trimethylammonium analogues (BTM) with 3 to 20 methylene groups between the two nitrogen atoms were studied on the frog's rectus abdominis muscle. Type of action, activity and affinity constant (K_a) of the compounds were determined.

2. The relation between the type of action of the compounds on a skeletal muscle and their affinity constant (K_a) to the acetylcholine receptor was established. The compounds with a low K_a (BTM-4—BTM-6; $K_a = 3.3 - 10 \times 10^5$) proved to be weak antagonists; compounds with intermediate K_a value (BTM-7—BTM-14; $K_a = 3.3 \times 10^4 - 2.1 \times 10^6$) were partial agonists; and the compounds with high K_a values (BTM-15—BTM-20; $K_a = 6.7 \times 10^6 - 2.9 \times 10^7$) acted as strong antagonists. The affinity constant of the agonist BTM-3 was not determined.

3. The results obtained showed that any BTM compound bound strongly enough to the receptor exerts an acetylcholine-like action. In other words, the dissociation constant of an agonist-receptor complex has to be near the optimum. The compounds readily forming a drug-receptor complex are strong antagonists, while those with a slight affinity are weak antagonists.

4. The mechanism whereby affinity constants modify the type of action of a compound on the acetylcholine receptor, is discussed.

A trimethylammonium structure of the cationic group of mono- and bis-quaternary compounds was found to be the best for depolarizing skeletal muscles (BARLOW and ING 1948; ING 1949; HOLTON and ING 1949; ARIENS and DE GROOT 1954; BARLOW 1955; CAVALLITO 1967; BARLOW et al. 1971). Strong agonists such as polymethylene have a lepto (BOVET et al. 1951) structure with an intercationic chain on the whole length or, at least, in the neighbourhood of the cationic group (CAVALLITO and GREY 1960; DANILOV et al. 1966, 1968, 1972a, b). This chemical character may provide optimal conditions for the quaternary nitrogen atom to approach the anionic site of the receptor and "neutralize" its charge (VAN ROSSUM and ARIENS 1959; ARIENS et al. 1964). The direct bond of a methonium-cationic group with a pachi-structure leads to a decrease or loss of the depolarizing properties, even if the middle part of the chain remains in lepto form. The result is the same if the volume of the cationic group is increased (BARLOW et al. 1971). There are, however, methonium compounds with lepto-structure which are devoid of depolarizing property. For example, a non-depolarizing type of action is produced by the higher homologues of alkyltrimethylammonium

(PHILLIPOT and DALLEMAGNE 1951; ARIENS et al. 1956; PATON 1961), by the polymethylene-bis-trimethylammonium series (BARLOW and ZOLLER 1964), and by penta- and hexamethylene-bis-trimethylammonium (PATON and ZAIMIS 1949; THESLEFF and UNNA 1954; BARLOW and ZOLLER 1964). The non-depolarizing property of higher homologues of these series may be explained by the fact that these compounds form a stable complex with the receptor (PATON 1961).

It is not clear why penta- and hexamethylene-bis-trimethylammonium have no depolarizing effect. Earlier we had suggested (DANILOV 1968) that an agonist must strongly adsorb to the receptor. In order to check this supposition the type of action of polymethylene-bis-thimethylammonium (BTM) compounds has been studied on the frog rectus abdominis muscle. The affinity constants of the compounds were also determined. The results obtained are presented in this paper.

Methods

The rectus abdominis muscle of the frog *Rana temporaria* was used. Nine or ten muscles were mounted in an organ bath containing 200 ml of frog-Ringer's solution at 20—22 °C. Neostigmine in a concentration of 2×10^{-6} g/ml was added to the solution which was bubbled with air. The ability of each compound to produce muscle contraction was examined. Acetylcholine-like compounds were added at different concentrations and the contractions produced were recorded isotonically and read on a millimetre scale. Cumulative dose–response curves (ARIENS 1964) were plotted and the concentration required to produce half of the maximum concentration (EC_{50}; D_2 in terms of ARIENS [1954]) was calculated.

Some of the compounds studied proved partial agonists, therefore the maximum response was expressed as a fraction of the maximum response produced by 0.1 N KCl. This fraction was characteristic of the intrinsic activity (ARIENS 1954, 1964) or efficacy (STEPHENSON 1956) of the compounds.

Since depolarizing drugs are able to produce desensitization, and this was, in fact, expected in those experiments where the doses were added cumulatively, therefore with drugs like BTM-7, BTM-11 and BTM-14, the maximum response and the EC_{50} value were determined also by a non-cumulative technique. In these experiments the drugs was washed out after each concentration tested and an interval of 30 min was kept before the next concentration was added.

In another series of experiments the affinity constant (K_a) was also determined on the rectus muscle. Since all the compounds tested except BTM-3 proved partial agonists, their K_a values were determined by the methods of STEPHENSON (1956), and of BARLOW et al. (1967). In cases where the maximum response was less than 0.5, the affinity constant of the compounds was determined by both methods (see BARLOW et al. 1967). If the maximum response amounted to 0.5 or higher, the affinity constant of the partial agonists was determined by the method of BARLOW et al. (1967). Tetramethylammonium (TMA) or acetylcholine (ACh) were used as full agonists. Muscle contraction was registered 20 min after the addition of a partial agonist and 10 min after the administration of TMA or ACh. The K_a value of BTM-3 was not determined, this compound being a full agonist. The antagonistic activity was studied in 9—10 muscles simultaneously. The value for A_2 (SCHILD 1957) that concentration of the compound which doubled the concentration of ACh to produce the same response as in the absence of the antagonist, was also estimated. The antagonist was left in contact with the tissue for 20 min, while the agonist (ACh) for 10 min. The K_a value for the compounds studied were calculated from the values of A_2.

The compounds BTM-3, BTM-4, BTM-5, BTM-7, BTM-8 and BTM-9 (see Table I) were synthetized by I. Ya. KVITKO (Technological Institute, Leningrad). The compounds BTM-11 to BTM-20 were kindly supplied by Prof. R. B. BARLOW. In addition, hexamethonium (BTM-6) and decamethonium (BTM-10) were used.

Results

Type of action. Of the whole BTM series, only BTM-3 proved a full agonist. The other compounds did not contract the muscle or failed to produce a maximum response. The compounds with the shortest (except BTM-3) intercationic chain (BTM-4, BTM-5, BTM-6), or with the longest intercationic chain (BTM-15—BTM-20) were antagonists. The compounds BTM-4, BTM-5 and BTM-6 turned to be weak antagonist ($A_2 = 1—3 \times 10^{-4}$ M) and the compounds BTM-15 and BTM-20 very strong ones (A_2 from 10^{-7} M to 3.5×10^{-8} M). However, the compounds with 7 to 14 methylene groups in the inter-cationic chain possessed acetylcholine-like properties. All of them proved to be partial agonists. The highest muscle contraction was produced by BTM-12. The average maximum response with this compound was 0.84. A decrease or increase of the number of methylene groups in the intercationic chain was accompanied by a lowering of the maximum response (Table I).

Table I

Cholinomimetic and cholinolytic activity of polymethylene-bis-trimethylammonium compounds on frog rectus abdominis muscle

$(CH_3)_3 — \overset{+}{N} — (CH_2)_n — \overset{+}{N} — (CH_3)_3$

Compound	n	EC_{50}	Max. resp.	A_2	K_a
BTM—3	3	$1.2 \pm 0.12 \times 10^{-4}$ (9)	0.96		
BTM—4	4			$3.1 \pm 0.6 \times 10^{-4}$ (9)	$3.3 \pm 0.14 \times 10^{3}$ (9)
BTM—5	5			$1.2 \pm 0.1 \times 10^{-4}$ (9)	$8.3 \pm 0.15 \times 10^{3}$ (9)
BTM—6	6			$1.0 \pm 0.04 \times 10^{-4}$ (9)	$1.0 \pm 0.009 \times 10^{4}$ (9)
BTM—7	7	$1.2 \pm 0.09 \times 10^{-4}$ (9)	0.42		$3.3 \pm 0.9 \times 10^{4}$ (3)
BTM—8	8	$4.0 \pm 0.24 \times 10^{-5}$ (9)	0.34		$6.0 \pm 0.0 \times 10^{4}$ (2)
BTM—9	9	$1.3 \pm 0.12 \times 10^{-5}$ (10)	0.37		$4.8 \pm 1.5 \times 10^{4}$ (3)
BTM—10	10	$5.1 \pm 0.46 \times 10^{-6}$ (11)	0.72		$8.2 \pm 2.1 \times 10^{4}$ (4)
BTM—11	11	$4.1 \pm 0.13 \times 10^{-6}$ (10)	0.71		$4.6 \pm 1.7 \times 10^{4}$ (7)
BTM—12	12	$3.3 \pm 0.25 \times 10^{-6}$ (12)	0.84		$1.4 \pm 0.3 \times 10^{5}$ (5)
BTM—13	13	$3.8 \pm 0.4 \times 10^{-6}$ (9)	0.62		$3.4 \pm 0.6 \times 10^{5}$ (4)
BTM—14	14	$2.9 \pm 0.51 \times 10^{-6}$ (7)	0.30		$2.1 \pm 0.15 \times 10^{6}$ (2)
BTM—15	15			$1.5 \pm 0.08 \times 10^{-7}$ (9)	$6.7 \pm 0.07 \times 10^{6}$ (9)
BTM—16	16			$1.3 \pm 0.02 \times 10^{-6}$ (10)	$7.8 \pm 0.05 \times 10^{6}$ (10)
BTM—17	17			$3.45 \pm 0.2 \times 10^{-8}$ (10)	$2.9 \pm 0.04 \times 10^{7}$ (10)
BTM—18	18			$3.5 \pm 0.38 \times 10^{-8}$ (9)	$2.86 \pm 0.07 \times 10^{7}$ (9)
BTM—20	20			$1.0 \pm 0.76 \times 10^{-7}$ (10)	$1.0 \pm 0.4 \times 10^{7}$ (10)

Numbers indicate mean values ± standard error. The number of estimations is shown in parentheses

The agonistic effect may lead to inactivation (desensitization) of the receptors (THESLEFF 1955; KATZ and THESLEFF 1957; GISSEN and NASTUK, 1966; RANG and RITTER 1970). Using the cumulative dose–response method an inactivation of the receptors can be expected.

It is also known that, as a result of the influence of the compounds with different structure, the rate and the degree of desensitization of the receptors may be different (RANG and RITTER 1970). Therefore, the rate of weaking of the effectivity of the studied compounds might be different as well. It was impossible to exclude the possibility that the decrease of the stimulating effect of higher BTM homologous resulted from a more rapid development of stronger desensitization. For this reason, the compounds from BTM-11 to BTM-14 and BTM-7 were studied under conditions where a discrete dose–response was registered, i.e. the muscle was washed out after application of each concentration of the compound. Under these conditions only a slight receptor desensitization was observed.

The results of these experiments are presented in Table II. The maximum responses to all partial agonists was somewhat higher than the maximum responses observed with the cumulative dose–response method. However, the difference did not prove significant. In addition it was of the same degree for BTM-7 and for the compounds with a longer intercationic chain.

Hence, if an inactivation of receptors took place, it still did not explain the decrease or loss of acetylcholinomimetic activity of the higher homologues of the series.

Affinity of the compounds to the receptor. As expected (STEPHENSON 1956; PATON 1961) with the increase of the number of methylene groups,

Table II

Acetylcholinomimetic action of some polymethylene-bis-trimethylammonium compounds determined by cumulative and non-cumulative techniques

Compound	Cumulative curve		Non-cumulative curve	
	EC_{50}	Maximum response (MR)	EC_{50}	Maximum response (MR)
BTM—7	$1.2 \pm 0.09 \times 10^{-4}$	0.42	$3.5 \pm 0.3 \times 10^{-5}$	0.43
BTM—11	$4.1 \pm 0.13 \times 10^{-6}$	0.72	$2.5 \pm 0.2 \times 10^{-6}$	0.80
BTM—12	$3.8 \pm 0.40 \times 10^{-6}$	0.84	$1.6 \pm 0.11 \times 10^{-6}$	0.86
BTM—13	$3.3 \pm 0.25 \times 10^{-6}$	0.62	$1.2 \pm 0.07 \times 10^{-6}$	0.76
BTM—14	$2.9 \pm 0.51 \times 10^{-6}$	0.30	$9.9 \pm 0.16 \times 10^{-7}$	0.36

the affinity constant of the compounds also increased (K_a; Table I). Moreover, there was a great difference in the value of K_a of the extreme members of the series; K_a of BTM-17 and of BTM-18 was higher by four orders than those

of BTM-4, BTM-5 and BTM-6. Such a degree of increase in K_a can be reached, when the addition of one methylene group leads to a twofold increase of K_a. It is apparently caused by an increase in the Van-der-Waals forces. But a uniform increase in K_a along with the lengthening of intercationic chain did not always occur, and sometimes even a decrease of K_a was observed. The decrease of the K_a of BTM-20 as compared with that of BTM-18 might have been caused by an excessive rise in the hydrophobic property of the molecule and by a decrease of its water solubility. It is also possible that, in spite of the flexibility of the molecule, the distance between the cationic groups of BTM-20 did not correspond to the mutual disposition of the anionic sites of the neighbouring receptors (KHROMOW-BORISOV and MICHELSON 1966). The pronounced inconsistency concerning changes in K_a was also observed with compounds BTM-10, BTM-11 and BTM-13, i.e. partial agonists with a maximum response of more than 0.6. The method used for the definition of K_a of such partial agonists may not have been quite adequate, but there could be no doubt concerning the correctness of the K_a values for the other compounds, especially for antagonists.

Thus, the type of action of the BTM compounds on the frog rectus abdominis muscle was modified together with the change of K_a. The compounds with low K_a proved to be weak antagonists. An increase in the value for K_a was accompanied with a change in the character of the molecule and weak antagonists (BTM-4 to BTM-6), partial agonists (BTM-7 to BTM-14) and strong antagonists (BTM-15 to BTM-20) were produced. The maximum response increased gradually up to 0.84 (BTM-12) and with the compounds BTM-13 and BTM-14, it decreased to 0.62 and 0.30, respectively.

