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G. ÁDÁM, P. BÁLINT, SZ. DONHOFFER, E. ERNST, B. ISSEKUTZ SEN.
L. KESZTYŰS, J. KNOLL, F. OBÁL, J. PÓRSZÁSZ

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## TIME AS A FACTOR IN METABOLIC STUDIES OF PEROMYSCUS

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Oxygen consumption of individual deer mice was recorded hourly for a continuous period of about 50 hours in a closed system maintained in constant darkness and at thermoneutrality. The oxygen consumption showed a marked circadian rhythm which reflected the dark-light cycle to which the mice were exposed prior to the experiment. Regardless of the time of day the experiment started, handling of the animal and the novelty of the metabolic chamber resulted in a high initial metabolic rate during the first 4—5 hours, but did not seem to affect the circadian clock. The data indicate that duration of fasting, length of experiment and the phase of the circadian cycle are all important factors which must be standardized if reproducible reference values of oxygen consumption are to be obtained. Since fasting combined with introduction into a new environment tends to increase activity, it is suggested that the period of adjustment to the chamber and the period of fasting be combined and determination be made during the $\varrho$-time, 24 hours after the mouse has been placed into the respiratory chamber.

The energy metabolism of an animal reflects the overall effect of many environmental factors and physiological variables. When the numerical values of these factors and variables are identical, the metabolic rates will also be the same. The converse, however, is not necessarily true, i.e., identical metabolic rates do not necessarily mean identical configurations of the variables and factors involved. Therefore the definition of a reference or standard level of metabolism is essentially a definition of a given configuration of factors and variables determining the oxygen consumption. The reproducibility of such a standard level depends on how precisely a chosen configuration can be realized in practice. Environmental factors, such as ambient temperature, light, partial pressure of oxygen, etc., are relatively easy to keep at a constant level, whereas physiological variables, such as locomotor activity or body temperature are more difficult to control. These variables depend on the animal and undergo

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circadian variations (ASCHOFF 1965; ASCHOFF and POHL 1970; HALBERG et al. 1958; HALBERG 1960). Therefore, identical configurations are most likely to occur only at certain hours of the day, with time becoming an important parameter in metabolic studies (HEUSNER 1963; KAYSER and HEUSNER 1967). Relatively little attention has been paid to such factors as the hour of the day at which measurements are made, the minimum duration an experiment should be run, and how long an animal should be fasted. Little consideration also has been given to the question whether the experimentation itself is a significant factor influencing the subsequent course of the oxygen consumption, and how long such a possible influence might last. The objective of this investigation is to determine the most suitable phase of the circadian rhythm for metabolic measurements in *Peromyscus*, the optimum duration of fasting and the minimum length of an experiment in order to obtain reproducible reference values which lend themselves to intraspecific as well as interspecific comparisons.

## Methods

Twenty-four adult male deer mice, *Peromyscus maniculatus sonoriensis*, were used in this study. Body weight ranged from 17 to 40 g and age from 6 to 18 months. These mice were born and raised at $26 \pm 1$°C and subjected to a 12 : 12 hour dark-light cycle, with light on from 6:00 to 18:00 hr. Food (Simonsen's white diet) and water were available ad libitum.

During the experiments the mice were placed in constant darkness in their thermoneutral zone, which during our preliminary trials, was found to range from 30 to 33°C; these temperature limits are similar to those published for this species (MCNAB and MORRISON 1963; MORRISON 1948; MURIE 1961; ROBERTS et al. 1966). Only one group of three mice had free access to food during the measurements, but the remaining 21 mice were deprived of food at the beginning of the experiments. Water was available to all animals in the respiratory chamber.

The oxygen consumption of each deer mouse was measured individually during one continuous period of about 50 hours. To allow for temperature equilibration of the metabolic apparatus, the recording started one hour after the deer mouse had been introduced into the respiratory chamber. Subsequently, the volume of oxygen consumed every hour was monitored with a closed system apparatus (HEUSNER et al. 1970). In order to provide the mouse with maximum running space compatible with the precision of the measurement, a ringshaped respiratory chamber was used, in which the mouse was free to run in a circular track 50 mm wide and 50 mm high on a screen floor which was elevated to keep the mouse dry. The $CO_2$ absorbant was located in the center of the respiratory chamber, thus minimizing the diffusion distance of the $CO_2$. The oxygen consumption has been expressed in ml s.t.p.d. per hour. The precision of a single measurement is 3%. The mice were tested at random and were separated into groups with respect to body weight only for purposes of statistical analysis. Most experiments were performed in June and July.

## Results

Fig. 1 shows the course during two consecutive days of the computed average hourly oxygen consumption and its standard deviation for two groups of deer mice of similar weight. Group 1 (upper curve) consists of six mice, the average weight of which was $18.5 \pm 1.1$ g (S.D.), placed into the respiratory chamber at 10:00 hr but on different days; Group 2 (lower curve) was com-

prised of 8 mice (20.6±1.3 g) put into the respiratory chamber at 17:00 hr on different days. In both groups the oxygen consumption displays a striking daily variation, reflecting the pattern of activity induced by the dark–light

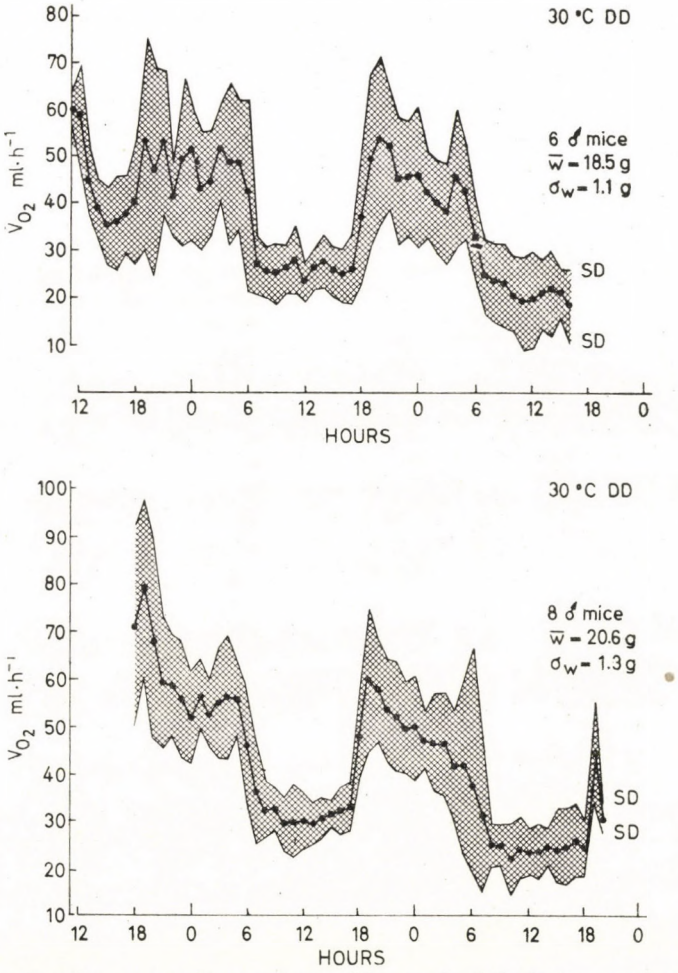


*Fig. 1.* The mean hourly oxygen consumption and its standard deviation as a function of time. The upper curve represents experiments started at 10:00 hr, the lower curve, experiments started at 17:00 hr

cycle to which the mice were exposed before the experiment. After midnight (0:00 hr) following the beginning of the experiment the two curves are very comparable, demonstrating a synchronous variation of the oxygen consumption in both groups.

From 6:00 to 18:00 hr, or $\varrho$-time for this group of mice according to the nomenclature of ASCHOFF and POHL (1970), the oxygen consumption

(ϱ-level) is low and its confidence interval small. The ϱ-time for individual mice may vary a few hours in either direction from the indicated hours. From 18:00 to 6:00 hr or α-time, the oxygen consumption (α-level) is about twice as high and its confidence interval large.

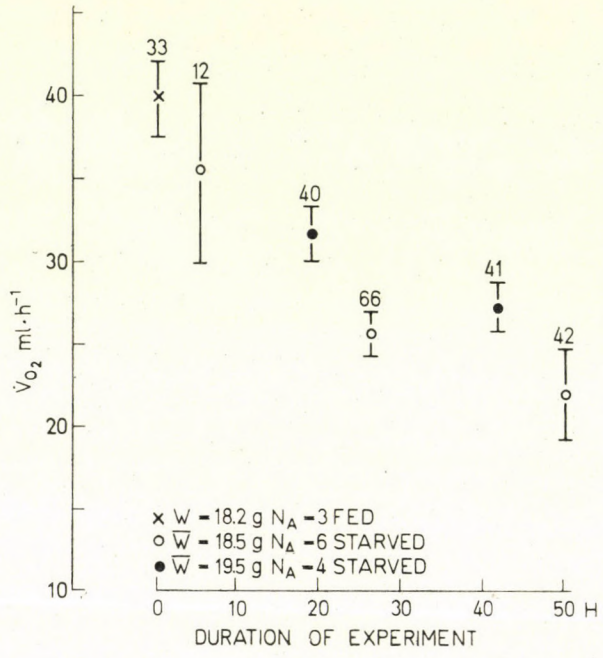


*Fig. 2.* The ϱ-oxygen consumption as a function of the duration of fasting (duration of the experiment); confidence interval (95%) as well as number of determinations are shown

In both groups the oxygen consumption is maximal at the beginning of the experiment, regardless of the hour at which the measurements begin, and then decreases during the following four to five hours until it reaches the ϱ-level in Group 1 and the α-level in Group 2. The handling of the mouse and the novelty of the new environment in the metabolic apparatus increases the oxygen consumption by 70% above the ϱ-level in Group 1 and 45% above the α-level in Group 2. The absolute increase of the hourly oxygen consumption is about the same in both cases, the difference in the relative increase in the two groups being due mainly to the difference between the ϱ and α-levels. Since the daily variations in both groups are synchronous the next day, there is no evidence of a resetting or phase shift of the circadian rhythm. Therefore the effect of the handling and the environmental change on the oxygen consumption is limited to 4—5 hours following the beginning of the experiment and does not seem to affect the clock itself. Very similar results have been obtained with each of 3 mice put into the respiratory chamber at 14:00 hr.

**Table I**

*ϱ-level oxygen consumption of a fasting deer mouse measured for a period of three consecutive days*

| | Body weight | $\varrho$ $O_2$ ml/h | Standard error | N* | t |
|---|---|---|---|---|---|
| Day 0 | 36.2 | 62.1 | 1.63 | 4 | — |
| Day 1 | — | 51.4 | 1.40 | 11 | 4.48 |
| Day 2 | — | 44.0 | 1.13 | 11 | 3.88 |
| Day 3 | 29.9 | 39.3 | 0.97 | 3** | 2.79 |

* N represents also the duration of the ϱ-time in hours
** This short ϱ-time is due to the fact that the experiment was stopped

The increased oxygen consumption at the beginning of the experiment has been observed individually on more than 50 mice, both males and females, during previous metabolic studies.

Examination of Fig. 1 also reveals that the ϱ-level of oxygen consumption during the last day is lower than that recorded the day before. This difference is highly significant in both groups, as demonstrated by Fisher's *t*-test: Group 1, $t = 2.77$, $N = 108$; Group 2, $t = 7.29$, $N = 162$.