Discussion

The affinity constant (K_a) is defined as the ratio of the rate constant of association (K_1) and dissociation (K_2):

$$K_a = \frac{K_1}{K_2}.$$

Theoretically, an increase of K_a can be the consequence of an increase of K_1 or a decrease of K_2, or of a simultaneous increase of K_1 and decrease of K_2. The rate of association of the ammonium ion with a cholinoreceptor is apparently determined by the strength of ionic attraction of the cationic group to the anionic site of a receptor. Since the structure of the cationic groups of the compounds studied is the same, therefore the strength of their ionic attraction can not differ essentially. It is difficult to understand, why the

addition of methylene groups to the intercationic chain should lead to an increase in K_1. With the increase of the number of methylene groups a decrease of K_a is expected. In the guinea pig ileum, the K_1 values for alkylmethylammonium compounds did not change considerably, but the dissociation constants decreased by 250% on lengthening of the alkyl radical (PATON 1961).

One may consider that the increase in K_a accompanying the lengthening of the intercationic chain in the BTM series is a result of the decrease of the dissociation constant but not of the increase in K_1.

The "occupation" theory does not consider the influence of the affinity on the type of action of a compound and from the point of view of this theory, the efficacy ("intrinsic activity") does not depend on the association and dissociation constants (ARIENS 1954; STEPHENSON 1956; ARIENS et al. 1957; ARIENS and SIMONIS 1968). According to the rate theory (PATON 1961, 1964, 1972; PATON and WAUD 1961), the type of action of a compound on the receptor is defined by the dissociation constant of the drug–receptor complex. For all that the higher the K_2, the higher the efficacy. A compound with a low K_2 value is an antagonist, with an intermediate value it is a partial agonist. On the grounds of the rate theory, the loss of acetylcholinomimetic properties of compounds from BTM-15 to BTM-20 is easy to understand. These compounds possess the highest affinity to the receptors and form, apparently, the most stable complex with the receptors. It is also understandable that the maximum response to the compounds BTM-13 and BTM-14 is lower than that to BTM-12. However, on the basis of this theory it is difficult to explain the absence of acetylcholinomimetic properties of the compounds BTM-4, BTM-5 and BTM-6, as their K_a values are the smallest and they seem to form the least stable complex with the receptors.

It is also difficult to understand the successive increase of the maximum response to the compounds from BTM-7 to BTM-12 with the increase of the number of methylene groups in the intercationic chain and with the successive increase in K_a.

The acetylcholinomimetic property of the BTM was observed in the compounds with a moderate (but not minimal) K_a. This was in agreement with our earlier finding (DANILOV 1968) that the depolarizing actionis characteristic of such ammonium compounds which are able to absorb enough to a cholinoreceptor of subsynaptic membrane with Van-der-Waals forces or with other short-radius bonds. The stability of the adsorption, however, is not excessive. Such a dependence of the type of action upon the life of a drug–receptor complex is understandable provided the dissociation constant is one of the factors which exert an influence on it. The dissociation constant affects the ability of the molecule to induce a transition of the receptor into the "excited" state (possibly, the transition to conformation which opens the

ionic channels), and the quantity of free receptors and the number of compound–receptor complexes which are formed in a time-unit, as it was supposed by the kinetic theory.

The process of the transition of a receptor from the resting state into the "excited" one seems to take some time. The life of an agonistic receptor complex may correspond to the duration of this process. This interaction depends on the dissociation constant of the complex. Since the ionic forces are too short-acting, they can not bring about this interaction. They have to be supplemented with a necessary quantity of short-radius forces or bonds, e.g. by Van-der-Waals forces. If they are still not strong enough, the "excitement" of the receptor fails to occur. In this case the compound is deprived of its cholinomimetic property. BTM-4, BTM-5 and BTM-6 are the compounds of such type. The addition of methylene groups to the intercationic chain (BTM-7, BTM-8, etc.) leads to an increase of the life-time of a complex providing a longer period for the development of the "excitement".

The stronger the relation between the period of existence of a drug–receptor complex and the time needed for transforming the receptor from the resting state into a complete "excitement", the stronger is the cholinomimetic activity; and the maximum response increased with the compounds from BTM-7 to BTM-12. Of course, not only the compounds which are able to adsorb enough to a cholinoreceptive surface can be agonistic but also those which have a lepto-structure.

The trigger role of the receptor in depolarization of the postsynaptic membrane lasts for some time and occurs independently of the fact whether or not the receptor–agonist complex exists. In the first case the receptor remains occupied and does not function, while in the second case it is ready to repeat complex formation with the agonist and to produce "excitation".

The further course of events will be limited and determined by the dissociation constant in accordance with PATON's rate theory. If a compound is able to "excite" the receptor, then depolarization of the postsynaptic membrane takes place only in those cases when a necessary number of receptors is excited simultaneously. Therefore, a minimal number of free receptors is necessary. During the action of higher homologues of the BTM series, in the period of equilibrium, the number of occupied receptors becomes so great, that not enough new complexes are formed in a time unit and therefore a threshold depolarization of the postsynaptic membrane can not be reached although the combination of a molecule with a receptor leads to "excitation" of a receptor. Before the equilibrium is attained in the system, the major part of the receptors is free, therefore the threshold depolarization is not yet reached. This is probably due to the fact that the molecules of the compound added to the intact tissue fail to reach the receptors simultaneously. Excitation of the receptors at different times does not produce threshold de-

polarization. This hypothesis seems to contradict the observation that the shortest compound, BTM-3, proved to be a full agonist. This compound, unlike the others from this series, might interact with cholinoreceptive surface so as tetramethylammonium (TMA) does, i.e. by a single cationic group. All three methyl radicals of the cationic group may adsorb to the hydrophobic area of the anionic site of the receptor.

If the second cationic group of a longer molecule comes into ionic interaction with the neighbouring receptor or with the negatively charged group of a cholinoreceptive surface which is not the anionic site of the receptor (GILL 1959), then probably only two methyl radicals of one or two cationic groups adsorb to the anionic site of the receptor, and the firmess of the adsorption may be insufficient (BTM-4, BTM-5, BTM-6) if it is not intensified by the Van-der-Waals forces of the chain. The importance of the adsorption of one methyl radical is obvious if we compare the activity of ACh with its tertiary analogue [$(CH_3)_2N-CH_2-CH_2-O-CO-CH_3$] on the isolated electroplax preparation (ROSENBERG et al. 1960) and on the frog rectus abdominis muscle (FRUENTOV 1962) at pH 7.0. At this pH the compound is completely ionized at the expense of the addition of hydrogen proton. Nevertheless, it proved to be 200 times weaker than ACh, owing apparently to the absence of one methyl radical in its cationic group. The second cationic group of the compounds BTM-4, BTM-5, BTM-6 changed the property of the molecules so that they fail to exert an acetylcholinomimetic activity. Similar findings have been published concerning alkyl-trimethylammonium compounds [$(CH_3)_3\overset{+}{N}-(CH_2)_nCH_3$; STEPHENSON 1956; PATON 1961; ARIENS 1964]. Moreover, butyl-TMA and pentyl-TMA were the strongest agonists.

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OESTROGEN CONTAINING ORAL CONTRACEPTIVES AND ANTITHROMBIN III ACTIVITY

By

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A total of 67 women taking oral contraceptives containing different agents was studied and compared to healthy controls. Contraceptive drugs containing oestrogen caused a decrease in antithrombin III (AT III) activity. This decrease was more frequent in women taking pills of high oestrogen content (100 μg) and was more consistent when using serum in the presence of heparin. The phenomenon may serve as a screening method revealing a tendency to decrease of AT III activity. The decrease in AT III activity in itself does not necessarily mean thromboembolism, but in the presence of other predisposing factors it may become an important cause of thrombosis.

Jordan (1961), when reporting on the occurrence of pulmonary embolism in a woman who had oestrogen-progestagen therapy for endometriosis, suggested that the use of these hormonal agents might predispose to thromboembolic episodes. Since then, numerous cases of venous and arterial thrombotic disease have been reported (Kilpatrick et al. 1968; Köhler 1971; Gaszner et al. 1974) and statistical studies (Markush and Siegel 1969; Doll and Vessey 1970; Westerholm 1971) showed that the incidence of thromboembolic disturbances increased in those taking the pill. The manner in which oral contraceptives cause thromboembolic disease is not completely understood.

In the present investigation, AT III activity in plasma and serum of women taking contraceptive pills of different composition has been studied and compared to that of healthy controls who had never taken oral contraceptives or had any form of steroid treatment.

Methods

Table I shows our material. Of the 67 women, 31 were taking Infecundin®, a pill with a high oestrogen content, and 36 Bisecurin®, a pill with less oestrogen.

The thrombin-inactivating capacity of plasma and serum was estimated after incubation with a thrombin solution of constant activity, according to the medhod of Rák (1969). In addition to this basic activity, AT III was determined in the presence of 0.25 IU/ml heparin which normally increases thrombin inactivation. However, according to our preliminary results, with samples of a decreased basic AT III activity a paradoxical reaction occurs in

that heparin causes a further decrease in thrombin inactivation (RÁKÓCZI et al. 1974). This phenomenon may, in some cases, precede the decrease in basic activity observed in women taking oral contraceptives.

The χ^2 test was used to assess the significance of the results.

Results

The changes of plasma AT III activity in our material are summarized in Table II. In the control group a decreased basic activity was found in one case and in 3 cases a paradoxical heparin effect occurred. In the plasma of

Table I

Patients

Group	No. of patients	Mean age, years	Mean duration of drug administration, months
Control	33	27 (19—36)	—
Infecundin® 2.5 mg norethynodrel + 0.1 mg mestranol	31	26 (21—35)	5 (2—20)
Bisecurin® 1.0 mg ethynodiol diacetate + 0.05 mg ethyniloestradiol	36	28 (20—38)	8 (2—14)

Table II

Antithrombin III activity in plasma

Group	Normal	Decreased	Paradoxical Heparin effect
Control n = 33	32 97%	1 3%	3 9 %
	$x^2 = 4.8$; $p < 0.05$		
Infecundin® n = 29	23 79%	6 21%	5 17%
	$x^2 = 3.5$; $p \approx 0.05$		
Bisecurin® n = 36	34 94%	2 6%	10 28%

$x^2 = 4.2$
$p = 0.05$

women taking Infecundin® basic AT III activity decreased in 6 cases out of 29. This was significantly different from the control group at the 5% level, and was on the borderline of significance from the controls and of the Bisecurin® group. There was no difference in the basic AT III activity between the Bisecurin® group and the controls, whereas the paradoxical effect of heparin was more frequent in the Bisecurin® group.

The results were more consistent with serum AT III (Table III). While its activity was normal in all the controls and except for one single case in the Bisecurin® group, it decreased in 13 out of 29 cases in the Infecundin® group. The difference was highly significant as compared to the controls, and significant at the 1% level vs. the Bisecurin® group. The same tendency could be observed in respect of the paradoxical heparin reaction which is

Table III

Antithrombin III activity in serum

Control	Normal	Decreased	Paradoxical heparin effect
Control n = 26	26 100%	0	3 11%
	$x^2 = 13.9$; $p < 0.001$		$x^2 = 115.4$; $p < 0.001$
Infecundin® n = 29	16 55%	13 45%	16 55%
	$x^2 = 9.2$; $p < 0.01$		$x^2 = 3.8$ $p < 0.05$
Bisecurin® n = 20	19 95%	1 5%	7 35%

supposed to be an earlier and more sensitive sign than the decreased basic activity of AT III.

Fig. 1 demonstrates an individual case observed continuously before starting, during use, and after discontinuation of Bisecurin®. During the first cycle after starting the pill, basic activity decreased somewhat but remained in the normal range, whereas the heparin effect became paradoxical. In the

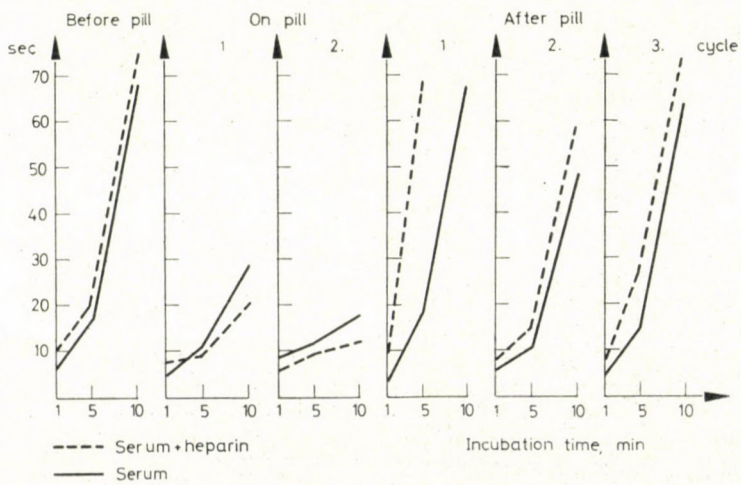

Fig. 1. Serum AT III activity in a women before, during and after taking the pill

second cycle, both basic AT III activity and heparin effect showed a severe disturbance in thrombin-inactivation. After having discontinued the pill, in the first cycle there was already a rebound effect both in the reaction to heparin and in basic activity. The original values, observed before the pill, were restituted in the 3rd cycle.

Discussion

The mechanism leading to thrombotic phenomena during the use of oral contraceptives is not understood. Many changes in coagulation factors have been reported in patients taking oral contraceptives; they include changes in procoagulants, in platelets, and in the velocity of blood flow. Increases in clotting factors I, II, VI, VII, IX and X (HODGES 1968; DUGDALE and MASI 1971), and a decrease in fibrinolytic activity have been reported (DUGDALE and MASI 1971; ASBECK et al. 1971).

Platelet function may also be effected and especially platelet aggregation is accelerated (POLLER et al. 1969; DUGDALE and MASI 1971).

Changes in the velocity of blood flow and lesions of the vascular intima are believed to be important factors in predisposing to thrombosis formation. Endothelial proliferation and subintimal fibrosis have been observed in the arteries of some women who while on the pill died of pulmonary thromboembolism (IREY et al. 1970). The distensibility of peripheral veins increases and the linear velocity of venous flow is reduced in women receiving oral contraceptives (GOODRICH and WOOD 1964).

Recently, several authors (FAGERHOL et al. 1970; VON KAULLA et al. 1971; ZUCK et al. 1971; LARSSON-COHN et al. 1972; ZUCK and BERGIN 1973) have suggested the importance of the decreased AT III activity in the development of thromboembolic episodes. AT III, an alpha-2-globulin of a molecular weight of about 65.000 is the main physiological clotting inhibitor (ABILDGAARD 1967) acting on thrombin (ABILDGAARD et al. 1970) as well as on the activated factor X (X_a), which is the principal enzyme in blood coagulation (MARCINIAK 1973).

Individuals with a congenital deficiency of AT III have a tendency to thromboembolism (EGEBERG 1965). Low antithrombin III values are frequently observed in patients with thromboembolic disease (VON KAULLA 1967), in disseminated intravascular coagulation (LASCH et al. 1961) as well as in severe liver cirrhosis (MANNUCCI et al. 1973). Antithrombin III concentrates injected intravenously prior to thromboplastin administration prevent intravascular clotting (MANN et al. 1969).

We have now found that the pills containing oestrogen cause a decrease in AT III activity. This decrease is more frequent in woman taking pills of

a high oestrogen content, and more consistent when using serum in the presence of heparin. The phenomenon may serve as a screening method for revealing a tendency to the decrease of AT III activity. Some authors have reported a connection between the dose of oestrogen and the degree of decrease in serum AT III activity (HOWIE and PRENTICE 1973). The so-called "low dose progestagen pill", which is probably devoid of the risk of thrombosis, did not lead to any reduction in the AT III level. Further studies have in fact shown that such pills increase the AT III level (BERGSJÖ et al. 1972).

The decrease in AT III activity in itself does not necessarily lead to thromboembolism. A fall of AT III activity only means a loss of protection against the consequences of thrombin traces present in the circulation. This loss of protection together with a rise induced by oral contraceptives in the activity or concentration of various clotting factors does not imply a formation of intravascular clots, but it might generate a certain propensity to it. If, however, the homeostatic equilibrium is further disturbed by prolonged stasis, vascular endothelial damage, fever, postsurgical hypercoagulability, etc., intravascular clotting may well be precipitated. In such instances the increased AT III activity may become an important factor in the manifestation of thrombosis.

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Communicationes breves

QUANTITATIVE RECORDING OF TURNING BEHAVIOUR INDUCED OR POTENTIATED BY AMPHETAMINE AFTER UNILATERAL BRAIN LESIONS IN THE RAT

By

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An apparatus has been constructed for the quantitative recording of drug-induced or electrical stimulus-evoked turning behaviour in the rat. It differentiates between rotations in different directions and provides a complete record of the animal's turning behaviour without the need for supervision. Its electronic component consists of logic units which are commercially produced as behavioural programming equipments.

Unilateral lesions in the nigrostriatal dopaminergic pathway of the rat induce mild asymmetric posture (ANDÉN et al. 1966a; UNGERSTEDT 1971) which can be converted into vigorous rotation toward the lesioned side of the brain by administration of amphetamine (UNGERSTEDT and ARBUTHNOTT 1970; MARSDEN and GULDBERG 1973; CHRISTIE and CROW 1971, 1973) or ephedrine (CHRISTIE and CROW 1971). Unilateral damage to the norepinephrine-containing pathways of the dorsal mesencephalic tegmentum produces contralateral asymmetry which is potentiated by amphetamine into an active rotation toward the non-lesioned side (MARSDEN and GULDBERG 1973). Since the unilateral lesions of these structures cause dopamine and norepinephrine depletion (ANDÉN et al. 1966a, b; FAULL and LAVERTY 1969), and because amphetamine causes the release of these catecholamines from the central neurons (CARLSSON 1970), ipsilateral and contralateral turning behaviour induced or potentiated by amphetamine and ephedrine are caused by dopamine and norepinephrine release by the non-lesioned side of the brain.

Measurement of this amphetamine-induced turning behaviour is important when the effect of various drugs on the function of the catecholamine-containing neurones is studied. An electromechanical apparatus for quantitative estimation of this behavoiur has been described by UNGERSTEDT and

ARBUTHNOTT (1970). However, with their apparatus the animals must be observed constantly if the direction of the rotation is an important variable. BARBER et al. (1973) have improved this apparatus to differentiate automatically between rotations in different directions.

We have worked out a similar method for the simultaneous measurement of clockwise and counter-clockwise turning behaviour and to provide

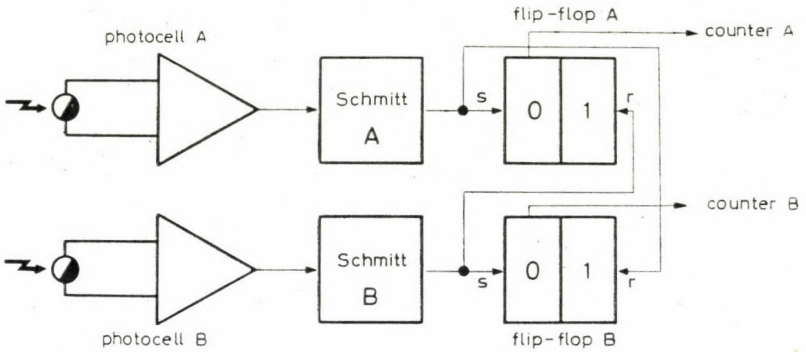

Fig. 1. Block diagram of the electronic unit

a complete record of the direction of rotations without the need for supervision. The advantage of this apparatus over that described by BARBER et al. 1973) is that its electronical unit can be built by using logic units which are commercially produced as behavioural programming equipments (e.g. Massey Dickinson Co.; Digi Bit logic packages of BRS Foringer, etc.). In addition, the method allows a quantitative evaluation of turning behaviour elicited by subcortical electrical stimulations (GRASTYÁN et al. 1968; SZABÓ 1972).

The apparatus consists of two main components, viz. (a) an electronic unit (Fig. 1), and (b), an experimental cage in which the animal is harnessed and connected through a coaxial cable to a freely rotating holed disk (Fig. 2). The coaxial cable is about 1 m in length and can be used to connect the stimulator with the brain when intracranial electrical stimulations are applied. Since coaxial cables cannot be twisted around their longitudinal axis but bend easily in each direction, the harnessed animal move rather freely in the cage. The electronic unit contains two photocells, two Schmitt triggers and two bistable multivibrators ("memory unit") with static reset. The outputs of the electronic unit are fed into a two-channel chart recorder and they can also be used to operate electromechanical or decadic counters giving separate totals of the number of clock-wise and counter-clockwise turnings.

The timing sheet of the electronic unit is shown in Fig. 3. When the holed rotating disk (Fig. 4) moves clockwise, it interrupts first the light beam activating photocell A and then photocell B. When the rotating disk moves

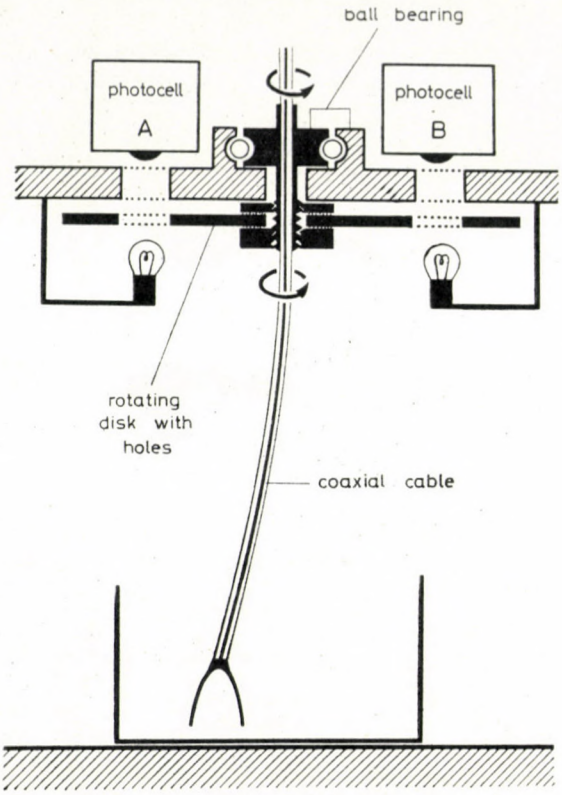

Fig. 2. Schema of the apparatus

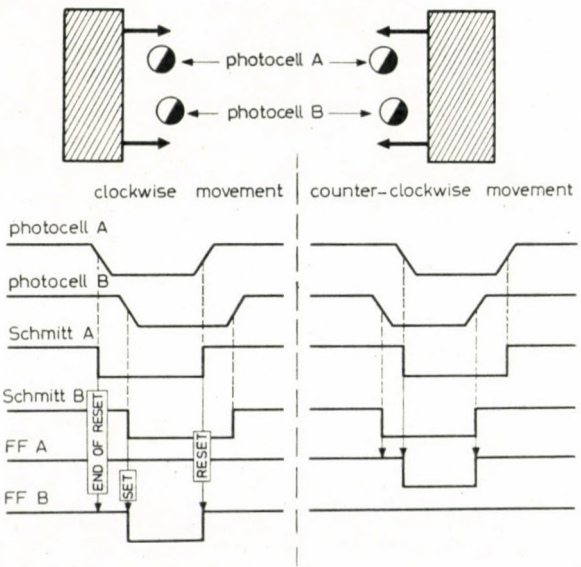

Fig. 3. Timing sheet of the electronic unit

counter-clockwise, the output of photocell B will be at first zero, and then after a delay the output of photocell A will decrease. The photocells are fixed in such a manner that the two beams let through the holes of the rotating disk are interrupted consecutively, and in reversed order if the direction of rotation is reversed. The outputs of the photocells operate Schmitt triggers to obtain

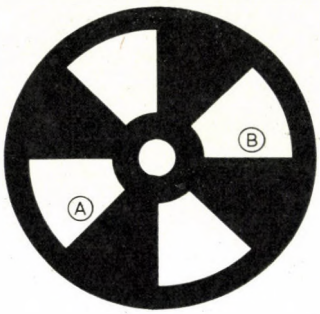

Fig. 4. Rotating disk and optimum position of the photocells in respect to the holes of the disk

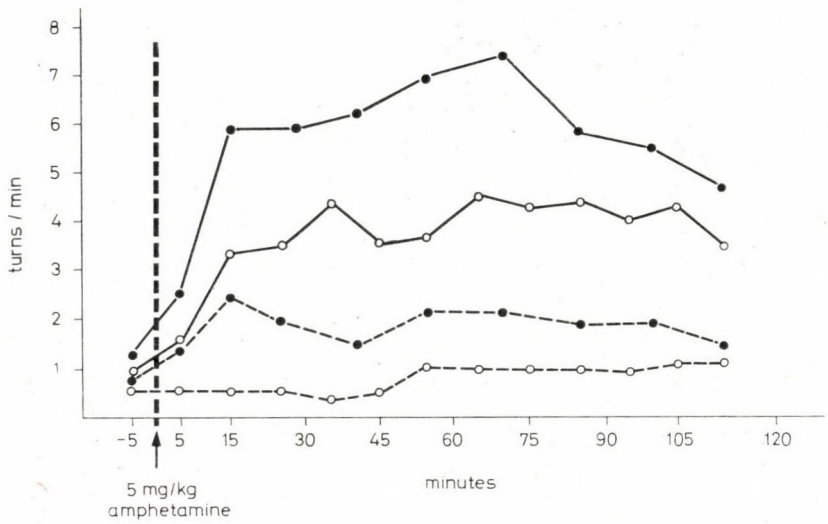

Fig. 5. Turning behaviour of satiated (filled circles) and food deprived (open circles) rat after 5 mg/kg amphetamine given following unilateral lesions of globus pallidus. Solid line: turnings toward the lesioned side of the brain. Dotted line: turning toward the non-lesioned side of the brain

a sudden change in voltage of the output signal when no sufficient amount of light arrives to the photocells (Fig. 3). The output of each Schmitt trigger is connected to the input of a direct-coupled bistable multivibrator (Fig. 2, flip-flop A and flip-flop B), respectively. The static reset of flip-flop A is given

by photocell B through Schmitt trigger B and that of flip-flop B is provided by Schmitt trigger A. When no light arrives to photocell A, the static reset of flip-flop B does not operate. Therefore, this flip-flop can be set by the Schmitt trigger B when the light beam for photocell B is also interrupted as the rotating disk moves clock-wise. In the case of counter-clock-wise rotation, flip-flop A will respond, since only this multivibrator does not receive a static reset signal when the rotating disk interrupts the light beam which activates photocell B. Therefore, flip-flop A can be set at the moment when no sufficient amount of light arrives to photocell A.

The apparatus has been found to be convenient and reliable in routine use. Spurious counts recorded may, however, be whenever the animal — and thus the rotating disk — stops in such a position that one photocell is covered but the light beam belonging to the other photocell is not yet interrupted and the rotating disk oscillates due to head movements. The number of such false counts can be minimalized if the rotating disk has not more then four holes and the photocells are fixed so that 1/16th of a turn is needed for interrupting both beams consecutively. This photocell arrangement is shown in Fig. 4.

A typical result obtained with the apparatus is shown in Fig. 5. After unilateral pallidal lesions, dl-amphetamine (5 mg/kg intraperitoneally) induces turning toward the lesioned side of the brain, but the number of these drug-induced turns of the same rat considerably decrease following food deprivation for 24-hours.

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FACILITATION OF AVOIDANCE CONDITIONING BY NICOTINE: A FUNCTION OF INDIVIDUAL LEARNING ABILITY

By

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A conditioned avoidance response has been elaborated in automatic shuttle boxes in rats and the effect of post-trial nicotine treatment on learning performance was measured. The animals were individually qualified as poor or good learner according to their first day, i.e. pretreatment, performance. Nicotine in a dose of 0.05 mg/kg had no significant effect, while in doses of 0.10—0.20 mg/kg it facilitated the acquisition of the conditioned response, however, only in the group of good learners.

Nicotine is claimed by many authors to enhance acquisition of new behaviour in animals. Its learning-facilitating effects seems, however, to be dependent on many experimental variables. The dose applied (Bättig 1969; Erickson 1971), the interval between administration and experiment (Essman 1969), the fact whether the nicotine was given before or after training (Erickson 1971), the strain of the animals (Bovet et al. 1966) and the learning paradigm itself (Garg 1969) are relevant in determining or modifying the behavioural effect. In our experiments carried out for testing some of the above mentioned results concerning the nicotine effect on avoidance conditioning, we encountered a further factor which seemed to be crucial in determining the drug's effect, namely the animals individual learning capacity.