Fig. 2 shows the relationship between the duration of the experiment and the ϱ-level. In order to minimize the effect of differences in body weight, only data on deer mice of comparable body weight were used to draw this graph. This is why a subgroup of 4 mice (solid circles) has been formed out of Group 2. Each point represents the average oxygen consumption of a group of deer mice during their ϱ-time. In addition the confidence interval of the mean and the number of values (hourly oxygen consumption) are shown. The 0 marks the beginning of the experiment and the time of a given ϱ-level corresponds to the hour around which the ϱ-time is centered. The ϱ-level plotted at time 0 (x) was obtained from three mice which had access to food during the experiment. In order to avoid the effect observed at the beginning of each experiment, the ϱ-level recorded the following day has been considered. This value obtained with fed mice is an estimate of the ϱ-level of deer mice of comparable body weight at the beginning of fasting, and therefore has been plotted at time 0.

The two groups of fasted deer mice considered in this graph differ with respect to the hour the experiment began: time 0 corresponds to 10:00 hr in Group 1 (open circles) and to 17:00 hr in Subgroup 2 (solid circles). Throughout the experiment, the ϱ-level decreases in the fasted deer mice. However, during the ϱ-time of a given day, the ϱ-level stays remarkably constant with a decrease occurring only one circadian period later (see Fig. 1). This indicates that the decrease, which proceeds by steps, is related to the circadian rhythm. This

phenomenon is not a statistical artifact due to the averaging of values from different deer mice, but can be observed on almost all the individual recordings. This is illustrated by the following data obtained from a fasted deer mouse tested for three days (Table I). All the differences between $\varrho$-levels of successive days are significant ($p = 0.02$), but the decrease in the $\varrho$-level is largest between day 0 and day 1. This phenomenon is also illustrated in Fig. 2.

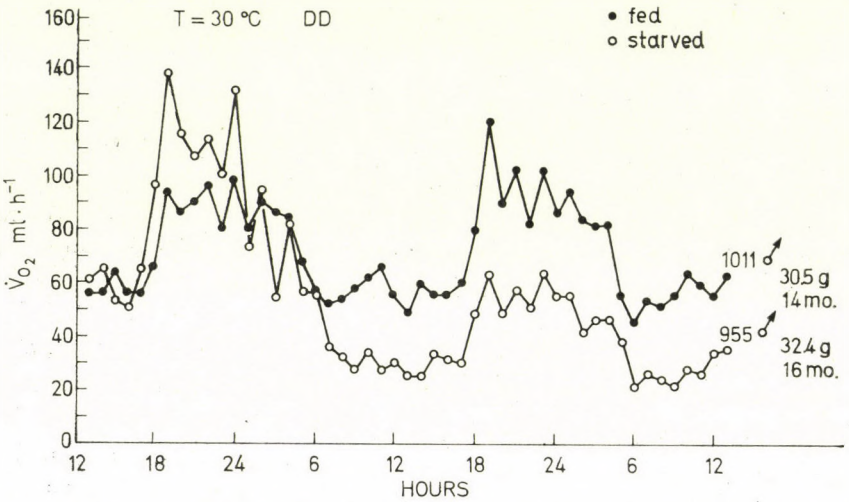


*Fig. 3.* The hourly oxygen consumption of a fed and fasting deer mouse as a function of time

During the 3-day experiment summarized in Table I the deer mouse lost 17.4% of its body weight but its $\varrho$-level dropped by 36.6%. Similarly, in group 1, body weight loss amounted to 12.43% ($t = 3.4$, $N = 12$) and in group 2 to 14.6% ($t = 4.43$, $N = 16$). In both groups the change in $\varrho$-level, about 45% as estimated from Fig. 2, is greater than what one might predict from the loss in body weight alone.

Fig. 3 shows two 48-hour recordings of oxygen consumption, one obtained on a deer mouse provided with food throughout the entire experiment (#1011) and the other on an animal deprived of food from the beginning of the run (#955). After the two mice have been adjusted to the respiratory chamber (the preliminary adjustment period not shown on this graph) their metabolic levels ($\varrho$-level) are closely similar at the beginning of the recording, but differ sharply in all the subsequent measurements. During the first night ($\alpha$-time) the oxygen consumption of the fasted mouse is significantly higher than that of the fed mouse. This demonstrates that the general activity of the hungry deer mouse is enhanced during this $\alpha$-phase of the cycle. After 6:00 hr the following morning and throughout the rest of the experiment, the oxygen consumption

of the fasted mouse is then consistently lower than the one of the fed mouse. Since body weight of the fasted deer mouse was sligthly higher than that of the fed mouse, the lower oxygen consumption cannot be ascribed to body weight. The course of the oxygen consumption in the fasted mouse shows clearly that prolonged fasting does not increase the general activity, the initial increase in activity being limited to the first night. This is probably due to an increase in

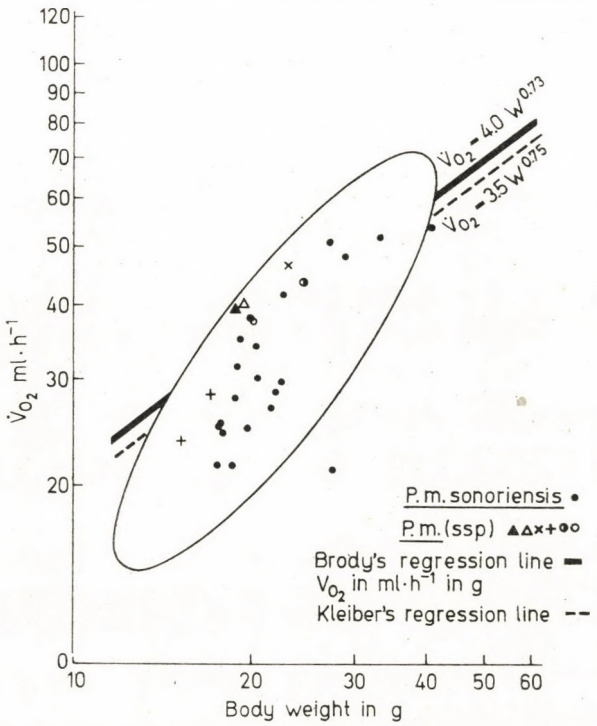


*Fig. 4.* Bivariate scatter diagram of logarithm of the $\varrho$-oxygen consumption recorded after 24 hours of fasting and logarithm of body weight. The different symbols represent basal metabolism of the following subspecies of *Peromyscus maniculatus: artemisiae* (×, 10), *austerus* (△, 10), *nebrascensis* (▲, 5), *oreas* (○, 5) (HAYWARD 1965) and *gracilis* (+, 1) (MORRISON 1948). The figure after the symbol indicates the number of animals for which the average was taken

the exploratory activity, the hungry mouse being in search of food. Fig. 3 as well as Fig. 1 show that the circadian rhythm persists even in the absence of food.

Fig. 4 shows the relationship between body weight and the $\varrho$-level recorded on day 1, for experiments begun on day 0. The logarithms of the $\varrho$-values have been plotted against the logarithms of body weight (solid circles). An estimate of body weight after the first 24 hours of fasting was obtained by averaging the initial and final body weight. The validity of this procedure has been tested

by weighing fasting deer mice at regular time intervals and comparing the interpolated values to the measured ones. The correlation between body weight and $\varrho$-values is highly significant: $r = 0.73$, $N = 21$, $p > 0.01$, though only 53% of the total variance of oxygen consumption is due to body weight. The weight exponent is 0.93; its confidence interval $0.51 > b > 1.35$ ($p > 0.05$) includes the 2/3 weight exponent as well as BRODY's (1945) and KLEIBER's (1961) interspecific weight exponents.

The ellipse plotted in Fig. 4 corresponds to a bivariate confidence interval within which the symbol representing the body weight and $\varrho$-value of a new deer mouse may be expected to fall ($p = 0.05$). This ellipse was computed by a procedure derived from Hotelling's $T^2$-test, the multidimensional generalization of Student's "*t*" (ANDERSON 1958; JOLICOEUR and MOSIMANN 1960; JOLICOEUR and HEUSNER 1970; MORRISON 1967).

In order to compare the $\varrho$-values with the values of basal metabolism of the deer mouse as reported in the literature, these data were also plotted on Fig. 4. The different symbols represent basal metabolism of the following subspecies of *Peromyscus maniculatus: artemisiae* (×, 10), *austerus* (△, 10), *nebrascensis* (▲, 5), *oreas* (○, 5) (HAYWARD 1965) and *gracilis* (+, 1) (MORRISON 1948). The figure after the symbol indicates the number of animals for which the average was taken. As shown in Fig. 3 the ellipse of the $\varrho$-values contains the values of basal metabolism reported in the literature for this species. Since the values from the literature are mean values and no standard deviation or standard error is given, it is not possible to make a more precise comparison.

Fig. 4 also show BRODY's (1945) and KLEIBER's (1961) interspecific regression lines between the logarithm of body weight and basal oxygen consumption. With respect to these lines the $\varrho$-values are distributed as follows: the $\varrho$-values of deer mice whose body weight is greater than 20 g are within the range of prediction of both interspecific regression lines, however, the $\varrho$-values of smaller deer mice are consistently lower.

## Discussion

The foregoing data demonstrate that the duration of fasting, the duration of the experiment itself and the phase of the circadian cycle of the mouse during which the determinations are made, are important factors which should be standardized in order to obtain reference values of oxygen consumption, which are reproducible within a small range of variation.

The length of fasting determines the $\varrho$-level, but food deprivation leads to increased restlessness (HITCHCOCK 1928; FINGER 1951; THOMPSON 1953; CAMPBELL and SHEFFIELD 1953; FEHRER 1956; ALDERSTEIN and FEHRER 1955; ZIMBARDO and MONTGOMERY 1957) with activity becoming then a disturbing

factor for the study of rest metabolism. The more pronounced fall in the ϱ-level during the first 24 hours of fasting makes this period less desirable for basal (reference or standard) metabolic studies. During this period the combined effect of loss in body weight and the disappearing caloric effect of food explain the decline in the ϱ-level. Periods of fasting longer than 24 hours will furthermore reduce the ϱ-metabolic level. Since the reduction cannot be entirely attributed to the loss in body weight, another variable must come into play. Several lines of evidence point to body temperature as being the factor involved: First, in fasting mice the body temperature is lower than in fed ones (CHEVILLARD 1935). Second, the amplitude of the daily rhythm of body temperature increases, the ϱ-value of temperature being markedly lower in fasted mice (HALBERG et al. 1965). Third, the oxygen consumption of small deer mice, consistently lower than predicted from interspecific regression lines, demonstrates their tendency to become hypothermic after 24 hours of fasting. This tendency also explains the relatively high intraspecific weight exponent found in this species.

The duration of the experiment itself has to be determined so that the deer mice are given time enough to adjust to the novelty of the respiratory chamber before the measurements are made. Since deprivation of food enhances the activity if the animal is subjected to an environmental change, the probability that the mouse remains quiet during the hours after it has been placed into the respiratory chamber is very low. On the other hand the exploratory activity in a restricted unfamiliar situation declines as a function of time (THOMPSON 1953) and prolonged starvation in a familiar environment does not increase activity, as shown by our data and by MONTGOMERY (1953). Under these circumstances the most practical solution is to place the fed mouse during the ϱ-time of the daily cycle into the respiratory chamber, so that the animal is fasting during the period of adjustment to the new environment. Proceeding in this way the deer mouse remains very quiet during the ϱ-time of the following daily cycle and activity is no longer a disturbing factor for the study of rest metabolism. The ϱ-values of oxygen consumption, obtained by dividing the total amount of oxygen consumed during the entire ϱ-time by the ϱ-time are not true rest-metabolic values but comparable to the energy metabolism at minimal levels of spontanenous muscular activity as defined by BRAMANTE (1968). The question arises then whether the ϱ-values will be accepted as representing a useful base line for metabolic studies. The answer to this question raises some problems and demands some qualifications. In the deer mouse there is no significant difference between the ϱ-values and basal metabolism, provided the ϱ-values are measured after 24 hours of fasting, in animals placed at thermoneutrality and given time to adapt to the new environment of the respiratory chamber. Under these circumstances the lowest value recorded during the same ϱ-time is still included in the ellipse of equal probability com-

puted for the average oxygen consumption during the ϱ-time. On all the individual recordings it is quite obvious that the ϱ-level represents the minimum steady state metabolism which can be recorded within a circadian period. No attempt was made to measure the activity of the deer mouse, nor to restrain its movements. On the contrary, the deer mouse was given maximum running space and time to adapt to the new environment. In this way the difficulties due to the animal's activity during measurements (KLEIBER 1961) can be overcome. Admittedly there still remain doubts as to whether some activity occurs during the measurement of the minimum oxygen consumption. However, previous research on basal metabolism in rats involving simultaneous recording of oxygen consumption and activity (BRAMANTE 1968; HEUSNER 1963), has shown that the minimum hourly oxygen consumption recorded in 24 hours does not seriously differ from the true resting oxygen consumption.