A total of 100 male Long-Evans hooded rats was used. The animals were conditioned in automatic shuttle boxes according to Bovet et al. (1966). They were given 75 trials per session on 5 consecutive days. In each trial, the intertrial interval (15 sec) was followed by a 15 sec conditional light stimulus (CS) overlapping a 5-sec scrambled foot-shock (1 mA). The rats avoided the shock by running into the adjacent compartment within 10 sec after the onset of the stimulus. Nicotine hydrogene tartrate dissolved in saline was injected in doses of 0.05, 0.10, or 0.20 mg/kg intraperitoneally immediately after the daily sessions (doses are expressed as base). Nicotine was administered post-trially to control its effect on memory consolidation. In the course of statistical evaluation animals were divided into two groups according to their performance in the first session, before the first nicotine injection. A 7% performance criterion halved the population; those performing above the criterion on the first day were qualified as good learners, the others as poor learners.

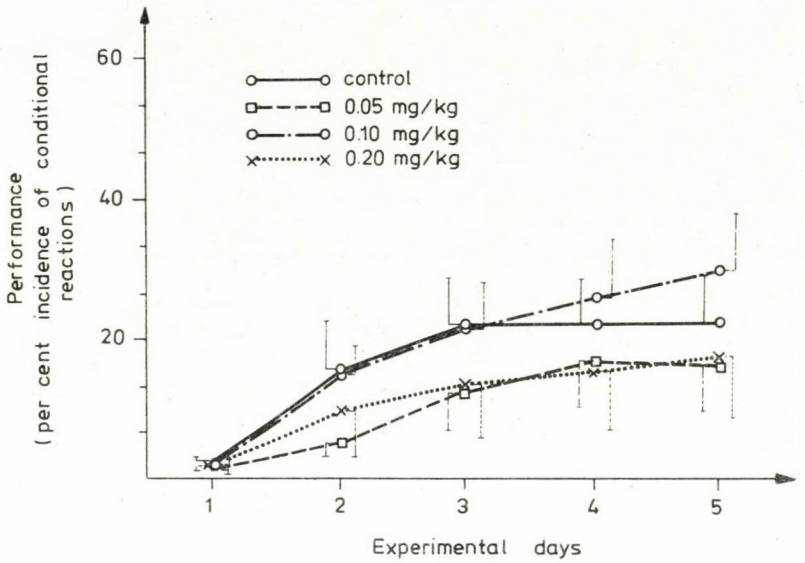

Fig. 1. Effect of nicotine on performance of poor learners. Ordinate: per cent of conditional reactions within the session. Conditional reaction means passing into the safe compartment of the shuttle box upon onset of conditional light stimulus. Mean ±S.E.

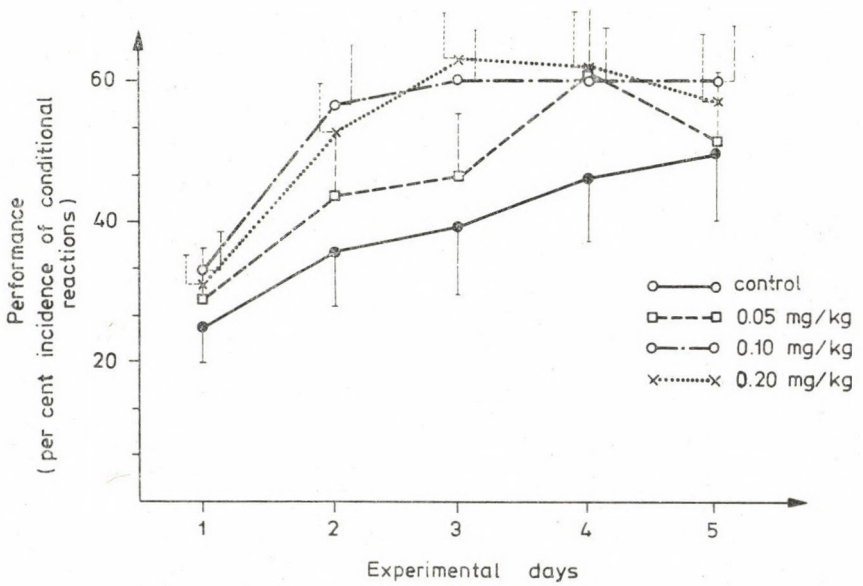

Fig. 2. Effect of nicotine on learning performance of good learners. For further explanation see Fig. 1

No preselection was applied. Animals were randomly assigned into the control or one of the treated groups 3 days before the first session and qualified as good or poor learner only for statistical evaluation. The 7% criterion was chosen because taking this value the whole population could be halved, 49 respectively 51 animals were qualified as poor or good learners on the basis of the first day performance, i.e. according to their learning capacity displayed before the first treatment, which took place after the first session.

As Fig. 1 shows, nicotine failed to facilitate the learning performance of the poor learners. Moreover, animals treated with 0.05 or 0.20 mg/kg showed a slightly lower avoidance level than the controls, but this difference was not significant statistically. On the other hand, nicotine treatment with higher doses improved the performance of the good learners, i.e. of the animals displaying an above-average performance already on the first day (Fig. 2; $0.001 < p < 0.01$ according to Student's *t*-test comparing the groups treated with 0.10 or 0.20 mg/kg nicotine to the controls; *t* was calculated from the pooled data of the 2nd to the 5th day. With the animals treated with 0.05 mg/kg of nicotine, the difference observed was not significant).

At present we cannot give any comprehensive explanation of the unexpected finding. According to some data (ORSINGHER and FULGINTI 1971, 1973) nicotine elicits its behavioural effect through central and peripheral sympathetic stimulation. If the poor learners are fearsome, overstimulated individuals, an additional excitation brought about by nicotine will only deteriorate the conditional reflex performance, while in others the same stimulation may improve it.

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SELF-STIMULATION AND ADRENOCORTICAL ACTIVITY IN YOUNG RATS

By

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Stress response and self-stimulation behaviour of 12 to 17-day-old rats were investigated. Stressors, such as are intraperitoneal injection of histamine in a dose of 4 mg/kg b.w. or electric footshock, increased plasma corticosterone level 20 min after application in animals older than 15 days. Self-stimulation could be elicited in the medial forebrain bundle (MFB) as early as on the 14th—15th day of life. Self-stimulation for 20 min in 15 to 17-day-old rats, and 20-min "passive" electrical stimulation of MFB with similar stimulus parameters in 12 to 14-day-old animals caused a significant increase in plasma corticosterone level. Since both the stress response and self-stimulation behaviour appear at the same age it can be assumed that the brain structures, and the connection between brain stem and hypothalamus in particular involved into the organization of stress response and self-stimulation, are, either identical at least in part, or display a parallel postnatal development. Direct electrical stimulation of the hypothalamic structures is capable of activating the hypothalamo–pituitary–adrenocortical system also in 12 to 14-day-old animals.

In the last years several findings suggested an interaction to exist between the hypothalamo–pituitary–adrenocortical system and self-stimulation. Increased adrenocortical activity was observed during self-stimulation in rats (URETSKY et al. 1966; SADOWSKI et al. 1972) and monkeys (MCHUGH et al. 1967), but a decreased one in cats (ENDRŐCZI et al. 1967). On the other hand, corticosteroid treatment or adrenalectomy influence the rate of self-stimulation; the frequency decreases after injection of the hormone and transiently rises following the removal of the adrenals (HARTMANN and KOLTAY 1971). On the basis of the data mentioned above the hypothalamo–pituitary–adrenal system has been supposed to play a modulatory role in the organization of self-stimulation behaviour.

In rats, however, the responsiveness of the hypothalamo–pituitary–adrenal axis is questionable as for as the first two weeks of life are concerned. The different stressors (cold, adrenaline, histamine, ether, electric shock, etc.) used for testing adrenal function proved to be effective at different ages (IRWIN et al. 1950; JAILER 1949, 1950; ENDRŐCZI and TÓTH 1955; RINEFRET and HANE 1955; BACO and FISCHER 1956; ESKINE 1957; GREGOIRE 1957; SCHAPIRO et al. 1962; BACA and CHIODI 1965; HALTMEYER et al. 1966; LEVINE et al. 1967; ZARROW et al 1968; MILKOVIČ and MILKOVIČ 1969; GRAY 1971). Direct electrical stimulation of the rat's tuber cinereum elicited ACTH release

only from the 10th day of life on (ENDRŐCZI et al. 1957). Since earlier experiments have been shown that the 14 to 15 days postnatal represent the youngest age in rats when the self-stimulation can be induced in the lateral hypothalamic region (HARTMANN 1970), the present experiments were carried out in order to analyse how the function of the hypothalamo–pituitary–adrenal system would be influenced by histamine-induced or electric stress, lateral hypothalamic self-stimulation and the „passive" electrical stimulation of the same brain structures. In addition, the experiments aimed at revealing possible correlations between the time of the first appearance of self-stimulation and stress response in young rats.

Methods

The experiments were carried out on 12 to 17-day-old R-Amsterdam rats of both sexes. The date of birth was recorded with an accuracy of ± 12 hours. To ensure homogeneity of weights and rates of development only litters of 8—10 per cage were used. Each litter was housed individually, in air-conditioned room with 12-hour light and dark periods. Standard laboratory food pellets (LATI, Gödöllő) and water were available ad libitum. From the seventh day of life on the offsprings were handled for some minutes every day. Of the youngest rats only those with opened eyes were used.

Implantation of electrodes. This procedure was performed in each age-group on the day before the test. The animals were anaesthetized with pentobarbital (Nembutal) and the head fixed with a special head-holder applied to a stereotaxic apparatus. A bipolar stainless steel electrode insulated except for the tip was introduced into the medial forebrain bundle (MFB) in the antero-lateral hypothalamus and fixed to the skull with acrylate. After the operation the rats were placed back to their cage.

The stereotaxic coordinates were checked in every age-group by making several electrolytic lesions in various hypothalamic areas and locating them in frozen sections.

Establishment of stimulus parameters. Early in the morning on the day after operation the animals were trained to press a lever for rewarding brain stimulation (0.1—0.2 sec train of rectangular 0.2 msec impulses at a frequency of 100 Hz). The amplitude of impulses was adjusted individually to a value slightly higher than the threshold of self-stimulation. This test lasted 5—10 min and served to determine the parameters adequate for stimulation. The youngest rats, 12 to 13-day-old ones used in the experiments were also trained, but they did not display any pedal pressing; only searching behaviour was observed upon stimulation in some cases.

Experimental test. The experiments were performed on the day following operation between 12.00 and 14.00 hr. The rats of each age-group were divided into four groups with at least two animals from each litter.

Group I: Intact animals. After the intraperitoneal injection of 0.1 ml physiological NaCl the rats spent 20 min in the test-box and were immediately decapitated afterwards.

Group II: Histamine-treated animals. A dose of 4mg/kg b.w. of histamine (Peremin®, Chinoin) was injected intraperitoneally. After 20 min spent in the test-box the rats were decapitated.

Group III: Operated controls. The cables were connected to the implanted electrodes, but no stimulation was applied. The animals were placed into the test-box for 20 min and then decapitated.

Group IV/A: Self-stimulating animals. After 20 min self-stimulation with the individually adequate stimulus parameters determined in the morning session the rats were decapitated.

Group IV/B: This sub-group included younger (12 to 14-day-old) animals who showed no pedal pressing. The animals were stimulated externally with stimulus parameters similar to those used in Group IV/A, and designated as "passively" stimulated rats. The animals were decapitated after 20 min.

In order to check the effectiveness of histamine, some of the 13-, 15- and 17-day-old animals were subjected to an electric footshock (5 mA, 5 sec) as a stressor. They were decapitated after 20 min spent in the test-box.

Corticosterone determination. The blood collected after decapitation was centrifuged and plasma corticosterone level determined with the spectrofluorimetric method of PURVES and SIRETT (1965).

Histological control. The brains were fixed in 10% formol. Localization of electrode was made in frozen sections according to the method of GUZMAN-FLORES et al. (1958). Only animals with accurate electrode localization were included into the statistical evaluation.

Statistical evaluation was made according to Student's *t*-test.

Results

Self-stimulation behaviour

In full agreement with our earlier results 12- to 13-day-old rats did not show self-stimulation. Only two out of the 10 animals of 14 days pressed the pedal, but even those with very low frequency only. From the age of 15 days on every rat pressed the lever for rewarding stimulation and the frequency of pedal-pressing gradually increased with increasing age ($\approx$200—600 responses per hour; Table I).

Table I

Frequency of self-stimulation in young rats

Age in days	No. of animals	Mean of pedal-pressing per 20 min	Minimum and maximum of pressings per 20 min
14	2	77	54 and 100
15	8	90	66 and 109
16	8	113	75 and 187
17	8	124	75 and 194

Effect of intraperitoneal histamine injection

Twenty min after intraperitoneal histamine injection the plasma corticosterone level did not change in 12 to 13-day-old animals when compared with NaCl-treated controls (Group II *vs.* Group I). A slight, but not significant increase was found in 14-day-old rats. The elevation of plasma corticosterone level became significant at the age of 15 days, and was more pronounced in older animals (Table II).

Effect of electric footshock

In order to exclude the possibility that young rats are insensitive to the histamine dose applied 13-, 15- and 17-day old animals were stressed with an electric footshock. There was no significant difference in changes of plasma corticosterone level following histamine injection or electrical stress (Table II).

Table II

Effect of intraperitoneal histamine (4 mg/kg b.w.) and electric footshock on plasma corticosterone level (μg/100 ml plasma)
Mean ± S.D.

Age in days	Group I, i.p. phys. NaCl	Group II, i.p. histamine [electric footshock]*	p
12	6.6±1.3 (8)	7.2±1.0 (8)	N.S.
13	6.5±1.2 (8)	6.5±1.5 (8) [6.9±1.9 (6)]*	N.S.
14	8.3±0.7 (8)	10.3±2.0 (9)	$0.05 < p < 0.1$
15	7.7±1.5 (8)	12.3±2.4 (8) [12.1±2.1 (6)]*	<0.01
16	7.8±1.7 (8)	14.0±2.0 (8)	<0.001
17	8.3±1.3 (8)	19.0±3.0 (8) [18.0±1.8 (6)]*	<0.001

In brackets: number of animals
* plasma corticosterone level after electric footshock
N.S.: not significant

Effect of "passive" stimulation and self-stimulation

When compared with the operated but not stimulated group, both "passive" electrical stimulation and self-stimulation of MFB significantly increased plasma corticosterone level (Group IV *vs.* Group III; Table III). Table III also shows the plasma corticosterone level of two self-stimulating 14-day-old rats. The plasma corticosterone level of the 16 to 17-day-old oper-

Table III

Effect of "passive" stimulation and self-stimulation in MFB on plasma corticosterone level (μg/100 ml plasma) in young rats
Mean ±S.D.

Age in days	Group III, operated control animals	Group IV, stimulated animals	p
12	7.5±1.5 (8)	11.9±1.5 (8)*	<0.01
13	7.9±1.7 (8)	11.1±1.4 (8)*	<0.01
14	9.0±2.4 (8)	13.6±2.4 (8)* 12.8 and 14.4 (2)	<0.01 —
15	10.1±2.7 (8)	14.1±2.0 (8)	<0.05
16	9.9±0.7 (8)**	12.9±2.5 (8)	<0.05
17	10.7±1.6 (8)**	20.0±2.5 (8)	<0.001

In brackets: number of animals
* "passively" stimulated animals
** significantly higher ($p < 0.05$) *vs.* Group I (see Table II)

ated control animals was significantly higher than that of the NaCl-treated controls (Group I), presumably because of the stressor effect of the electrode implantation performed on the day before.

Discussion

Data concerning the age at which stress response of rat's hypothalamo–pituitary–adrenal system appears are contradictory. In our investigations an elevation of plasma corticosterone level after i.p. histamine injection could be first observed in 14- to 15-day-old, and a long-term stressor effect of the operation in 16-day-old animals. The possibility that one of the components of hypothalamo–pituitary–adrenal system in younger rats fails to secrete the required hormone (CRF, ACTH, corticoids) can be excluded. SCHAPIRO et al. (1962) suggested that i.p. ACTH injection increases plasma corticosterone level in the rat from the first day of life. It was also shown that the pituitary gland secreted ACTH as early as in the first postnatal week (JAILER 1951; SCHAPIRO 1962). HIROSHIGE and SATO (1970, 1971) revealed the eminentia mediana to contain CRF in 2-day-old rats, but the increment of the CRF activity after stress became significant on the 14th postnatal day only. According to our own data the electrical stimulation of MFB region elicited a significant increase of plasma corticosterone level in age-groups in which histamine or the electric footshock proved to be ineffective. These data and the ineffectiveness of both stressors renders it improbable that it is only the low sensitivity of younger rats to histamine which is responsible for the absence of elevated plasma corticosterone level; rather, the findings indicate a lack of other mechanisms involved in the stress response in adults. Although the structures through which histamine elicits its stressor effect are not accurately known it can be supposed from the ineffectiveness of electric footshock that the afferent pathways from brain stem to hypothalamus are immatured at this age. Some other findings also support this assumption. The circadian rhythm of the hypothalamo–pituitary–adrenal system in the maintenance of which the subcortical structures and connections play an important part (GALICICH et al. 1965), also appears at the third postnatal week in the rat (HIROSHIGE and SATO 1970). The immaturity of mesencephalic–basal forebrain connections in rats before 14 to 15 days of age was demonstrated by ENDRŐCZI and HARTMANN (1968) in studies on the appearance and maturation of evoked potentials in brain stem and forebrain.

On the other hand, the importance of brain stem–forebrain connections in the organizaton of self-stimulation behaviour was emphasized by many authors (DEUTSCH and HOWARTH 1963; ENDRŐCZI et al. 1967; etc.). The present observations, according to which 14 to 15 days represent the youngest age

at which pedal-pressing self-stimulation will develop in MFB, can also be explained with the maturation of this connections.

On the basis of the coincidence that both self-stimulation behaviour and stress response of hypothalamo–pituitary–adrenal system to intraperitoneal histamine injection or electric footshock appear on the 14 to 15th postnatal day one can assume that brain structures and connections involved in the two processes either come to maturity parallelly at identical point of time, or, that the same structures play a part both in self-stimulation and stress mechanism.

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Recensiones

Zsuzsa HOLLÁN

Haemoglobinok és haemoglobinopathiák

280 pages, with 128 figures and 24 tables.
Akadémiai Kiadó, Budapest 1972

In the preface of her book the authoress points at the increasing gap separating basic from clinical research, although the unfamiliarity with the results of basic research is a hinderance in diagnostic and therapeutic work. One of the aims of the book is to bridge over this gap, and this intention is tackled in an exemplary way. The book alloys the most abstract molecular-biological data with practical observations seemingly insignificant at first sight.

Chapter 1 deals with the structure, development and function of the haemoglobin molecule, and the second one with its polymorphism. They summarize the pertinent knowledge in such a way as to make it understandable even for readers without any particular expertness in either biology or haematology.

Chapter 3 describes the haemoglobinopathies, among them the rare cases of beta-thalassaemia recognized by the authoress in Hungary.

Chapter 4 deals with labile haemoglobins; these deviations rare forms are of importance in both research and practice as they lead to haemolytic anaemia. Mainly on the basis of own investigations, the pathomechanism of inclusion formation is described together with the clinical course and treatment of labile-haemoglobin disease.

Chapter 5 discusses the haemoglobinopathies causing congenital cyanosis, and M-haemoglobin disease. Characteristics of the cases observed in Hungary are described in detail. It is really exciting to follow the discovery of a new haemoglobin-anomaly starting from cyanosis as the only symptom.

Chapter 6 describes the haemoglobinopathies causing polyglobulia, diseases which have been recognized in the last years. Correct diagnosis in these cases is of importance not only for the fate of patients; the authoress was the first to obtain evidence of the alpha-chain being coded by at least two genetic locus-pairs also in human haemoglobin. This is a discovery of considerable molecular-genetic significance.

Chapter 7 deals with the geographical distribution of haemoglobinopathies and with population-genetic problems. All the haemoglobinopathies, which concern several million individuals, are beta-chain variants. The study of these deviations has created geographic haematology, a branch of important in future anthropological research.

Finally, Chapter 8 shows the perspectives of genetic research. The idea does not seem unreal that just these or similar molecular-biological and genetic research will lead to the elaboration of a causal therapy of hereditary diseases by means of a transfer of normal genetic information ("genetic engineering").

This monograph on haemoglobins and haemoglobinopathies fills a real gap and its reading is enjoyable from beginning to end.

The book is indispensable for the clinician and all those interested in molecular biology and genetics. The beautiful illustrations deserve particular mention; they greatly contribute to the value of the text.

Ibolya NAGY

K. LISSÁK (Ed.)

Hormones and Brain Function

Publishing House of the Hungarian Academy of Sciences, Budapest, and Plenum Press, New York 1973. 529 pages

The volume presents the Max REISS memorial lecture and forty-five papers delivered during the Second Congress of the International Society of Psychoneuroendocrinology organized by Professor K. LISSÁK, and held in Budapest, Hungary, from July 1 to 3, 1971.

An outstanding assembly of internationally respected scientists reports on some of the most crucial aspects of the exciting field of psychoneuroendocrinology in this volume. The advances disclosed are of remarkable importance. Starting with the neonatal and ontogenetic aspects of the brain–pituitary system, it has been fascinating to learn of the uncommitted nature of its embryonic matrix and the role of perinatal hormonal actions in determining its development and subsequent sexual polarization or disturbances in maturity. Sections II, III, IV and V deal with the control, biosynthesis, and release of pituitary hormones: psychopharmacology, and neurochemical bases of drug actions; hormonal influences on brain functions; and clinical neuroendocrinology. Reports on these topics and other clinical areas of research show the current status and concepts of the field and the vigour with which the Society attempts to fulfil its mission of bringing psychiatry and neuroendocrinology together to ensure an improved approach to the treatment of mentally disturbed patients.

The papers and bibliographies will be most helpful to workers in this field, and the very well produced volume is a reference book indispensable in every self-respecting medical library.

B. FLERKÓ

E. BROMSER, A. KLEINZELLER (Eds)

Current Topics in Membranes and Transport

Volume 4. Academic Press, New York, London 1973.
XXII + 351 pages, with 21 figures and 14 tables.
Price: US $ 28.50; £ 13.70

This volume is dedicated to the memory of Aharon KATZIR-KATCHALSKY (1913—1972). The biographical appraisal is written by S. R. CAPLAN; it contains the bibliography of the principal publications of KATCHALSKY on membrane phenomena.

The four chapters of the volume contain: The Genetic Control of Membrane Transport by Cosolyn W. SLAYMAN; Enzymic Hydrolysis of Various Components in Biomembranes and Related Systems by Manhendra KUMAR JAIN; Regulation of Sugar Transport in Eukaryotic Cells by Howard E. MORGAN and Carl F. WHITFIELD; Secretory Events in Gastric Mucosa by Richard P. DURBIN. The volume ends with an author and subject index.

This 4th volume of the series Current Topics in Membranes and Transport will be an important source of information for all research workers interested in biological transport in the fields of biochemistry, biology and physiology.

K. LISSÁK

Olga HUDLICKÁ

Muscle Blood Flow, Its Relation to Muscle Metabolism and Function

Swets and Zeitlinger N. V., Amsterdam 1973. VIII +
219 pages, 47 figures. Price: Dutch Fl 54.—

In the six chapters of the monograph, the authors discuss in Chapter 1, the Anatomy and histology of muscle circulation; Chapter 2: Basic mechanisms regulating muscle blood flow: adaptation of muscle blood flow to systemic circulatory changes; Chapter 3: Humoral mechanisms affecting muscle circulation; Chapter 4: Nervous regulation of muscle circulation;

Chapter 5: Muscle metabolism and blood flow; Chapter 6: Disturbances of muscle circulation. The volume concludes with abundant references and an author and subject index.

The monograph provides a large number of references from the last century up to 1972. The book will be interesting for physiologists, biochemists and pathophysiologists.

K. Lissák

T. Kawai

Clinical Aspects of the Plasma Proteins

Igaku shoin LTD, Tokyo, and Springer-Verlag, Berlin, Heidelberg, New York 1973.
XVI + 464 pages, with 278 figures, 90 tables and 20 colour plates.
Price: DM 136.—; US $ 52.40

This book is the partly revised English edition of the monograph "The Plasma Proteins, Their Fundamental and Clinical Aspects" published in Japanese in year 1969. The first part discusses the fundamental and physiological properties of plasma proteins, but the most significant part deals with the pathophysiology of the plasma protein abnormalities. They are well-known to cause functional disorders of the tissues and, inversely, the pathologic changes of tissues may result in plasma protein alterations.

The book consists of four sections. Section I, Introduction discusses in Chapter 1 the Fundamental Structure of Proteins, and in Chapter 2 the General Principles of Protein Fractionation. Section II, Properties of Individual Plasma Protein Components. In Chapter 3, Plasma Proteins Included in the Albumin Fraction are discussed; in Chapter 4, Plasma Proteins Included in the α_1-Fraction; in Chapter 5, Plasma Proteins Included in the α_2-Fraction; in Chapter 6, Plasma Proteins Included in the β-Fraction; in Chapter 7, Fibrinogen and its Degradation Products; in Chapter 9, Glycoproteins and Lipoproteins. Section III deals with the Metabolism of the Plasma Proteins. Chapter 10, General Survey of the Plasma Protein Metabolism; Chapter 11, Synthesis of the Plasma Proteins; Chapter 12, Bodily Distribution of the Plasma Proteins; Chapter 14, External Loss of the Plasma Proteins. Section IV discusses the Diagnosis and Pathogenesis of Plasma Protein Abnormalities. Chapter 15: Diagnostic Approaches in Plasma Protein Abnormalities; Chapter 16, Variations in the Measurement of the Plasma Proteins, Chapter 17, Interpretation of Serum Protein Patterns; Chapter 18, Plasma Protein Changes in Malnutritional Conditions; Chapter 19, Plasma Protein Changes in Protein-losing Conditions; Chapter 20, Plasma Protein Changes in Hepatic Disorders; Chapter 21, Plasma Protein Changes in Acute Phase Responses; Chapter 22, Plasma Protein Changes in Polyclonal Hyperimmunoglobulinemia; Chapter 23, Plasma Protein Changes in M-Proteinemic Type; Chapter 24, Abnormal Plasma Proteins; Chapter 25, Defect Dysproteinemias; Chapter 26, Hyperlipoproteinemia; Chapter 27, Plasma Protein Changes in Pregnant and Fetal Periods. Each section is complete with abundant references. The volume ends with a subject index.

The volume will be a very valuable source of references for workers in the clinical laboratory, clinical pathology and biochemistry.

K. Lissák

B. Jirgensons

Optical Activity of Proteins and Other Macromolecules

Molecular Biology, Biochemistry and Biophysics, Vol. 5. Second,
revised and enlarged edition. Springer-Verlag, Berlin, Heidelberg, New York 1973.
IX + 199 pages, with 71 figures. Price: DM 59.80; US $ 23.10

With the application of physical methods in the study of the structure of proteins and other biological macromolecules, great advances have been made in protein chemistry. Particularly the optical rotatory dispersion (ORD) proved successful in solving structural problems. The purpose of the monograph is to introduce the reader in the use of spectropolarimetric methods and to their applications in the problems of molecular biology. The first edition

of the monograph was published by Springer-Verlag in 1969, under the title: Optical Rotatory Dispersion of Proteins and Other Macromolecules. During the last ten years, optical rotatory dispersion and circular dichroism (CD) have established themselves fully as leading methods in the study of conformational transitions of peptides, proteins, and various other lyopolymers in solution.