## Acknowledgement

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# EFFECT OF PHYSOSTIGMINE ON SODIUM EFFLUX OF FROG MUSCLE

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1. In normal Ringer's solution, 25% of the efflux of labelled sodium from sodium loaded muscle is blocked by physostigmine.

2. The physostigmine-sensitive component of the sodium efflux is markedly increased if the sodium in the external medium is replaced by lithium or choline. In half sodium Ringer the physostigmine-sensitive part of the sodium efflux accounts for about 45% of the total; in sodium-free Ringer about 60% of the sodium efflux is physostigmine-sensitive.

3. The inhibitory effect of physostigmine on sodium efflux depends on the fibre sodium concentration. If the internal sodium level of muscle is less than 10 mM, the total sodium efflux is physostigmine-insensitive.

4. The inhibitory effect of physostigmine on sodium efflux from loaded muscle resembles in many respects that of ouabain. Physostigmine failed to affect the ouabain-insensitive exchange diffusion component of sodium efflux, whereas it can block the ouabain-sensitive net transport.

For more than two decades it has been known that physostigmine as well as other cholinesterase inhibitors have some effect upon ion transport in peripheral nerve fibres (BULLOCK et al. 1946, 1947; WRIGHT 1956), in the giant axon (ROTHENBERG 1950), in red blood cells (TAYLOR et al. 1952), and in frog skin (KIRSCHNER 1953). As to the skeletal muscle, VAN DER KLOOT (1956, 1958) was the first, who demonstrated that the net loss of intracellular sodium from the frog muscle soaked in an isotonic Ringer's solution containing 55 mM of NaCl could be blocked by physostigmine or hexaethyl pyrophosphate. As these compounds inhibited ethyl chloroacetate hydrolysis in the muscle cell, it was concluded that some connection might exist between the activity of the sodium pump and that of the intracellular cholinesterase in muscle fibres.

It has been shown previously that physostigmine reduced both the net loss and gain of sodium in isolated frog muscle (KOVÁCS and SZABÓ 1966) and it was supposed that the exchange diffusion was hindered by physostigmine. On the other hand VARGA and HOROWICZ (1963) in tracer experiments on the frog sartorius presented evidence suggesting that potassium influx and efflux were inhibited and chloride permeability was enhanced by physostigmine under steady state conditions.

The aim of the present experiments was to obtain data on the effect of physostigmine exerted on the efflux of $^{24}Na$ under various conditions. The

sodium efflux of muscle fibre consists of two components (KEYNES and STEINHARDT 1968). One of them is an exchange diffusion component, reduced upon transfer of a muscle to Na-free medium, saturating at relatively low internal sodium concentration, the magnitude of the exchange diffusion flux is unaffected by changes in external potassium concentration. The second component described as a net transport or sodium pump is saturated only at a much higher internal concentration of sodium, increased in Na-free medium and only this component is sensitive to external potassium and can be blocked by ouabain.

The present experiments were carried out on sodium-loaded muscles, to study the dependence of the inhibitory effect of physostigmine on internal and external concentration of sodium.

## Methods

Frog sartorius muscles from *Rana esculenta* were used. The muscles were dissected and threads were tied to the tendons at each end. The threads served to tie the muscles to hooks of glass rods to maintain the muscles at resting length. Then the muscles were immersed in 10 ml of K-free Ringer (Table I) and held at 2—3°C from 12 to 16 hours. At the end of this loading period they were placed in K-free Ringer containing $^{24}Na$ for 2 to 3 hours at 20°C. Subsequently, the muscles were soaked for carefully timed intervals in a series of 10 ml samples of various inactive solutions in numbered test tubes which were chosen to fit into a Gamma well-type crystal scintillation detector. Transfer of muscles through the various experimental solutions could be effected by moving the muscles and glass rods as a unit and to ensure proper exposure to the solutions they were raised and lowered by motor every five seconds. At the end of the experiment the muscles were disintegrated in 0.5 ml of 50% nitric acid and made up to 10 ml for determination of their residual radioactivity.

The method adopted for calculation of results and to plot rate constants against time was similar to that described by CALDWELL and KEYNES (1969).

The composition of the experimental solutions is summarized in Table I. The radioactive isotope $^{24}Na$ was supplied by the Isotope Institute of the Hungarian Academy of Sciences as the chloride salt in acid solution and, after neutralization, was added to Ringer's solution, the total sodium concentration having been adjusted to 120 mM. Physostigmine sulphate (BDH) was dissolved in inactive Ringer's solutions. The experiments were carried out at room temperature of 20—22°C.

**Table I**

*Composition of solutions**

| Designation | NaCl | LiCl | Choline Cl | KCl | $CaCl_2$ | $NaH_2PO_4$ | $Na_2HPO_4$ | TRIS—$PO_4$ | Sucrose |
|---|---|---|---|---|---|---|---|---|---|
| | mM/litre solution | | | | | | | | |
| Normal Ringer | 115.0 | — | — | 2.5 | 1.8 | 0.85 | 2.1 | — | — |
| Half Na-Ringer | 57.5 | — | — | 2.5 | 1.8 | 0.85 | 2.1 | — | 115.0 |
| K-free Ringer | 117.5 | — | — | — | 1.8 | 0.85 | 2.1 | — | — |
| Lithium Ringer | — | 120.0 | — | 2.5 | 1.8 | — | — | 5.0 | — |
| Choline Ringer | — | — | 120.0 | 2.5 | 1.8 | — | — | 5.0 | — |

* The pH of all solutions was 7.2

## Results

### *Effect of physostigmine on $^{24}Na$ efflux in normal Ringer*

In the first group of experiments the effect of physostigmine on sodium efflux was observed in Ringer's solution at a sodium concentration of 120 mM as illustrated in Fig. 1. The rate coefficient curve reveals that after the washout

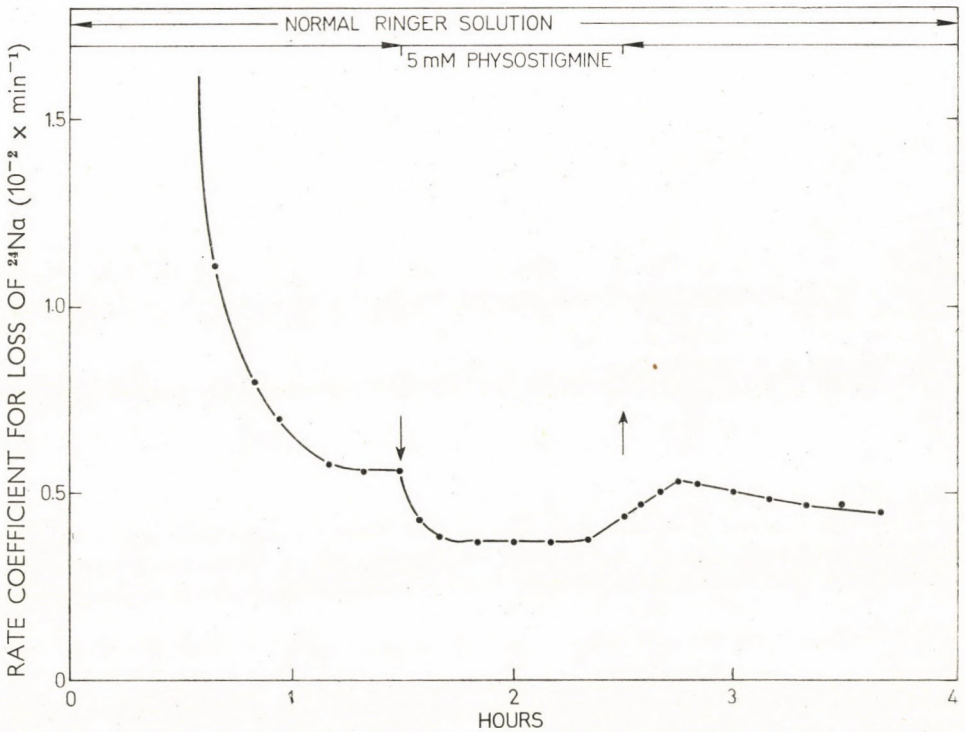


*Fig. 1.* Time course of the decline of the rate coefficient of an individual muscle in normal Ringer's solutions. The muscle was kept for 17 hrs in inactive K-free Ringer at 2°C, then 3 hours in K-free $^{24}Na$—Ringer at room temperature. The rate coefficients from 35 minutes to 220 minutes were plotted. At 130 minutes the addition of 5 mM physostigmine sulphate caused a sudden marked decrease in the rate coefficient, and this persisted in the continued presence of physostigmine. Experiment C-05

of $^{24}Na$ from the extracellular space, sodium efflux reached a relatively steady value. In the Ringer's solution containing physostigmine the rate coefficient was rapidly reduced and a value of 0.0037 $min^{-1}$ was found in 30th minute of treatment in comparison with the mean value of rate coefficients (0.0052 $min^{-1}$) measured before and after the treatment (in 80th and 190th min of experiment). Thus physostigmine inhibited the $^{24}Na$ efflux by 30%. Table II summarizes all the measurements made under similar conditions and confirms

that in Ringer's solution containing $5 \times 10^{-3}$ M of physostigmine the mean $^{24}Na$ efflux from the loaded muscle was reduced by 25%. Physostigmine at lower concentrations (e.g. $10^{-3}$ M) had a weaker inhibitory effect, the efflux was cut down by less than 20%. No correlation was found between the degree of inhibition and the period in which physostigmine was applied.