The contents of the volume are as follows. Chapter I, The Realm of Proteins. Structural Features. The Phenomenon of Optical Activity. Historical Highlights. Chapter II, The Phenomena of Optical Activity. Terms and Definitions. Theoretical Considerations. The Drude and Moffitt Equations. Chapter III, Polarimeters and Spectropolarimeters. The Measurement of Optical Activity. Chapter IV, Optical Activity of Amino Acids, Peptides, and Proteins. Chapter V, The Optical Rotatory Dispersion of Polyamino Acids and Proteins. Measurements in the Visible and Near Ultraviolet Spectral Zones. Chapter VI, The Far Ultraviolet Cotton Effects of Synthetic Polyamino Acids. Chapter VII, The Cotton Effects of Conformations of Proteins. Chapter VIII, Cotton Effects and Conformations of Nonhelical Proteins. Chapter IX, Optical Activity of Structural Proteins. Chapter X, Optical Activity of Nucleoproteins, and Histons. Chapter XI, Optical Activity of Glycoproteins and Lipoproteins. Chapter XII Concluding Remarks.

The volume ends with a list of references and a subject index.

The book is a very important reference work for workers and postgraduate students of molecular biology, biochemistry and biophysics.

K. Lissák

T. Ando, M. Yamasaki, K. Suzuki

Protamines: Isolation, Characterization, Structure and Function

Molecular Biology, Biochemistry and Biophysics, Vol. 12.
Springer-Verlag, Berlin, Heidelberg, New York 1973.
IX + 114 pages, with 24 figures. Price: DM 48.—; US $ 19.70

The history of nucleoproteins dates back to over a century. In the nucleoproteins nucleic acids combine in some manner with proteins. They are found in most living cells. The term "protamine" was given by Miescher (1874), but he failed to observe the protein nature of protamine, and after 20 years Kossel and his collaborators found complexes of nucleic acid with various kinds of protamine. Protamine was considered important in view of its occurrence in cell nuclei. It proved to have the simplest amino-acid composition among the proteins. After the second World War, a remarkable progress was made in chemical methods and techniques, which revolutionized the field of protein chemistry. The Japanese research group of Tokyo under the leadership of Professor T. Ando was able to add new points to the results concerning the chemical structure of protamines. The studies of this group have established a general means of determining the primary structure of each component of protamine.

The contents of the volume are: Chapter I, Introduction. Chapter II, Distribution of Nucleoprotamines and Protamines. Chapter III, Preparation of Nucleoprotamines and Protamines. Chapter IV, Composition. Chapter V, Molecular Weight. Chapter VI, Chemical Structure of Nucleoprotamines and Protamines. Chapter VII, Heterogeneity of Protamines and Homogeneous Molecular Species of Protamines. Chapter VIII, Chemical Structure of Homogeneous Molecular Species of Protamines. Chapter IX, Physical Structure of Nucleoprotamines and Protamines. Chapter X, Properties and Functions.

The volume ends with abundant references and with a subject index. It will be interesting for molecular biologists, biochemists and biophysicists.

K. Lissák

V. NEUHOFF (Ed.)

Micromethods in Molecular Biology

Molecular Biology, Biochemistry and Biophysics, Vol. 14.
Springer-Verlag, Berlin, Heidelberg, New York 1973.
XV + 428 pages, with 275 figures and 23 tables.
Price: DM 98.—; US $ 40.20

The book contains contributions by G. F. BAHR, P. DÖRMER, J. E. EDSTRÖM, U. LEEMANN, G. M., LEHRER, F. RUCH, H. G. ZIMMER; it is a treasury of selection of up-to-date practical methods of molecular biology, based on the authors own experience in whose laboratories the methods described are performed routinely.

The contents of the book are as follows. Chapter 1: Micro-Electrophoresis on Polyacrylamide Gels by V. NEUHOFF; Chapter 2: Micro-Determination of Amino Acids and Related Compounds with Densyl Chloride by V. NEUHOFF; Chapter 3: Micro-Determination of Phospholipids by V. NEUHOFF; Chapter 4: Micro-Diffusion Techniques by V. NEUHOFF; Chapter 5: Capillary Centrifugation by V. NEUHOFF; Chapter 6: Micro-Electrophoresis for NNa and DNA Base Analysis by J. EDSTRÖM E. and V. NEUHOFF; Chapter 7: Determination of the Dry Mass of Small Biological Objects by Quantitative Electron Microscopy by G. F. BAHR; Chapter 8: The Construction and Use of Quartz Fiber Fish Pole Balances by G. M. LEHRER; Chapter 9: Microphotometry by H. G. ZIMMER; Chapter 10: Cytofluorometry by F. RUSH and U. LEEMANN; Chapter 11: Quantitative Autoradiography at the Cellular Level by P. DÖRMER; Chapter 12: Micro-Dialysis by V. NEUHOFF; Chapter 13: Micro-Homogenisation by V. NEUHOFF; Chapter 14: Wet Weight Determination in the Lower Milligram Range by V. NEUHOFF, Chapter 15: Micro-Magnetic Stirrer by V. NEUHOFF: Chapter 16: Production of Capillary Pipettes by V. NEUHOFF. The volume is complete with a subject index.

This book is a very important help and reference work in the daily work of molecular biology laboratories.

K. LISSÁK

P. R. STEWART, D. S. LETHAM (Eds)

The Ribonucleic Acids

Springer-Verlag, Berlin, Heidelberg, New York 1973.
XV + 268 pages, with 56 figures and 14 tables.
Price: DM 45.40; US $ 20.50

This volume is based on a postgraduate course organized by the University of Canberra "to attempt to provide a comprehensive, though not excessively detailed outline of biological roles of RNA".

The eleven chapters of the book deal with the most important basic knowledge related to ribonucleic acids (RNA), with the aim of supplying the postgraduate student with directives in the vast literature on RNA. The chapters give the whole material of the course.

Chapter 1: RNA in Retrospect by D. S. LETHAM, P. R. STEWART and G D. CLARK-WALKER; Chapter 2: Transcription by G. M. POLYA; Chapter 3: Nuclear RNA by H. NAORA; Chapter 4: Messenger RNA by A. J. HOWELLS; Chapter 5: Transfer RNA and Cytokinins by D. S. LETHAM; Chapter 6: Ribosomal RNA by L. DALGORNA and J. SHINE; Chapter 7: Translation of Messenger RNA by G. D. CLARK-WALKER; with an Appendix on the Inhibitors of Translation by P. R. STEWART; Chapter 8: Mitochondrial RNA by P. R. STEWART; Chapter 9: Chloroplast RNA by P. R. WHITFELD; Chapter 10: Viral RNA by A. J. GIBBS and J. J. SKEHEL; Chapter 11: Isolation, Purification and Fractionation of RNA by P. R. STEWART; Subject Index.

The book is a useful basic text for postgraduate students of biology, biochemistry, biophysics and physiology, but will be a good introduction for everybody interested in the problems of molecular biology.

K. LISSÁK

E. K. F. BAUTZ, P. KARLSON, H. KERSTEN (Eds)

Regulation of Transcription and Translation in Eukaryotes

Springer-Verlag, Berlin, Heidelberg, New York 1973.
VII + 349 pages, with 131 figures.
Price: DM 68.—; US $ 27.90

The volume contains the proceedings of the 24th Mosbach Colloquium held in April, 1973, in Mosbach, Germany, under the auspices of the Gesellschaft für Biologische Chemie.

The 18 papers presented by a group of internationally outstanding scientists are pointed in five main chapters: Chromosome Structure and Function, Transcription I, Transcirption II, Translation I, Translation II. Each paper was followed by discussions which greatly increase the value of the book. Instead of a broad topic the subjects discussed at the Symposium were concentrated on a few aspects of gene expression in reasonable detail. Dr BAUTZ pointed out that "the symposium was concentrating on four questions, which are most basic to an understanding of the mechanism of transcription and translation and for which fragmentary but nontheless reliable experimental results have become available within the last few years. These are the structure of chromatin, the synthesis of messenger RNA, the structure of the active ribosome, and the role of inactivation factors in protein synthesis".

The book presents an informative survey of what is known presently and also what we need to know in order to understand the molecular mechanisms by which gene expression is regulated. This very important source of informations will be an indispensable reading for biochemists and biologists interested in the recent problems of molecular biology.

K. LISSÁK

H. PRECHT, J. CHRISTOPHERSEN, H. HENSEL, W. LARCHER (Eds)

Temperature and Life

Springer-Verlag, Berlin, Heidelberg, New York 1973.
XX + 779 pages, with 263 figures. Price: DM 142.—; US $ 58.30

The present volume is a revised and enlarged English version of the German book "Temperatur und Leben" by H. PRECHT, J. CHRISTOPHERSEN, H. HENSEL. The main theme of the book is the adaptation of organisms to changing temperatures. The present book was written with the contributions by K. BRÜCK, D. M. GATES, B. HAVSTEEN, U. HEBER, I. L. INGRAHM, H. D. JANKOWSKY, H. LAUDIEN, K. NAPP-ZINN, A. PISEK, P. PATHS, K. A. SANTORIUS, A. VEGIS; it presents a complete survey of the whole subject of thermoregulation of living organisms, microorganisms, plants and homeothermic organisms including man. Each chapter concludes with abundant literature and the volume ends with a subject index.

The book is very important especially for biologists working in microbiology, botany, zoology and medicine, and interested in thermoregulation.

K. LISSÁK

W. WIESER (Ed.)

Effects of Temperature on Ectothermic Organisms

Ecological Implications and Mechanisms of Compensation.
Springer-Verlag, Berlin, Heidelberg, New York 1973.
XI + 298 pages, with 126 figures. Price: DM 66.—; US $ 27.10

The book contains the Proceedings of a Symposium held at Obergurgl, Austria, from 4 to 8 September, 1972. The concept on which the Symposium was based on the witty draft "there are two sides to the coin mechanism and ecology". The nearly sixty participants presented 26 papers in the fields of mechanism, ecology and cold resistance.

The book is an important source of informations and references for research workers and postgraduate students in physiology, zoology and ecology, interested in the field of thermoregulation.

K. Lissák

H. Bartels, K. Riegel, J. Wenner, H. Wulf

Perinatale Atmung

Physiologische Grundlagen und therapeutische Konsequenzen.
Springer-Verlag, Berlin, Heidelberg, New York 1972.
XII + 101 pages with 50 figures. Price: DM 22.—; US $ 7.—

The oxygen needed by the fetus is transported through the maternal respiratory system, maternal blood and the placenta before it reaches the fetal blood. There are differences in the process of gas-exchange in the lung and placenta. To understand the dramatic physiological changes taking place when fetal circulation and breathing are switching over from the intrauterine type to that existing in extrauterine life, the analysis of the details of gas transport in maternal and fetal blood is of a vital importance.

The first chapter of this book is a review of the physiology of gas-transport in the lung and blood, analyzing the buffer capacity of the blood, the role of circulation in gas-transport, and the regulation of respiration. The second chapter deals with pulmonary ventilation during pregnancy, the functions of maternal and fetal blood in ventilation; the mechanism of gas exchange in the placenta. The details of uterine blood flow are also discussed. A separate chapter is devoted to the problems of gas exchange and gas transport in the placenta during labour. The most detailed part of the book analyzes the factors of gas exchange in the immediate postnatal period and the fifth chapter summarizes the special details of treatment indicated in various cases of perinatal respiratory failure.

Without oxygen, there is no human life. Without transport, there is no oxygen for the human fetus. Without adequate therapeutic measures, there is no future for the newborn suffering from respiratory failure. The book deals with the up-to-date answers to the majority of questions related to the problems mentioned above. It is therefore an excellent and indispensable guide for those, who have to take the responsibility for human life in utero, and also immediately after it.

G. Illei

K. Eckoldt, C. Pfeiffer, R. Winter (Eds)

Physiologie und Pathophysiologie des Warmehaushalts

Ergebnisse der experimentelle Medizin, Vol. 11.
Verlag Volk und Gesundheit, Berlin 1973. 257 pages.
Price: M 28.15

The volume contains the contributions presented at a symposium organized jointly by the Physiological and the Pathophysiological Societies of the German Democratic Republic in May, 1971. The contributions covering a wide field are published in extenso with the pertaining literature, but without the discussions. It is regrettable that two years elapsed between the symposium and the publication of its valuable material. Nevertheless, the volume is useful as a source of information on the work performed in the German Democratic Republic in this field. In addition, some contributions from other socialist countries are included.

Sz. Donhoffer

E. SCHÖNBAUM, P. LOMAX (Eds)

The Pharmacology of Thermoregulation

Proceedings of a satellite symposium held in conjunction with the fifth International Congress on Pharmacology, San Francisco 1972. S. Karger, Basel, München, Paris, London, New York, Sydney 1973. XIV + 583 pages, with 159 figures and 44 tables.
Price: DM 169.—; £ 23.80; US $ 58.30

As pointed out in the preface, the title of this volume fails to indicate the wide range of the contributions which cover beside pharmacological aspects many other problems of the physiology and pathophysiology of thermoregulation, including clinical observations. In addition to the fairly extensive references listed in the individual contributions, a cumulative list of references (1227 items), and a useful subject index of 16 pages enhance the value of the volume. Discussions are not included in detail. The main points which arose in the course of the discussions have been summarized by the editors in 5 pages. The contributions maintain a high level throughout the well edited and finely produced volume.

Sz. DONHOFFER

V. SIGUSCH

Ergebnisse zur Sexualmedizin

Wissenschafts-Verlag, Köln, Basel, München, Paris, London, New York, Sidney 1973.
2nd Edition. 186 pages, with 26 figures and 10 tables. Paperback.
Price: DM 12.—; US $ 4.05

Research of sexual behaviour had begun in the first decades of this century; it gained independence as a new branch of science in the forties. Before World War II its main fields were biology, zoology, endocrinology and neurophysiology; nowadays, the theory and practice of sexuality, determined by social relations and grounded biologically, is the common work of physicians, sociologists and psychologists.

From the middle 50's up to 1970 the leading personality of German sexology was Hans GIESE; his contributions were especially important in the field of psychopathology. A quite new, existentiallistic-phenomenological school with many psychiatrists has developed around GIESE. The author of the present book was also a pupil of this school, and now he is the leader of the Department of Clinical Sexology at the University of Frankfurt am Main. In his book, he gives a survey of modern sexual-pathological, physiological and sociological knowledge and the tasks of sexual medicine. His starting point is the fact that, while the number of patients with sexual problems, conflicts and behavioural disturbances is extremely high and continuously increasing, sexual-medical education and expertness of the medical students and physicians is still one-sided and imperfect.

Chapter 1 of the book surveys the reality of the creation of sexual medicine as an independent branch of science in the light of the most recent results. It outlines the 26 items of the author's two-semester university lectures which deal with the problem according to didactic points of view, without aiming at completeness. As starting basis the author lays down seven thesis concerning the relationship of medical science and sexuality.

1. The history of medicine is at the same time, the history of a struggle against sexuality.
2. In medicine, sexuality means malady, abnormality, perversities and criminology.
3. Medicine regards sexuality as first and foremost a reproductive function.
4. Medical science ignores the pleasure-giving function of sexual life.
5. Medical science does not strive after susceptibility to sexuality, after the emancipation of sexuality; it aims at its "conservation by elimination".
6. The sexual morals of medicine are oppressive, rigidly insisting upon traditions.
7. A study of sexuality within the frame of medical sciences is not desirable, and sexual medicine does not even exist.

These theses actually outline the prejudicial system developed within medicine and existing even at present; at the same time, the thesis serve as working hypothesis for the creation of sexual medicine.

Chapter 2 deals with the physiology and psychology of sexuality, differentiating the genital and extragenital sexual reactions of females and males. The human sexual-psychological reactions are, of course, different in the two sexes. It is especially difficult to approach the problem of female orgasm. No similar reaction occurs in animals. In women the orgasm is not coupled with such a well-defined physiological event as is the ejaculation of males. Further differences between the two sexes can be found in 1. the running-up curve of sexual excitation; 2. subjective experience of the orgasm; 3. content of the orgasm; 4. duration of the refractory period following orgasm; 5. sensation of satisfaction caused by consecutive orgasms. Female orgasm presumably takes place entirely within the nervous system, and its mechanism is of psychological nature.

At the end of this chapter the author describes some own experiments to demonstrate the differences in the reactions of the two sexes. The emotional and autonomic reactions evoked by psychosexual stimulation by means of sex films and diapositives are compared in male and female groups. The results refute the myth propagated even today concerning the unlike character of psychosexual excitability in the two sexes. The antisexual, dual morals of civilized countries punishes more severely and suppresses more strongly the sexual manifestations of girls than those of boys of similar age. No sexual interest and desire were allowed to develop in the woman; most girls of the traditional education became frigid. The mother opposing sexuality damages the sexuality of her son, which leads to homosexuality and other disturbances of sexual behavior; and she passes her own frigidity, her own emotional immaturity to her daughter.

Chapter 3 describes the investigations performed with sociological methods of V. SIGUSCH, G. SCHMIDT and H. GIESE. The basis of sexual sociology has been laid down by A. C. KINSEY in his book on American sexual behaviour. After KINSEY, comprehensive investigations have been started also in Europe; in 1953 L. FREIDEBURG published the results of a German analysis similar to the KINSEY report. SIGUSCH then describes the sexual behavioural patterns of three classes of society, based on analyses performed on workers and university students between the age of 20 and 21 as well as on secondary class students between 16 and 17 years of age. Investigation of the latter age group is of special significance since youth today also wittingly emphasizes the peculiar characteristics of the transition from childhood to adulthood. The special culture of the young, their contentions and system of value form the so-called "youth culture", the sexual standards of which are described in detail.

The closing part of the book deals with sexual deviations; it is the common work of G. SCHMIDT, E. SCHORSCH and V. SIGUSCH. SCHORSCH outlines the relationship of sexual deviations and the general attitude to illness as follows.

1. According to the traditional psychiatric concept any deviation in sexual behaviour is abnormal and pathological.

2. A sexual deviation is not necessarily an abnormity or illness but, first of all, such a rare behaviour which offends the moral laws of the society.

3. Some sexual deviations are only variants of sexual behaviour, with intact personality.

4. Since sexual deviation does not necessarily mean a psychopathological syndrome, the mere existence of a sexual deviation does not mean an indication for treatment.

5. When the individual experiences his sexual deviation as an "illness", this awareness of malady is often a reaction to the intolerance of the environment, or, the manifestation of the internalization of the operative morals.

6. Treatment of sexual deviants should aim at liberating the deviations sexuality and not at freeing them from it.

Finally, taken into account the points mentioned above, the authors make suggestions for a reform of the criminal sexual law. This detour also shows that the borders of sexual medicine as an independent branch of science can be outlined only by means of a complex approach.

The book's construction shows the author's endeavour to bring in harmony the conceptions of psychophysiology, psychoanalysis, dynamic psychiatry and personalistic psychology. At the same time it shows the complexity of approaching sexual problematics and calls attention to the prejudices of the medical and lay attitude.

A. STARK

INDEX

PHYSIOLOGIA

PHARMACOLOGIA

COMMUNICATIONES BREVES

RECENSIONES

International Union of Physiological Sciences

XXVIIth INTERNATIONAL CONGRESS OF PHYSIOLOGICAL SCIENCES

PARIS – 1977

The Congress will take place from Monday July 18th to Saturday July 23rd 1977. Reception desk will open on Sunday July 17th at the University Paris VI, quai Saint Bernard, Paris.

A new formula has been chosen for the general organizing scheme of the Congress. Its first part will be devoted to lectures covering the major fields of the Physiological Sciences. A few lectures will emphasize the relationship of Physiology with other biological disciplines and/or with socio-economical problemes.

The second part of the Congress begins on Wednesday the 20th. It aims to promote contacts between students working in the same area. Therefore the Scientific program will be divided into sections representing the different fields of the Physiological Sciences. For each section half-day symposia and free presentations (communications or posters) will take place, without any overlap between them in order to increase the attendance at the various events. Each section will have its proper conference rooms, amphitheaters and poster surfaces. Sections will meet in the same geographical area so that a close relationship between the different specialists might be preserved. Congress participants will be able to choose their section(s) of interest.

Before and after the Congress, Satellite Symposia will take place. A list of laboratories open to Congress participants interested in scientific contacts will be provided.

Any further information concerning the 1977 Congress can be obtained through each National Physiological Society or by writing directly to Congress Secretary:

Pr. J. SCHERRER, Secrétariat du XXVIIe Congrès International des Sciences Physiologiques, U.E.R. Pitié-Salpêtrière, Cedex 1300, F-75300 Paris — Brune, France

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ACTA PHYSIOLOGICA

ТОМ 45 – ВЫП. 3–4

РЕЗЮМЕ

ДИСК-ЭЛЕКТРОФОРЕТИЧЕСКОЕ ИССЛЕДОВАНИЕ БЕЛКОВ СЫВОРОТКИ МАТЕРИНСКОЙ КРОВИ, КРОВИ ПУПОЧНЫХ СОСУДОВ И ОКОЛОПЛОДНОЙ ЖИДКОСТИ

Г. ТАН, Г. ИЛЛЕИ и И. ЧЕХ

У 25 беременных женщин исследовали диск-элетрофорезом белки сыворотки во время беременности и через восемь недель после родов. Кроме того, анализировали состав белков амниотической жидкости и крови пупочных сосудов (артериальной и венозной). Количественные и качественные изменения, белков материнской крови, имевшие место во время беременности, отсутствовали в крови пупочных сосудов, в амниотической жидкости и в сыворотке крови той же женщины через восемь недель после родов. Считают, что количественные изменения белков, характеризующие беременность, сводятся к повышению содержания быстрых и медленных постальбуминов и трансферринов. В половине случаев в сыворотке материнской крови были обнаружены две белковые фракции (протеин$_1$ беременности и протеин$_2$ беременности) в области альфа-2-глобулин, не представленные ни у одного вида животных.

ЭФФЕКТ ПРОСТАГЛАНДИНА E_1 НА СОСТАВ СВОБОДНЫХ ЖИРОВЫХ КИСЛОТ В СЫВОРОТКЕ КРОВИ КРОЛИКОВ

М. Л. МИХАЙЛОВ

Исследовался эффект простагландина E_1 на состав свободных жировых кислот в сыворотке крови кроликов с помощью газовой хроматографии. Было установлено, что уровень ненасыщенных жировых кислот (линолевой, нефтяной и арахидоновой кислот повышался. В то же время пропорция насыщенных жировых кислот (за исключением стеариновой кислоты) уменьшалась. Результаты обсуждаются на основании известных метаболических эффектов простагландина E_1.

ДЕЙСТВИЕ ТЕНОТОМИИ НА СОСТАВ ЛИПИДОВ МЫШЦ С РАЗЛИЧНЫМИ БИОЛОГИЧЕСКИМИ ФУНКЦИЯМИ

Л. ХЕЙНЕР, Й. ДОМОНКОШ и М. ВАРГА

Через одну и две недели после тенотомии у крыс исследовали состав липидов тонической m. soleus, промежуточного типа (содержащей тонические и тетанические волокна) m. gastrocnemius и тетанической m. vastus lateralis. Через одну неделю после операции количество триглицеридов и фосфолипидов возросло только в тонических мышцах. Это возрастание было более выражено после двух недель. В это время количество вышеупомянутых липидов возрасло также в мышце промежуточного типа. С другой стороны в тетанической мышце состав липидов не изменился значительно даже через две недели после тенотомии. Количественные изменения фосфолипидов тоже могли быть обнаружены в тонической мышце через две недели после тенотомии: возросло содержание жирного альдегида холин-фосфатидов и не-холин-фосфатидов. Описанные изменения фосфолипидов в тонической мышце после тенотомии могли быть предотвращены выполненной одновременно с тенотомией денервацией.

ВЛИЯНИЕ ПОВЫШЕННОГО ВЕНОЗНОГО ДАВЛЕНИЯ КРОВИ НА ВНУТРИПОЧЕЧНУЮ ГЕМОДИНАМИКУ

Г. КЁВЕР, Л. Г. ХАРШИНГ и Л. ХАРШИНГ

В экспериментах на собаках под нембуталовым наркозом было исследовано действие повышения венозного давления на внутрипочечную циркуляцию крови. Венозное давление повышали до 30 мм рт. ст. путем частичного пережатия вены на левой стороне почки. Общий ренальный кровоток (TRBF) измерялся путем сбора вытекающей крови. Почечный, кортикальный и медуллярный кровоток измерялись экстракцией Rb из ткани (Rb—RBF). TRBF не изменялся, в то время как Rb—RBF уменьшался пропорционально уменьшению разницы артериально-венозного давления. Причем снижение кровотока, в медуллярном веществе было большим, чем в кортикальном. В контроле не было обнаружено такой разницы между TRBF и Rb—RBF. Было также установлено снижение GFR, количества мочи и выделения натрия. Как показала экстракция Rb, редукция кровотока может объясняться перераспределением крови из нутриционных капилляров в ненутриционные. Калькуляция сопротивления сосудов позволило предположить, что перераспределение кровотока может быть локализовано, главным образом, в постгломерулярном сегменте юкста-медуллярной циркуляции.

ВЛИЯНИЕ ГИПОКСИИ НА ТЕРМОРЕГУЛЯТОРНУЮ ТЕПЛОПРОДУКЦИЮ И ТЕМПЕРАТУРУ ТЕЛА У НОВОРОЖДЕННЫХ И МОЛОДЫХ МОРСКИХ СВИНОК

М. ФАРКАШ и С. ДОНХОФФЕР

На морских свинках в возрасте менее чем 48 часов, 3—6 дней, 7—9 дней, 10—20 дней и 30—60 дней при внешней температуре (T_a) 20°C исследовали изменение температуры в толстом кишечнике (T_a) и теплопродукции (VO_2), выдерживая животных при 12% O_2 и при 8% O_2. Для сравнения использовались данные, полученные в аналогичных условиях на взрослых морских свинках. Экспозиция при 12% O_2 не вызывала изменений VO_2 ни в одной из возрастных групп животных, однако после окончания экспозиции величина VO_2 значимо возрастала в группах от 7 дней. При экспозиции 12% O_2 средние величины изменений теплопродукции (ΔO_2), зарегистрированных в отдельных наблюдениях, показали значительное повышение теплопродукции в группах 3—6 дней и 7—9 дней. Было установлено, что распределение ΔO_2 при экспозиции 12% O_2 статистически отличается у групп животных в возрасте менее 10 дней от более старших возрастных групп. И наоборот, при экспозиции 12% O_2 имелось значительное снижение T_c во всех возрастных группах. Эта реакция была менее выражена в группах 3—6 и 7—9 дней по сравнению с младшими и старшими группами животных. Во всех возрастных группах T_c возрастала в высшей степени значимо после окончания экспозиции. Распределение ΔT_c в возрастных группах 3—6 дней и 7—9 дней значимо отличалось от такого в группах менее чем 48 часов, 10—20 и 30—60 дней, но не было обнаружено различий в распределении ΔT_c между тремя последними возрастными группами. При экспозиции 8% O_2 как T_c, так и VO_2 снижались в высшей степени значимо. После окончания экспозиции VO_2 возрастал выше предэкспозиционного уровня. Также имело место быстрое сопутствующее возрастание T_c. При экспозиции животных в 8% O_2 ΔVO_2 была значительно меньше в двух самых младших группах, чем в старших группах новорожденных. Распределение ΔO_2, вычисленное для трех младших групп, взятых вместе, отличалось от такового в общей группе новорожденных от 10—20 дней и от 30—60 дней. Наибольшая ΔT_c наблюдалась при экспозиции 8% O_2 и была почти одинаковой в возрастных группах менее чем 48 часов и в группе 10—20 дней, обе из которых значимо отличались от других возрастных групп. В последующие за окончанием экспозиции 15 мин ΔT_c была значимо выше в самой младшей группе, чем в других. В других группах новорожденных величина ΔT_c была идентичной, несмотря на то, что во время предшествующей гипоксии имело место ее падение, и уровень T_c к моменту окончания гипоксии значительно отличался в этих группах. В противоположность крысам и кроликам, у которых отличия между новорожденными и возрослыми животными имеют тенденцию к постепенному стиранию, у морских свинок ΔT_c животных в возрасте менее чем 48 часов напоминает таковую в группе 10—20 дней гораздо больше, чем в группах 3—6 или 7—9 дней. Терморегуляторная система по-разному реагирует на холод и гипоксию во

время неонатального периода. Тогда как падение T_c в ответ на холод было меньшим в возрасте менее чем 48 часов, падение T_c в ответ на гипоксию было значительно большим чем в группах животных в возрасте 3—6 и 7—9 дней.