**Table II**

*Effect of $5 \cdot 10^{-3}$ molar physostigmine on sodium efflux in normal Ringer*

| Experiment reference | Time of exposure to physostigmine | Rate coefficient for $^{24}Na$ loss ($min^{-1}$) | | | Rate coefficient ratio $\frac{K'}{K_m}$ * |
|---|---|---|---|---|---|
| | | in Ringer before physostigmine ($k_1$) | in Ringer with physostigmine ($K'$) | in Ringer after physostigmine ($k_2$) | |
| I—10 | 50— 80 | 0.0078 (40)** | 0.0044 | 0.0045 (120) | 0.72 |
| I—12 | 50— 80 | 0.0086 (40) | 0.0052 | 0.0067 (110) | 0.68 |
| | | | | Mean ± S.E. | 0.69±0.06 |
| K—01 | 70—100 | 0.0106 (60) | 0.0075 | 0.0098 (140) | 0.73 |
| K—02 | 70—100 | 0.0178 (60) | 0.0131 | 0.0140 (150) | 0.79 |
| | | | | Mean ± S.E. | 0.76±0.03 |
| C—04 | 90—150 | 0.0079 (80) | 0.0056 | 0.0073 (190) | 0.73 |
| C—05 | 90—150 | 0.0056 (80) | 0.0037 | 0.0047 (190) | 0.71 |
| C—06 | 90—150 | 0.0051 (80) | 0.0037 | 0.0049 (190) | 0.74 |
| | | | | Mean ± S.E. | 0.72±0.03 |
| K—11 | 150—210 | 0.0107 (140) | 0.0083 | 0.0099 (230) | 0.80 |
| K—12 | 150—210 | 0.0033 (140) | 0.0019 | 0.0022 (240) | 0.69 |
| K—07 | 150—210 | 0.0106 (140) | 0.0077 | 0.0102 (250) | 0.74 |
| K—08 | 150—210 | 0.0174 (130) | 0.0130 | 0.0154 (250) | 0.79 |
| | | | | Mean ± S.E. | 0.75±0.04 |
| K—03 | 180—240 | 0.0139 (170) | 0.0094 | 0.0120 (290) | 0.72 |
| K—04 | 180—240 | 0.0103 (160) | 0.0077 | 0.0094 (290) | 0.78 |
| | | | | Mean ± S.E. | 0.75±0.03 |

* The average of determinations before and after physostigmine treatment was used for calculating the ratio,

$$K_m = \frac{k_1 + k_2}{2}$$

** In brackets, duration (in minutes) of the period for which the rate coefficient was determined

### *Inhibition of $^{24}Na$ efflux by physostigmine in a medium of 55 mM Na*

If half of the NaCl is replaced by an amount of sucrose appropriate to maintain tonicity in external medium, an increase of $^{24}Na$ efflux from sodium loaded muscles will take place. When the muscle was transferred to normal

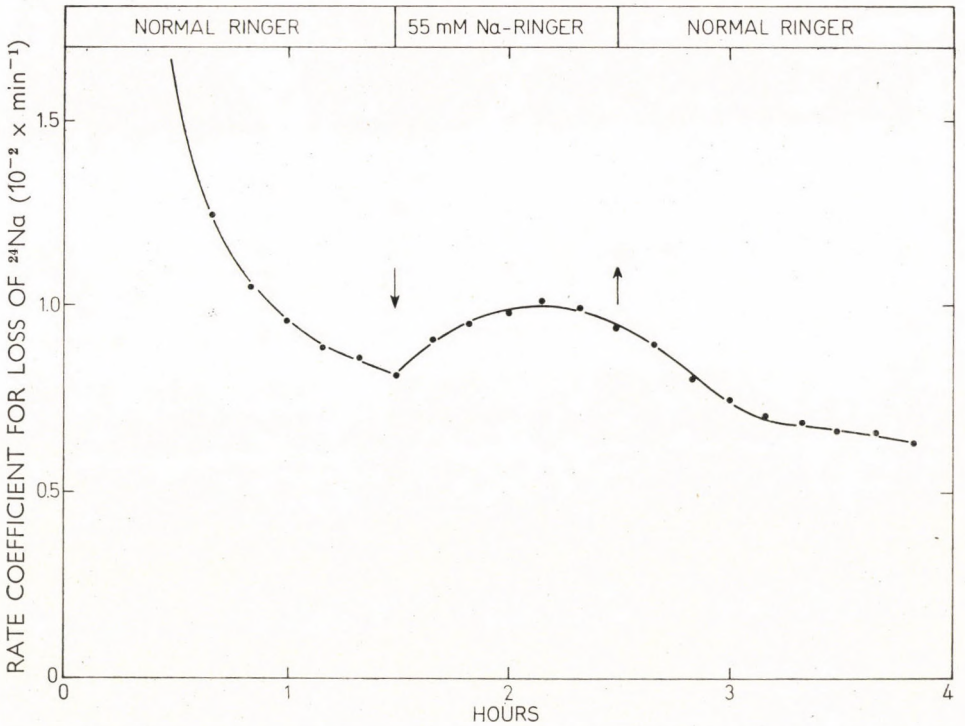


*Fig. 2.* Effect of half sodium Ringer on the rate coefficient for loss of labelled sodium from a sodium loaded frog sartorius muscle. Solutions were changed as shown. The reduction of external sodium caused a marked increase in the rate coefficient. Experiment E-02

**Table III**

*Effect of half sodium Ringer* on sodium efflux from loaded muscle*

| Experiment reference | Time of exposure to $[Na]_o = 55$ mM | Rate coefficient for $^{24}Na$ loss ($min^{-1}$) | | | Rate coefficient ratio $\frac{K'}{K_m}$ ** |
|---|---|---|---|---|---|
| | | in normal Ringer* ($k_1$) | in half Na-Ringer (K') | in normal Ringer ($k_2$) | |
| D—01 | 90—150 | 0.0069 (80)*** | 0.0073 | 0.0060 (180) | 1.14 |
| D—02 | 90—150 | 0.0081 (80) | 0.0097 | 0.0063 (200) | 1.36 |
| E—01 | 90—150 | 0.0071 (75) | 0.0080 | 0.0058 (180) | 1.24 |
| E—02 | 90—150 | 0.0085 (80) | 0.0097 | 0.0070 (190) | 1.25 |
| | | | | Mean ± S.E. | 1.25±0.05 |

* Composition of solutions, see in Table I

** The average of determinations in normal Ringer, before and after half sodium Ringer treatment, was used for claculating the ratio,

$$K_m = \frac{k_1 + k_2}{2}$$

*** In brackets, duration of the period for which the rate coefficient was determined

Ringer's solution containing 120 mM of Na, the efflux declined again (Fig. 2). Data in Table III show that the rate coefficient of $^{24}Na$ efflux proved to be about 25% higher than the mean value measured in normal Ringer before and after exposure to half sodium Ringer. It was found that in half sodium Ringer efflux was cut down by physostigmine in such a way that not only the increase

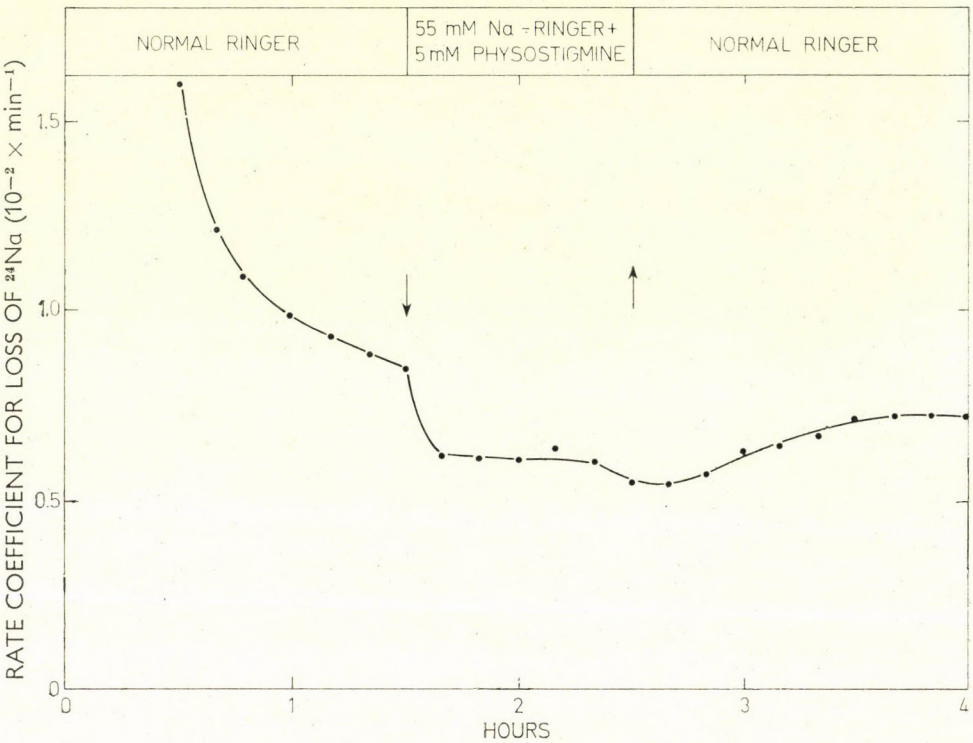


*Fig. 3.* Reversal of the half sodium Ringer effect in the presence of 5 mM physostigmine sulphate. The muscle was kept in an inactive K-free solution at 2°C for 15 hrs and then in a similar solution containing $^{24}Na$ for 3.5 hrs. The rate coefficients from 30 to 240 minutes were plotted. At 90 minutes the muscle was transferred to half sodium Ringer containing 5 mM physostigmine. Experiment D-03

of efflux induced by the reduction of sodium concentration was blocked but the rate coefficient itself was decreased (Fig. 3). As it can be seen in Table IV, the mean rate coefficient ratio ($K'/K_m$) in treated muscle was 0.62; i.e. sodium efflux was inhibited by about 40% if the half sodium Ringer contained physostigmine at a concentration of 5 mM. Data presented in Table IV suggest that the reduction of external sodium concentration to half of that in normal Ringer's solution caused the inhibitory effect of physostigmine to increase. Some experiments were carried out on pairs of sartorius muscle and one member of the pairs was exposed to half sodium Ringer containing physostigmine, the other member to half sodium Ringer without physostigmine. The rate coeffi-

**Table IV**

*Effect of half sodium Ringer with physostigmine on sodium efflux*

| Experiment reference | Time of exposure to half Na-Ringer with physostigmine | Rate coefficient for $^{24}Na$ loss ($min^{-1}$) | | | Rate coefficient ratio $\frac{K'}{K_m}$ |
|---|---|---|---|---|---|
| | | in normal Ringer before physostigmine ($k_1$) | in half sodium Ringer with physostigmine ($K'$) | in normal Ringer after physostigmine ($k_2$) | |
| D—03 | 90—150 | 0.0136 (80) | 0.0062 | 0.0081 (190) | 0.57 |
| D—04 | 90—150 | 0.0087 (80) | 0.0061 | 0.0077 (195) | 0.76 |
| E—01 | 90—150 | 0.0088 (75) | 0.0037 | 0.0055 (180) | 0.52 |
| E—02 | 90—150 | 0.0113 (80) | 0.0060 | 0.0067 (190) | 0.66 |
| | | | | Mean ± S.E. | 0.62±0.07 |

cients ratio in treated and control muscles was 0.62 and 1.25, respectively. Thus physostigmine had reduced the sodium efflux by about 40—60% in half sodium Ringer in contrast with the 25% inhibition found in normal Ringer.

*Effect of physostigmine on $^{24}Na$ efflux in Na-free Ringer*

As outlined under Methods, the muscles were exposed for several hours to K-free Ringer before and during $^{24}Na$ uptake. Under such conditions the muscles gained sodium and their intracellular concentration rose to 30—40 mM/kg intracellular water, in accordance with earlier data (DESMEDT 1953). It is generally accepted that in lithium or choline Ringer the Na for Na exchange diffusion is discontinued but net sodium efflux, especially from a sodium loaded muscle, is increased (KEYNES and SWAN 1959; MULLINS and FRUMENTO 1963; KEYNES and STEINHARDT 1968). To avoid the interference of the excessive net sodium loss and the loss of radioactivity from the extracellular space, the muscles were soaked in a K-free Ringer during the first 70 min of the desaturation period. Fig. 4 shows results of such an experiment. The curve reveals an initial rapid release of $^{24}Na$ from the muscle and a slower one decreasing at a constant rate between the 50—70th min. In such a way, the activity of the extracellular space was washed out without any considerable loss of intracellular sodium. When muscles were exposed to lithium-Ringer, the $^{24}Na$ efflux increased 3—4 fold within ten minutes and after reaching the peak the curve decreased steeply. At the same time $^{24}Na$ activity of the muscle dropped markedly (Fig. 4). Part of $^{24}Na$ efflux was found to be inhibited by physostigmine also in Na-free medium, this block proceeded rapidly, reaching the lowest rate coefficient value within 10—15 minutes and remained at a constant level for a long time (Fig. 5). However the transitional increase of rate coefficient after withdrawal of physostigmine could not be observed in every experi-

ment. Table V summarizes the results of the experiments in lithium Ringer and shows that about 60% of sodium efflux was inhibited by physostigmine. In contrast to the calculations tabulated in column $K'/k_1$, the data in column $K'/k_2$ indicate no significant difference in the rate coefficients during physostigmine treatment and 20—30 minutes after washing it out. It appears likely

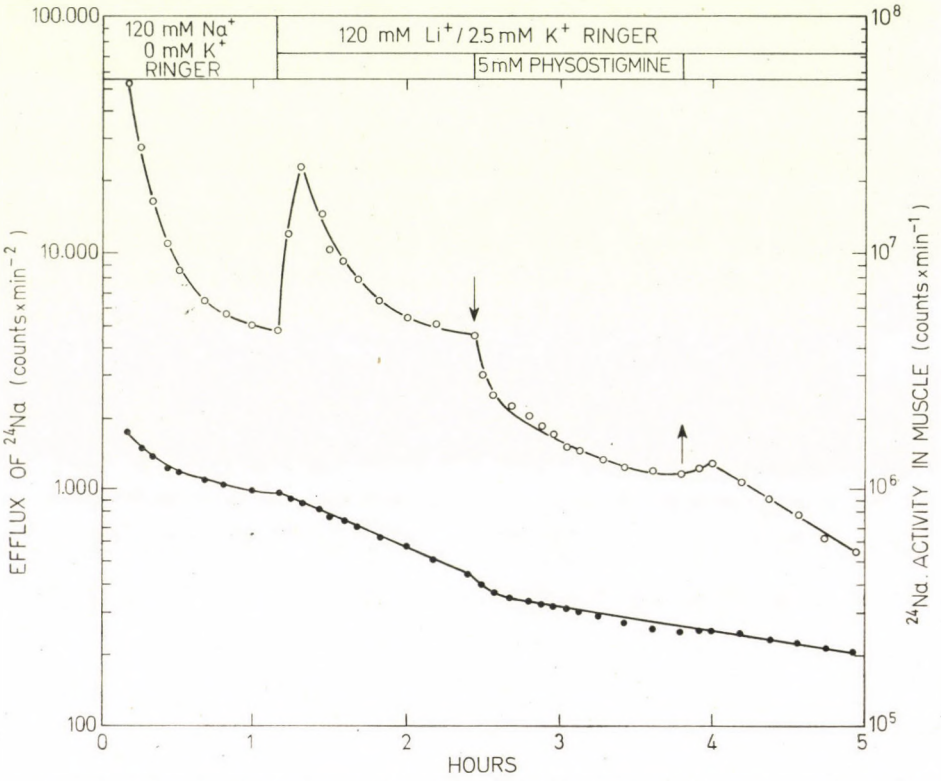


*Fig. 4.* Effect of physostigmine on sodium efflux in lithium Ringer. Upper curve: time course of decline of $^{24}Na$ efflux from an individual sodium loaded muscle in lithium Ringer after 70 minutes exposure to K-free Ringer's solution. Lower curve: time course of the decline of $^{24}Na$ content of muscle obtained from the same preparation as in upper curve. Solutions were changed as shown. Experiment M-04

that the degree of inhibition decreased with time if the muscles had been exposed to lithium Ringer. A comparison was carried out on the time course of block on $^{24}Na$ efflux from muscle, which had been soaked in choline Ringer and physostigmine was applied only for 30 minutes at various times. Data calculated from these experiments are summarized in Table VI. The results show that rate coefficient of $^{24}Na$ efflux was cut down by about 50% if physostigmine had been added between the 70th and 100th minute and by 20% on exposure between the 150th and 180th minute. The effect was much weaker or

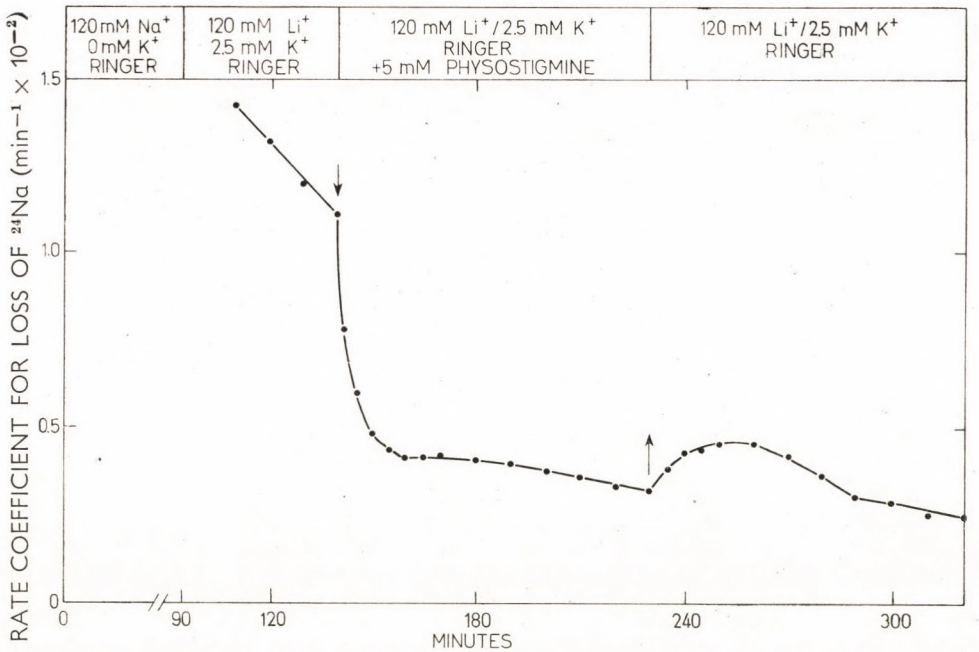


*Fig. 5.* Effect of physostigmine on the rate coefficient for loss of labelled sodium from a frog sartorius muscle during exposure to lithium Ringer. Counts taken during the first 2 hours in inactive K-free sodium as well as in lithium Ringer are not shown. Physostigmine was added as indicated by the arrow

**Table V**

*Effect of physostigmine on rate coefficient of $^{24}Na$ efflux in lithium Ringer*

| Experiment reference | Time of exposure to physostigmine | Rate coefficient ($min^{-1}$) | | | Rate coefficient ratio | | |
|---|---|---|---|---|---|---|---|
| | | before physostigmine ($k_1$) | during physostigmine (K') | after physostigmine ($k_2$) | $\frac{K'}{k_1}$ | $\frac{K'}{k_2}$ | $\frac{K'}{K_m}$ * |
| M—01 | 140—230 | 0.0087 (135)** | 0.0042 (185) | 0.0047 (265) | 0.48 | 0.89 | 0.62 |
| M—02 | 140—230 | 0.0094 (135) | 0.0043 (185) | 0.0035 (260) | 0.45 | 1.22 | 0.67 |
| M—03 | 140—230 | 0.0116 (130) | 0.0040 (185) | 0.0043 (265) | 0.35 | 0.93 | 0.51 |
| M—04 | 140—230 | 0.0069 (135) | 0.0033 (185) | 0.0035 (260) | 0.47 | 0.94 | 0.63 |
| | | | | Mean | 0.43 | 0.99 | 0.60 |
| | | | | S.E. | ±0.04 | ±0.09 | ±0.03 |

* Ratio calculated from the average of determinations of $k_1$ and $k_2$
** In brackets, period for which the rate coefficient was determined

negligible if physostigmine had been added after a longer incubation in choline Ringer's solution.

From Table VI it is evident that the degree of efflux inhibition was dependent upon the duration of exposure to sodium-free Ringer before the application of physostigmine. It may be recalled that the data in Table II did not indicate

**Table VI**

*Effect of $5 \cdot 10^{-3}$ molar physostigmine on sodium efflux in choline Ringer*

| Experiment reference | Time of exposure to physostigmine | Rate coefficient for $^{24}Na$ loss ($min^{-1}$) | | | Rate coefficient ratio $\frac{K' *}{K_m}$ |
|---|---|---|---|---|---|
| | | in choline Ringer before physostigmine ($k_1$) | in choline Ringer with physostigmine ($K'$) | in choline Ringer after physostigmine ($k_2$) | |
| K—05 | 70—100 | 0.0171 (60)** | 0.0068 | 0.0093 (130) | 0.51 |
| K—06 | 70—100 | 0.0152 (60) | 0.0056 | 0.0092 (130) | 0.46 |
| | | | | Mean ± S.E. | 0.48±0.03 |
| K—09 | 150—180 | 0.0048 (140) | 0.0033 | 0.0026 (210) | 0.90 |
| K—10 | 150—180 | 0.0066 (140) | 0.0037 | 0.0028 (215) | 0.78 |
| | | | | Mean ± S.E. | 0.84±0.06 |
| K—07 | 190—210 | 0.0061 (175) | 0.0058 | 0.0073 (250) | 0.86 |
| K—08 | 190—210 | 0.0094 (170) | 0.0108 | 0.0107 (250) | 1.08 |
| | | | | Mean ± S.E. | 0.97±0.11 |

* The average of determinations before and after physostigmine treatment was used for calculating the ratio,

$$K_m = \frac{k_1 + k_2}{2}$$

** In brackets, the period for which the rate coefficient was determined

any correlation between the measure of inhibition and the point of time when physostigmine was added during the efflux experiments in Ringer's solution containing 120 mM Na.

A more critical test of actual time course of physostigmine block was obtained by using pairs of muscle. One of a pair after washing out the extracellular space with K-free Ringer was soaked in choline Ringer and the other in choline Ringer containing physostigmine. After calculating the rate coefficients ratio in treated and control muscles, values were plotted against time in Fig. 6. It is evident that the inhibition of $^{24}Na$ efflux was greatest after 30 minutes of physostigmine application. From that point of time the $K_t/K_c$ ratio increased as a result of the reduction of physostigmine inhibition and this had ceased in the 200th minute so that the ratio became 1.0. On account of the net

loss of fibre sodium in choline Ringer, these results suggest that block of sodium efflux caused by physostigmine was markedly dependent upon the sodium concentration in the muscle fibre.

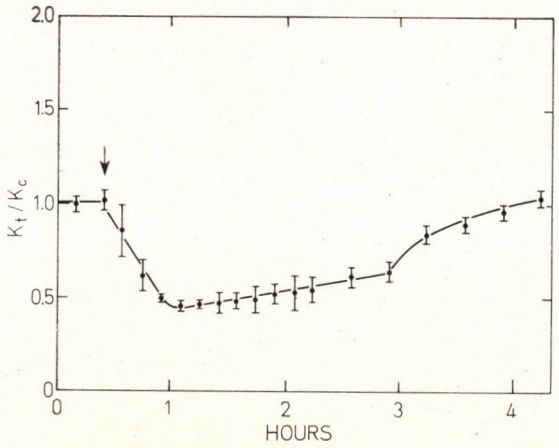


*Fig. 6.* Time course of decline of physostigmine inhibition of sodium efflux in choline Ringer. The rate coefficient for sodium efflux in the presence of 5 mM physostigmine at any given point of time, divided by the same coefficient found in choline Ringer without physostigmine is plotted as ordinate. Exposition of muscles to choline Ringer containing physostigmine was started at arrow. Vertical bars at each point represent the mean of four experiments and ± S.E.