ДЕЙСТВИЕ ФИЗИОЛОГИЧЕСКИХ ДОЗ ВАЗОПРЕССИНА И ОКСИТОЦИНА НА ИЗБЕГАТЕЛЬНОЕ И ИССЛЕДОВАТЕЛЬСКОЕ ПОВЕДЕНИЕ КРЫС

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Было изучено действие малых доз вазопрессина (30 мЕд/кг) и окситоцина (30 мЕд/кг) на активное и пассивное избегания и на исследовательскую активность. Ни вазопрессин, ни окситоцин не оказывали какого-либо влияния на выработку активного избегания, в то же время его угашение задерживалось вазопрессином и ускорялось под влиянием окситоцина.

Анализ реакции пассивного избегания электрошока (PSAR) позволил установить, что вазопрессин укорачивает PSAR в повторных испытаниях. Окситоцин не оказывал влияния на PSAR. Исследование поведения «открытого поля» показало, что вазопрессин значительно снижал только величину дефекации.

ВЛИЯНИЕ ПОВРЕЖДЕНИЯ RAPHE СРЕДНЕГО МОЗГА НА ДНЕВНЫЕ И ВЫЗВАННЫЕ СТРЕССОМ ИЗМЕНЕНИЯ СОДЕРЖАНИЯ СЕРОТОНИНА В ОТДЕЛЬНЫХ ОБЛАСТЯХ ЛИМБИЧЕСКОЙ СИСТЕМЫ И НА ФУНКЦИЮ АДРЕНАЛОВОЙ СИСТЕМЫ У КРЫС

И. ВЕРМЕШ, Г. ТЕЛЕГДИ и К. ЛИШШАК

На животных с повреждением raphe среднего мозга определялся уровень серотонина в среднем мозге, гиппокампе, своде, миндалевидном ядре и гипоталамусе, а также содержание кортикостерона плазмы в 8, 16 и 24 часа.

Последующее электролитическое повреждение ядер raphe среднего мозга привело к исчезновению дневных колебаний содержания серотонина в мозге и кортикостерона плазмы. Уровень серотонина снизился на 50—75% во всех исследованных областях мозга, а уровень кортикостерона плазмы увеличился примерно в три раза, по сравнению с обычным утренним уровнем.

Стрессовая реакция на эфир и электрический шок у животных с повреждением raphe среднего мозга были облегчены и возвращение к исходящему уровню — замедлено. Уровень гипоталамического серотонина был более низким, чем у контрольных, и не изменялся на стресс.

Работа позволяет предположить, что ядра raphe среднего мозга ответственны за ритмические колебания содержания серотонина в лимбической системе, которая находится в реципрокной связи с гипофизарно-надпочечниковой системой.

УРОВЕНЬ ^{3}H-МЕЛАТОНИНА В ЦЕРЕБРОСПИНАЛЬНОЙ ЖИДКОСТИ И СОСУДИСТОМ СПЛЕТЕНИИ ПОСЛЕ ВНУТРИВЕННОГО ПРИМЕНЕНИЯ РАДИОАКТИВНЫХ СОЕДИНЕНИЙ

Б. МЕШШ и Г. П. ТРЕНТИНИ

Взрослым самцам крыс внутривенно вводили ^{3}H-мелатонин и ^{3}H-N-ацетилсеротонин (NAS). В различные интервалы времени от 1 мин до 12 часов измеряли радиоактивность цереброспинальной жидкости (CSF), плазмы, сосудистого сплетения, гипоталамуса и среднего мозга. Была обнаружена высокая активность CSF. Пик активности наступал через 5 мин после инъекции, но значительная активность имела место и через 12 часов. Активность ткани сосудистого сплетения (на 100 мг) была в 3—12 раз выше, чем

остальных тканей мозга. Как показала перфузия мозга физиологическим раствором, высокий уровень радиоактивного мелатонина в сосудистом сплетении не был связан с содержанием крови органа. Подобно мелатонину ^{3}H-NAS имел одинаковую концентрацию в сосудистом сплетении и в CSF.

Делается заключение, что сосудистое сплетение поглощает индоламины из крови и выделяет их в CSF.

АКТИВНОСТЬ CORPUS STRIATUM КОШКИ ВО ВРЕМЯ ЕСТЕСТВЕННОГО СНА; КОРРЕЛЯЦИОННЫЙ АНАЛИЗ

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На 9 кошках в условиях хронического эксперимента проводили авто- и кросс-корреляционный анализ активности сенсомоторной коры (Mc), вентрального заднелатерального ядра (VPL) и вентролатерального ядра (VL) таламуса, каудатум (Cau), бледного шара (GP) и подушки (Pu) во время естественного сна.

Частота веретенообразных волн во время поверхностного и медленно-волнового сна была 12—17 кол/сек в сенсомоторной коре и в двух ядрах таламуса, от 10 до 15 кол/сек в бледном шаре и от 9 до 12 кол/сек в каудатум. Активность подушки характеризовалась довольно стабильной частотой и амплитудой как при бодрствовании, так и во время медленноволновой стадии сна.

Было обнаружено, что в каждой из исследованных структур при всех состояниях медленный и быстрый компоненты активности значительно отличались по их силе и связи. Практически, только в Mc имела место доминантная медленно-волновая активность 2—3 кол/сек. Изменения активности Mc и VPL были однотипными при различных состояниях, в то же время активность VL была похожей на активность первых двух структур а также на активность c. striatum. Во время REM-стадии сна была зарегистрирована идентичная активность VL, Cau, GP и Pu. VPL и VL активности обычно были синхронными с активностями других структур, фазовые отношения обычно были постоянными или изменялись незначительно. Фазовые отношения активностей GP, Cau, и Pu почти всегда были взаимосвязаны и изменялись однонаправленно по мере углубления сна. Наименьшие значения показателя взаимной согласованности активностей различных структур, особенно c. striatum, наблюдались во время SWS-стадии в механизмах сна

Обсуждается возможное функциональное значение веретено-образной волновой активности, медленных волн и базальных ганглиев в сна.

ВЛИЯНИЕ ИНГИБИТОРОВ ХОЛИНЭСТЕРАЗЫ НА ЧУВСТВИТЕЛЬНОСТЬ МЫШЕЙ К ПЕНТЕТРАЗОЛУ

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Физостигмин и параоксон увеличивают чувствительность мышей к пентетразолу в то время как неостигмин уменьшает ее. Малые дозы оксотреморина усиливают действие пентетразола, а большие — тормозят.

Антихолинергические средства атропин и мекамиламин тормозят облегчающее судорожную готовность действие физостигмина. Аналогичным эффектом обладают ингибиторы аминоксидазы ниаламид и транилципромин.

ЭФФЕКТ ДРОПЕРИДОЛА НА ЭЛЕКТРИЧЕСКУЮ АКТИВНОСТЬ КЛЕТОК ПРЕДСЕРДИЯ КРОЛИКА

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Электрическая активность ткани правого предсердия кролика зарегистрировалась внутриклеточными электродами при действии нейролептического агента дроперидол. Было установлено, что продолжительность потенциала действия, dV/dt, сокращалась, частота разрядов снижалась и сердце останавливалось после длительного влияния вещества. Ампли-

туда диастолической деполяризации и «overshoot» уменьшались в скрытых «pacemaker» клетках. Противоположно, в настоящих «pacemaker» клетках амплитуда повышалась, и соединение не оказывало влияния на их автоматизм.

Результаты показывают, что высокая концентрация или длительное влияние дроперидола вызывает блокад импульсов, генерированных в синусном узле.

ДЕПОЛЯРИЗУЮЩЕЕ ВЛИЯНИЕ ВЕРАТРИНА НА СКЕЛЕТНУЮ МЫШЦУ

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Механизм деполяризующего действия вератрина исследовался на портняжной мышце лягушки.

1. После удаления внеклеточных ионов хлорида вератрин продолжал оказывать деполяризующее действие на мышцу.

2. После удаления внеклеточного калия 0,1 мМ вератрина не вызывал деполяризации в течение 2—3 часов наблюдения, а если вызывал, то только в течение значительно более долгого периода времени, чем в растворе Рингера, содержащем 2,5 мМ калия.

3. Деполяризующее действие 0,1 мМ вератрина не проявлялось в гипертонических растворах различного состава (обычный раствор Рингера + 150 мМ NaCl; обычный раствор Рингера + 300 мМ сахарозы; обычный раствор Рингера + 300 мМ глюкозы) несмотря на то, что в этих условиях функция генерации спайков не нарушалась.

4. Единичный электрический стимул, примененный в соответствующую фазу латентного периода между аппликацией вератрина и развитием деполяризации мог послужить триггером появления вератриновой деполяризации.

ДЕЙСТВИЕ И СРОДСТВО К ХОЛИНОРЕЦЕПТОРАМ СКЕЛЕТНЫХ МЫЩЦ СОЕДИНЕНИЙ ПОЛИМЕТИЛЕНА-БИС-ТРИМЕТИЛАММОНИУМА

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1. На прямой мышце живота лягушки исследовали действие аналогов полиметилена-бис-триметиламмониума (ВТМ), содержащих от 3 до 20 метиленовых групп между двумя атомами азота. Определяли тип действия, активность и константу сродства (K_a) соединений.

2. Была установлена связь между типом действия соединений на скелетную мышцу и между константой сродства к ацетилхолиновым рецепторам. Соединения с низким значением K_a (ВТМ-4—ВТМ-6; $K_a = 3{,}3—10 \times 10^3$) оказались слабыми антагонистами, соединения со средним значением K_a (ВТМ-7—ВТМ-14; $K_a = 3{,}3 \times 10^4 — 2{,}1 \times 10^6$) были частичными агонистами, а соединения с высоким значением K_a (ВТМ15—ВТМ-20; $K_a = 6{,}7 \times 10^6 — 2{,}9 \times 10^7$) действовали, как строгие антагонисты. Константу сродства агониста ВТМ-3 не определяли.

3. Результаты показали, что любое соединение ВТМ, которое достаточно прочно связывается с рецептором вызывает ацетилхолиноподобный эффект. Другими словами, константа диссоциации комплекса агонист-рецептор должна быть близка к оптимуму. Соединения, легко образующие комплекс вещество-рецептор являются строгими антагонистами, в то время как соединения со слабым сродством — слабые антагонисты.

4. Обсуждается механизм, посредством которого константа сродства изменяет тип действия соединения на ацетилхолиновый рецептор.

ЭСТРОГЕНСОДЕРЖАЩИЕ ОРАЛЬНЫЕ КОНТРАЦЕПТИВЫ И АКТИВНОСТЬ АНТИТРОМБИНА III

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Было исследовано 67 женщин, принимавших орально контрацептивы различного состава, и полученные данные сравнивали с данными здоровых женщин. Эстрогенсодержащие контрацептивы вызывали снижение антитромбина III (АТ III). Такое снижение

более часто встречалось у женщин, принимавших пилюли с высоким содержанием эстрогена (100 мкг) и было более последовательным если для анализа использовали сыворотку крови в присутствии гепарина. Это явление может быть использовано как демонстрационный метод, вскрывающий тенденцию к снижению активности AT III. Само по себе снижение AT III активности не обязательно обозначает тромбоэмболическую болезнь, но в присутствии других предрасполагающих факторов может послужить в качестве одной из важных причин тромбоза.

КОЛИЧЕСТВЕННАЯ РЕГИСТРАЦИЯ ВРАЩЕНИЯ, ВЫЗВАННОГО ИЛИ ПОТЕНЦИРОВАННОГО АМФЕТАМИНОМ У КРЫС ПОСЛЕ ОДНОСТОРОННЕГО ПОВРЕЖДЕНИЯ МОЗГА

И. САБО и Л. НЕМЕТ

Описан аппарат для количественной регистрации поведения вращения у крыс, вызванного лекарственными веществами или электрической стимуляцией мозга. Аппарат дифференцирует направление ротации и осуществляет полную регистрацию поведения вращения, что устраняет необходимость визуального наблюдения. Его электронная часть состоит из логических схем, выпускаемых промышленностью.

ОБЛЕГЧЕНИЕ НИКОТИНОМ РЕФЛЕКСА ИЗБЕГАНИЯ: ФУНКЦИЯ ИНДИВИДУАЛЬНОЙ СПОСОБНОСТИ К УЧЕНИЮ

Й. И. СЕКЕЙ, Й. БОРШИ и И. КИРАЙ

На крысах с выработанным в специальной клетке (Shuttle-box) с автоматическим управлением избегательным рефлексом исследовался вопрос влияния введения никотина на протекание обуславливания. Перед введением никотина животные были разделены на хорошо и плохо обучавшиеся подгруппы по результатам первого дня тренировки. Никотин в дозе 0,05 мг/кг не оказал значительного влияния. В то же время такие дозы препарата, как 0,10—0,20 мг/кг облегчали появление условнорефлекторных ответов в группе хорошо обучавшихся животных.

САМОРАЗДРАЖЕНИЕ И АДРЕНОКОРТИКАЛЬНАЯ АКТИВНОСТЬ У МОЛОДЫХ КРЫС

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Изучены ответ на стресс и самораздражение у крыс в возрасте от 12 до 17 дней. Стрессоры, как введенный внутрибрюшинно гистамин (4 мг/кг веса тела), или электрический удар на ноги, через 20 мин повышали уровень кортикостерона в плазме у крыс, старше 15 дней. Самораздражение можно было помучить биполярными электродами в медиальном пучке переднего мозга (MFB) только с 14—15-ого дня жизни. 20-минутное самораздражение у крыс в возрасте от 15 до 17 дней, и 20-минутная пассивная электрическая стимуляция MFB с подобными импульсными параметрами, значительно повышали уровень кортикостерона в плазме у животных в возрасте от 12 до 14 дней. Так как, как ответ на стресс, так и самораздражение, появлялись в том же возрасте, авторы предполагают, что мозговые структуры, и, главным образом, связи между стволом мозга и гипоталамусом, включенные в организацию стрессового ответа и самораздражения, частично те же, или они показывают параллельное постнатальное развитие. Прямое электрическое раздражение гипоталамических структур может активировать гипоталамо-гипофизарно-адреналовую систему и в возрасте от 12 до 14 дней.

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