## Discussion

Our findings leave no doubt that one part of sodium efflux from muscle can be blocked by physostigmine. The lower the sodium concentration of external fluid the greater part of the efflux proved to be physostigmine-sensitive. In normal Ringer about 25% of the total efflux was inhibited by 5 mM physostigmine sulphate. In half sodium Ringer the physostigmine-sensitive part of sodium efflux accounted for about 45% of the total. In sodium-free medium about 60% of the total efflux was found to be physostigmine-sensitive.

A characteristic of the physostigmine effect was that in sodium-free Ringer the inhibition of sodium efflux was reduced with the net loss of sodium from muscle. From a final analysis for internal sodium and $^{24}Na$ of muscle and back addition of counts collected during efflux periods in choline Ringer, it is possible to reconstruct a curve for muscle fibre $Na^+$-concentration vs. efflux as shown in Fig. 7. The S-shaped curve of the estimated concentration–efflux relation demonstrates that if the sodium content of muscle is less than 15—18 mM, sodium efflux is physostigmine-insensitive. It may be stated that the inhibitory effect of physostigmine on sodium efflux depends on the fibre sodium concentration.

In general, the results seem to be consistent with previous data, that the outward transfer of sodium from frog muscle fibres may be regarded as consisting of two apparently independent components (HOROWICZ 1965; KEYNES and STEINHARDT 1968). The sodium efflux can therefore be divided into a sodium–potassium coupled component and a sodium–sodium coupled exchange. In their investigations of the inhibitory effect of cardiac glycosides and of sodium-free solutions on sodium efflux from frog sartorius fibres, HOROWICZ (1965) and KEYNES (1966) demonstrated that less than half of the efflux from a freshly dissected muscle can be blocked by ouabain; in sodium loaded muscle the ouabain-sensitive fraction of efflux increased to 75%. Sodium efflux can therefore be regarded as consisting of an ouabain-sensitive, sodium–potassium coupled net transport and a ouabain-insensitive, sodium–sodium coupled exchange diffusion. Under normal physiological conditions in freshly dissected frog muscle, the exchange diffusion components account for about 50% of the total sodium efflux. In sodium loaded muscles the proportion falls to 25% indicating that exchange diffiusion channels are saturated at low internal sodium level and become relatively less predominant when this level increases. Therefore the fraction of total sodium efflux which accounted to exchange diffusion is inversely proportional to internal sodium level and it has been reported previously, that at an internal sodium concentration of 21.5 mM, the exchange diffusion ceases (MULLINS and FRUMENTO 1963).

We have used physostigmine to block sodium efflux. Its effect on sodium efflux from loaded muscle during long exposure to sodium-free solution resembles in many respects that of ouabain (Fig. 7). Physostigmine reduced the $^{24}Na$ efflux only at a high sodium concentration in the fibre and failed to influence the efflux any further if in choline Ringer the internal sodium level fell to about 10 mM/kg intrafibre water. In conclusion, the present findings seem to indicate that physostigmine has no effect on the ouabain-insensitive exchange diffusion component of sodium efflux, whereas it can block the ouabain-sensitive net transport.

However, several points remain open at present. It is unknown whether physostigmine or ouabain can reduce the net transport by combining reversibly with the same kind of receptor or carrier site. Regarding the inhibition of sodium efflux from loaded muscle, in the present experiments about 60% of the total was blocked by 5 mM physostigmine sulphate, whereas 0.01 mM ouabain cut down about 75% of sodium efflux from loaded muscle (KEYNES and STEINHARDT 1968). Hence we can expect that ouabain has a greater affinity to the net transport system. The other possibility is that physostigmine inhibits the net transport by a process different from that of cardiac glycosides. It is undecided, whether the sodium-potassium coupled net transport consists of a physostigmine-sensitive component and of a ouabain-sensitive one or not. On the other hand, it has also been demonstrated that in the presence of 2.5

mM $K^+$, sodium influx from freshly dissected muscle was not significantly altered by ouabain (HOROWICZ and GERBER 1965; KEYNES and STEINHARDT 1968), and these data were regarded as a strong support for the concept, that ouabain-sensitive efflux does not have a substantial exchange diffusion component. It has, however, been shown that physostigmine reduces sodium influx in normal Ringer's solution (SZABÓ et al. 1966).

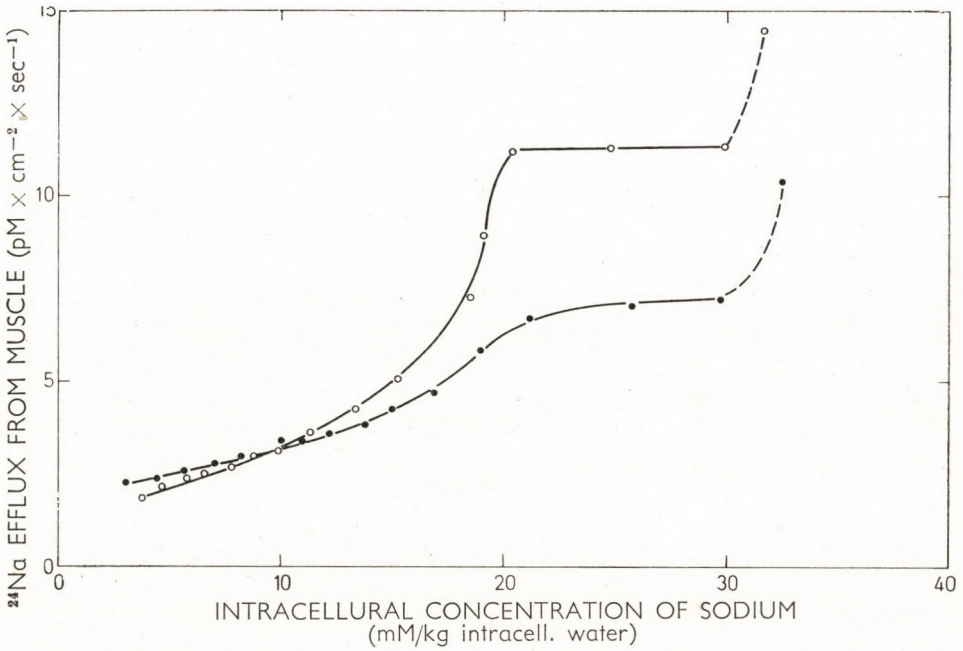


*Fig. 7.* Calculated efflux-concentration curves for paired sodium loaded frog sartorii in choline Ringer. The muscles were soaked in a K-free solution at 2°C for 17 hrs then in the same solution containing labelled sodium for 3 hrs. Before starting the collecting periods in choline Ringer, extracellular radioactivity was removed by soaking in the inactive K-free solution at room temperature for about 1 hr. Open circles: control muscles. Solid circle: muscle in 5 mM physostigmine. Experiment N-04/05

There are few data concerning the difference in the effect of cardiac glycosides and of physostigmine on $K^+$-transport. SJODIN and BEAUGÉ (1968) have shown that potassium efflux from the sodium-loaded muscle is increased while potassium influx is decreased by strophanthidin, whereas it has been demonstrated that physostigmine blocks both potassium efflux and influx of frog sartorius fibre (VARGA and HOROWICZ 1963).

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# EFFECT OF IONS ON RESTING AND ACTION POTENTIALS OF THE MYOCARDIAL FIBRES OF HELIX POMATIA L.

By

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The ionic mechanism of membrane and action potentials has been studied by microelectrodes in the myocardial cells of *Helix pomatia*. Mean membrane potential was 50.6±3.4 mV, action potentials varied between 18—40 mV and showed no overshoot. The duration of action potentials was 300—500 msec. Action potentials of the plateau-type and of the spike-type could be recorded from the control hearts. Reduction of external $Na^+$ concentration resulted in hyperpolarization of the membrane, complete removal of $K^+$ and $Ca^{++}$ from the medium in its depolarization. $Na^+$ excess produced no significant changes in membrane potential. On removal of 50% of $Na^+$, the plateau-type action potentials changed into spike potentials. In $Na^+$-free medium generation of action potentials ceased. In $Ca^{++}$-free medium, generation of action potentials first increased but prolonged $Ca^{++}$ deficiency had an inhibitory effect on potential generation. The ascending limb of action potentials of the Helix heart proved to be $Na^+$-dependent, thus the character of its ion mechanism resembled that of the mammalian heart and not that of other invertebrates or the muscle of Crustacea.

The regulation of cardiac action of molluscs has extensively been studied. However, the use of microelectrodes for the recording of bioelectric signals gained ground only in the sixties. These investigations were mainly concentrated on the heart of Pelecypoda (IRISAWA et al. 1961; NOMURA 1963, 1965; EBARA 1964; IRISAWA et al. 1969). No data are available as to the heart of Gastropoda. It has been shown that the ion mechanism involved in action potential generations and maintenance in the heart of some marine shell-fish differed from that observed in the heart of vertebrates (IRISAWA et al. 1968). These observations have induced us to study the ion mechanism of membrane and action potentials in the heart of *Helix pomatia* with the aim to add some new information to our earlier experimental results (S.-RÓZSA and PÉCSI 1967).

The heart of *Helix pomatia* is of the myogenic type and in this respect it shows a similarity to the heart of vertebrates (KRIJGSMAN and DIVARIS 1955). It differs from the vertebrate heart in its diffuse pacemaker nature and the lack of a specialized conduction system. Thus, it represents a lower level of organization in the evolution of myogenic hearts (PROSSER and BROWN 1962).

The aim of the present experiments was to measure membrane and action potentials of the *Helix* myocardium and to study the dependence of these potentials on $Na^+$, $K^+$ and $Ca^{++}$.

## Methods

The experiments were performed on the isolated heart of *Helix pomatia* L. The cells of the ventricular muscle measure 3—5 microns in diameter (NORTH 1963).

Glass microelectrodes filled with 2.5—3 M KCl were used for recording the membrane and action potentials. Resistance of the electrodes was 10—20 MΩ, their tip-potential never exceeded —7 mV. The microelectrodes were fixed to the movable arm of the micromanipulator.

The isolated heart was placed on a wax plate and tied down with a nylon net to reduce its movements to a minimum. Under these conditions spontaneous action potentials of myocardial cells could continuously be recorded for 5—15 minutes. In experiments of longer duration the electrodes were repeatedly inserted into the myocardial cells.

Frog Ringer's solution adapted to molluscan heart was used. Its composition was, NaCl, 6.5 g; KCl 0.14 g; $NaHCO_3$, 0.2 g; $CaCl_2$, 0.12 g; in 1 litre distilled water. The ions removed from the solution in the course of the experiment were replaced by saccharose in order to maintain osmotic stability.

The bioelectric signals were transmitted by a negative capacity amplifier of high input impedance ($10^{12}$ Ohm) made at the Institute onto the screen of a Dual Beam Oscilloscope EMG Type TR-4602 (VÉRÓ 1970). A DISA photorecorder was used to record the signals on film.

The experiments were made at room temperature (20—24°C) in the period October to May.

## Results

### *1. Membrane and action potentials of control hearts*

Resting potential was 30—55 mV corresponding to $50.6 \pm 3.4$ mV. Action potentials varied between 18—40 mV, they never showed an overshoot. Duration of action potentials was 300—500 msec.

Two types of action potential could be distinguished in the control hearts. In the first of these the initial fast repolarization turned to the delayed one and as a consequence plateau was formed, followed by a slower repolarization phase (Figs 1a, b, c). A uniform repolarization phase was characteristic of the second type of action potential with no plateau formation (Fig. 1d). The time course of the latter type of action potential was shorter and its shape resembled the spike potential of neurons. The two types of action potential had occasional after-potentials.

### *2. Effect of $Na^+$ and $K^+$ on resting and action potentials*

If $Na^+$ concentration of the normal physiological solution is reduced, the membrane becomes hyperpolarized. Accordingly, the membrane potential increases by 20% in a $Na^+$-free solution, and by 10% in a medium with 50% of the $Na^+$ removed (Fig. 2). In $Na^+$ excess, the membrane becomes slightly depolarized. However, at 150% $Na^+$ concentration depolarization is not significant (Fig. 2).

On a decrease of $Na^+$ concentration, the action potentials showed a change in shape. In a solution with 50% of the $Na^+$ removed, there was no plateau formation and the frequency of action potentials was increased

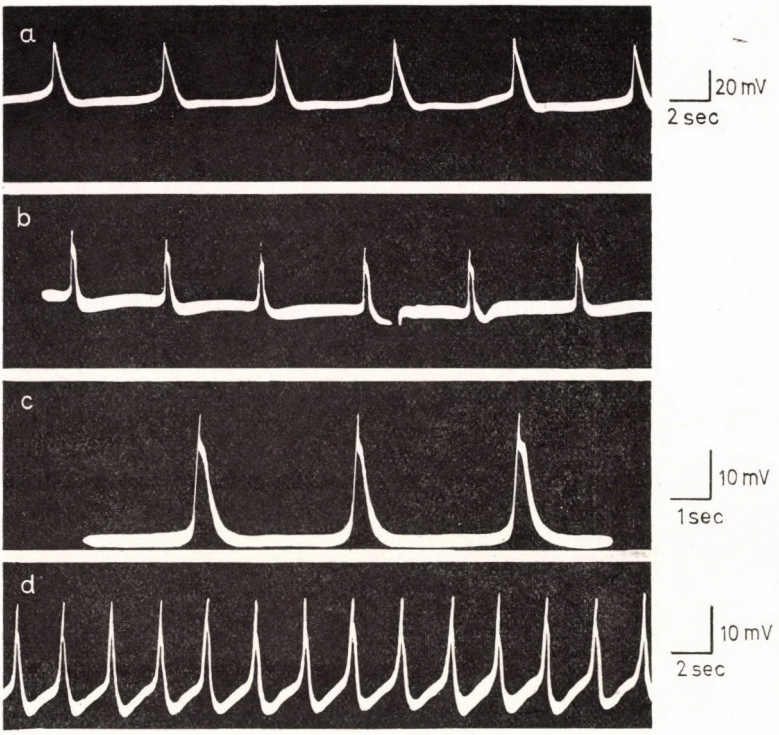


*Fig. 1.* Action potential patterns in control hearts. For details, see text

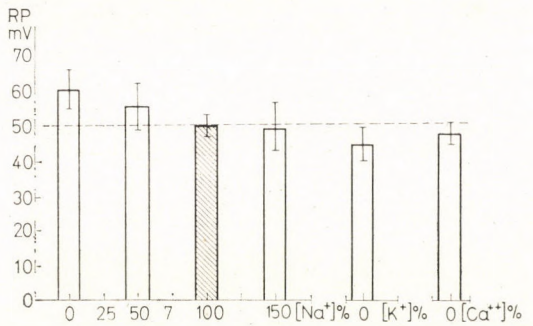


*Fig. 2.* Changes in resting potential in $Na^+$, $K^+$, $Ca^{++}$ deprivation and $Na^+$ excess. Hatched column: control values (100%). Vertical bars: SD of the mean. Ordinate: membrane potential in mV; abscissa: ion concentrations in per cent

(Fig. 3b). On restoration of the original $Na^+$ concentration, the increased frequency persisted but the potentials gradually changed into the plateau-type again. In a $Na^+$-free solution the generation of action potentials ceased 2—3 minutes and only minor oscillations persisted (Fig. 4b, c).

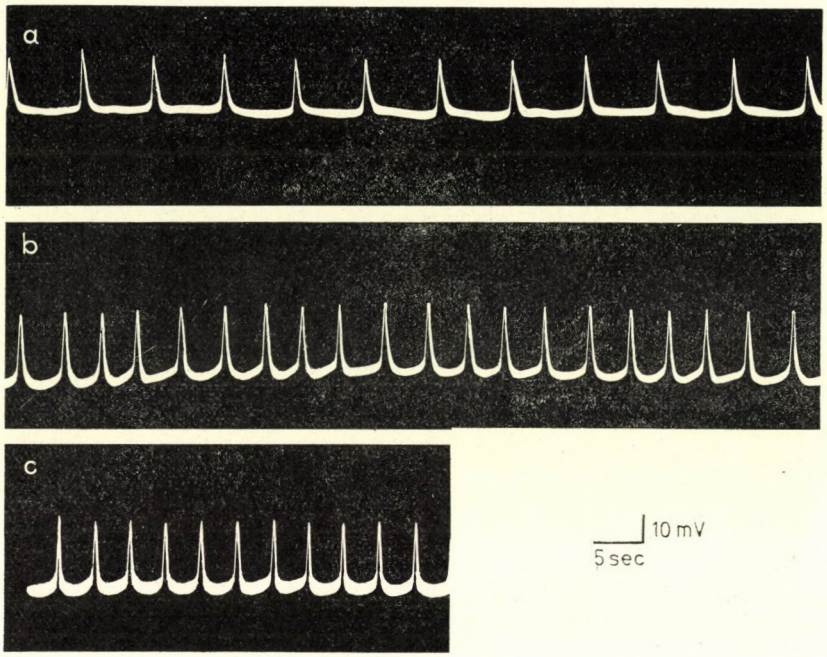


*Fig. 3.* Effect of 50% Na-deprivation on action potential: a) action potential in normal physiological solution; b) the same in 50% Na-deficiency; c) again in normal physiological solution

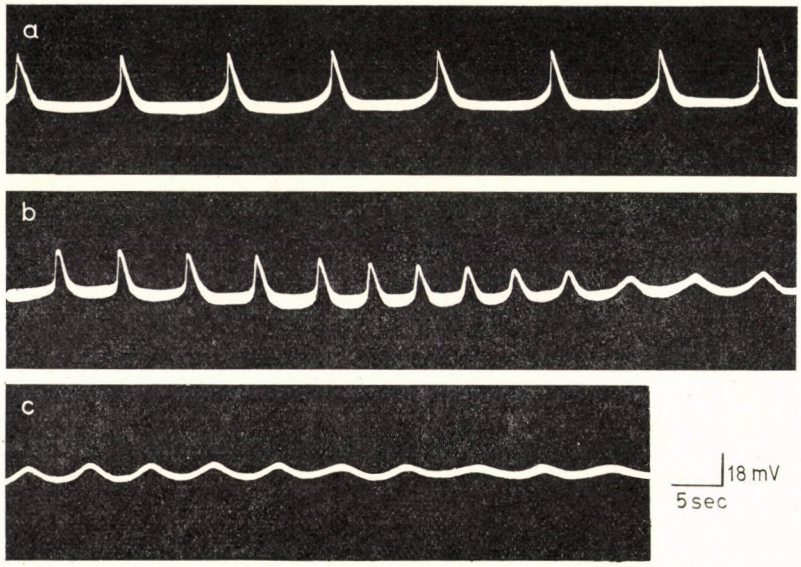


*Fig. 4.* Effect of Na-lack on action potential: a) action potential in normal physiological solution; b) and c) Na-free solution

In a $K^+$-free medium, the membrane potential of the myocardial cells decreased by approximately 10%, proving a depolarizing effect (Fig. 2). At the same time, the frequency of action potentials was unchanged but their amplitude decreased (Fig. 5).

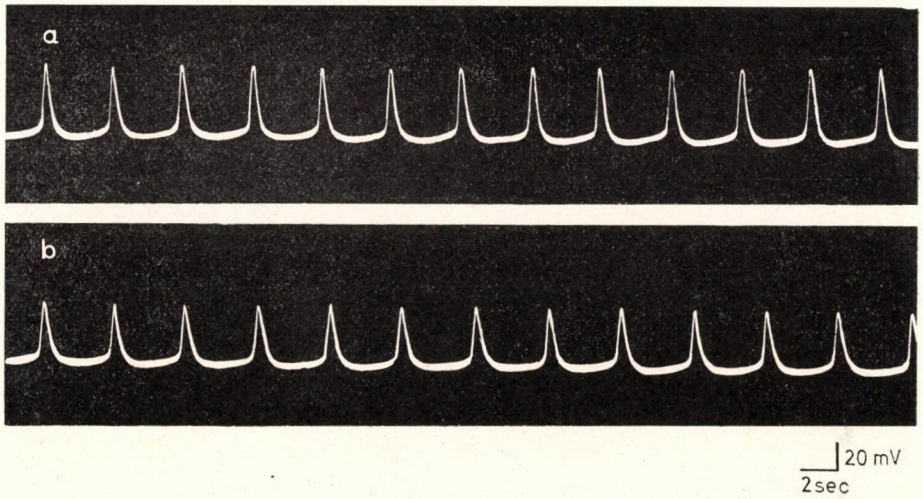


*Fig. 5.* Effect of K-lack on action potential: a) action potential in normal physiological solution; b) complete deprivation

### *3. Effect of $Ca^{++}$ on resting and action potentials*

Omission of $Ca^{++}$ from the physiological solution had a slight depolarizing effect and thus induced a 4% decrease in the membrane potential (Fig. 2).

In $Ca^{++}$-free medium, the action potentials showed two types of change. First, their generation increased, then the amplitude decreased and a complete inhibition of potential generation set in. This took usually 30—40 minutes after the exchange of the normal solution. In some cases, action potentials could be recorded even 1 hour later. On prolonged washing with a solution of normal composition, potential generation was restored (Fig. 6). The time needed for the restoration was almost two-and-a-half-times that needed for inhibiting the action potential.

If EDTA was added to the $Ca^{++}$-free solution with the aim of inactivating internal calcium reserves, the results were the same as described above, with the difference that initial increase in frequency of the action potentials was smaller or did not come about and generation of action potentials became

suspended as early as after 15—20 minutes (Fig. 7). Washing with a normal physiological solution restored the original frequency and amplitude of the potentials.

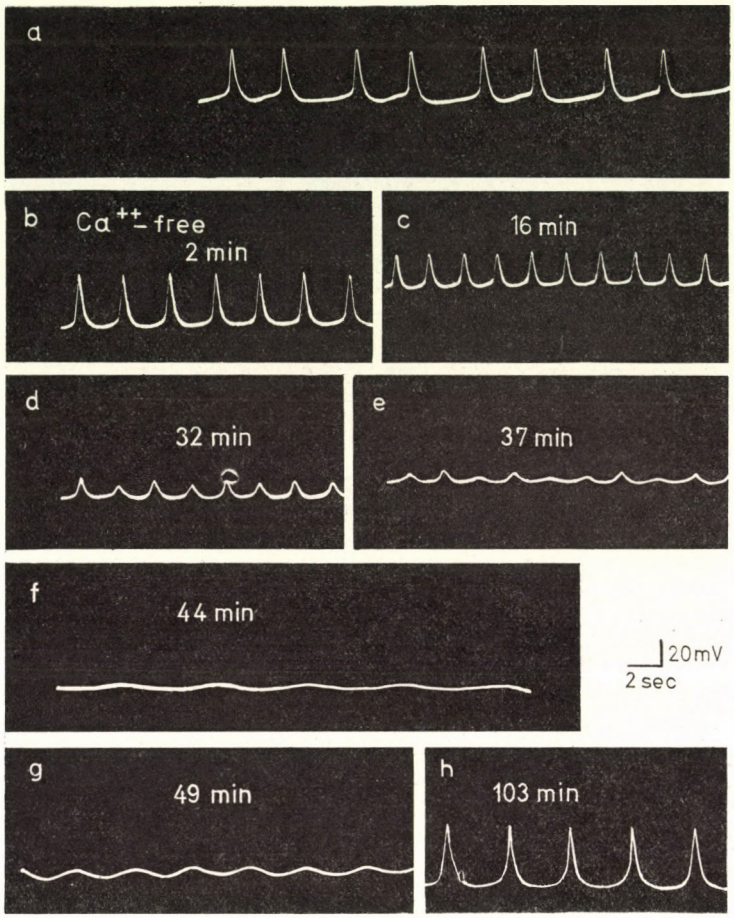


*Fig. 6.* Changes of action potential in $Ca^{++}$-free solution; a) control; b), c), d), e) and f) effect of $Ca^{++}$-free medium at different times after application; g) and h) again in normal physiological solution

## Discussion

The experiments have shown that low membrane and action potentials are characteristic of the cellular membrane in the myocardium of *Helix pomatia* ($50.6 \pm 3.4$ mV and 18—40 mV respectively). Both potentials are 20—30 mV higher in the heart of mammals. Potentials of similar magnitude as observed in the *Helix* heart were found in the Retzius cells of the leech (ECKERT 1963), in the myocardial cells of *Crassostrea*, *Mytilus* and *Venus* (IRISAWA and Ko-

BAYASHI 1964; IRISAWA et al. 1967; IRISAWA et al. 1969). The action potential of the *Helix* heart resembles that of marine Pelecypoda also in the respect that it never shows an overshoot.

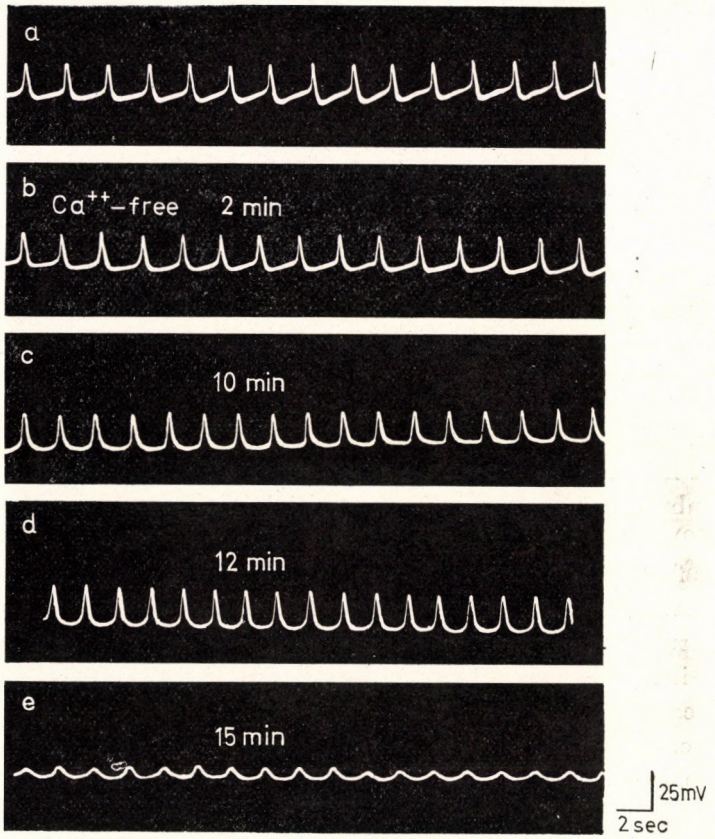


*Fig.* 7. Effect of $Ca^{++}$-lack on action potential in the presence of EDTA ($10^{-4}$M): a) control; b), c), d) and e) $Ca^{++}$-free solution $+10^{-4}$M EDTA

The action potential of the *Helix pomatia* heart is a characteristically myocardial one. A slow initial depolarization is followed by a rapid phase producing the steep rise of the ascending phase. The subsequent rapid repolarization phase is followed by plateau formation and the slowing down of repolarization (Fig. 1); in some cases a positive after-potential is also seen. The potential shape is similar to that observed in mammal hearts (CARVALHO et al. 1966). The two types of potential observed in the *Helix* heart may characterize cells in a different physiological condition and not cells different in function and structure. Action potentials without a plateau are sometimes called spike-like potentials and some authors consider the plateau-type potentials to result

from a fusion of the former ones (CHURNEY and OSHIMA 1963; OSHIMA 1969). These investigators obtained spike potentials only from heart fragments. In our experiments, both types of potential were observed in the intact heart; their actual relationship awaits, however, further clarification.

According to the present results, the action potential of the cells in the *Helix* myocardium is considerably $Na^+$ dependent. The Na ions participate also in the generation of the resting potential of the membrane as indicated by the hyperpolarization occurring in response to the partial or total removal of these ions (Fig. 2). A decrease in the $K^+$ equilibrium potential accounts for this effect, in agreement with the ion theory of excitation processes (HODGKIN and HOROWICZ 1959). The rapid depolarization phase of the *Helix* heart action potential proved to be $Na^+$ dependent. This is in contrast with the conditions observed in the shell-fish myocardium (IRISAWA and KOBAYASHI 1964; IRISAWA et al. 1967; IRISAWA et al. 1969), further in crustacean muscle and heart (FATT and GINSBORG 1958; VAN DER KLOOT 1966). In these species action potential generation is possible in $Na^+$-free solution and shows primarily a $Ca^{++}$ dependence. The ionic base of the *Helix* heart action potential differs also from that of the insect heart, where changes in $Na^+$ concentration have no effect either on the membrane or on the action potential (MCCANN 1964; MCCANN and SANGER 1969). Thus, the ionic mechanism of the action potential in the *Helix* heart differs from that of other invertebrates but is similar to that recorded in mammal hearts (CRANFIELD and HOFFMAN 1958).

Our results have not confirmed the multi-ionic character of the *Helix* heart potential as has been suggested in other Molluscs (IRISAWA et al. 1969). The Ca ions cannot substitute the Na ions in maintaining the membrane potential and in generating the action potential. We assume that, similarly to observation made in vertebrates (THORP and COBBIN 1967), the role of $Ca^{++}$ is primarily a regulation of the movement of univalent cations. Changes in the permeability of the membrane for $K^+$ and $Na^+$ may account for the arrest of action potential generation under the effect of prolonged $Ca^{++}$ omission (CARMELIET 1961; RÜBALTCHENKO 1970).

Our results suggest that different ion mechanisms are involved in the action potential generation in the myocardium of the various classes of Molluscs.

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# INTRALOBAR DISTRIBUTION OF HEPATIC BLOOD FLOW IN THE DOG, I

## EFFECT OF SOME VASOACTIVE AGENTS AND OF HAEMORRHAGIC HYPOTENSION

By

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The ratio of sinusoid volume and cell volume per unit liver tissue was calculated in areas of the lobe lying near (central) the hilus and distal from it (peripheral). The total volume of the sinusoids per unit liver substance was higher in the central than in the peripheral parts.

Corrosion preparations made from the central and peripheral parts showed that the sinusoids were the 4th to 6th branchings of the portal or hepatic venous trunks in the central and the 6th to 10th branchings in the peripheral parts. Thus, the course of blood flow is shorter centrally than peripherally.

Monoaminergic nerves could be demonstrated in the interlobular areas mainly in the peripheral part of the lobe by Falck—Hillarp's fluorescence method.

Circulatory changes in response to adrenaline, noradrenaline, isoproterenol, acetylcholine and histamine, further to haemorrhage (40—50 mm Hg blood pressure) were measured by Hensel's flexible thermoelements in the central and peripheral parts of a liver lobe. Adrenaline and noradrenaline elevated portal pressure but induced no difference in flow between the central and peripheral parts. Isoproterenol increased blood flow in the central and decreased it in the peripheral part of the lobe ($p < 0.05$).

Acetylcholine when given intraportally was ineffective but on intravenous administration it elevated portal pressure and diminished blood flow. Circulation was decreased less in the central than in the peripheral part of the lobe ($p < 0.05$).

Histamine injected intraportally or intravenously elevated portal pressure and decreased blood flow through the central and peripheral areas. The decrease was smaller in the central than in the peripheral tissue ($p < 0.05$).

In haemorrhagic hypotension (40—50 mm Hg) circulation decreased in both the central and the peripheral part of the lobe; the decrease was significantly higher peripherally ($p < 0.001$).

All the interventions which induce a major fall in systemic blood pressure (in normovolaemia or in hypovolaemic hypotension) elicit a so-called restrictive circulatory redistribution in the dog liver.

Modern circulation research has increasingly been focussed on the regional distribution of cardiac output, and on the distribution of blood flow within the individual organs (MELLANDER 1970). FISCHER et al. (1960) found the portal fraction and the hepatic arterial fraction of cardiac output to be extremely low in severe arterial hypoxia of dogs, indicating that hepatic circulation does not change parallel with the increase in cardiac output. TAKÁCS (1963) showed that in nephrectomized rats besides the skin fraction mainly the previously elevated intestinal and hepatic fractions decreased. Thus, the splanchnic region took over the role of the kidney in the circulatory shifting.

DANIEL and PRICHARD (1951a, b, c) observed two forms of circulation in the rat liver by means of serial portograms. In the majority of the animals the injected contrast material perfused the liver uniformly, the filling of the sinusoids showed the contours of the organ (diffuse form of circulation). In some animals the contrast material did not penetrate into the peripheral parts of the liver, the diffuse shadow appeared only near the hilus (restrictive form of circulation). These authors could prove in corrosion preparations that near the hilus the passage leading from the portal vein to the hepatic vein was shorter than in parts of the organ more distal from the hilus. They also observed that on the stimulation of the hepatic plexus and after the intraportal administration of adrenaline, the restrictive form of circulation developed more frequently. OLERUD (1953) examined the rabbit's hepatic circulation by portography and could observe the development of restrictive circulation when intraabdominal pressure had increased. HONIG (1960) used micro oxygen electrodes and heated thermoelements and assessed local fluctuation of blood flow in the different parts of the dog liver that could probably correspond to the variation of restrictive and diffuse forms of circulation observed in other species. ZÖCKLER and FRIEDEL (1970) measured blood flow in dogs and could differentiate "individual parenchymal regions" within the liver. Based on this finding they suggested that the liver may act as a "transflow reservoir".

The present studies were aimed at clarifying the following questions. 1. Are there any differences between the vascular system or the innervation of the liver substance nearer to the hilus (central part of the lobe) and of that lying more distally (peripheral part of the lobe)? 2. Do vasoactive agents like noradrenaline (Na), adrenaline, isoproterenol, histamine and acetylcholine (ACh) elicit a "restrictive form of circulation" in the dog liver? 3. Does haemorrhagic hypotension which is invariably associated with circulatory shifting in the dog, induce a restrictive form of liver circulation?

## Methods

A total of 33 mongrel dogs of either sex weighing 10—16 kg was used in the experiments.

(1a) Specimens were removed from parts lying near to and far from the hilus of the right medial liver lobe of 5 dogs sacrificed by an over dose of hexobarbital. The specimens were fixed in formalin, embedded in paraffin, sections 8 $\mu$ were made and stained with haematoxylin eosin. A square grid with $10 \times 10$ intersections was projected on the sections. The nuclear variation table of PALKOVITS and CSAPÓ (PALKOVITS 1962) was used for counting of sinusoids. Sinusoids coinciding with the intersections were counted in 100 visual fields of both the hilar and the peripheral tissue sections (Fig. 1).

(b) The liver of 5 dogs sacrificee by an over dose of hexobarbital was infused with 5% PVC solutions different in colour. The solutions were injected through the portal vein or retrograde via the caudal vena cava and the hepatic veins. The number of branchings of the portal and of the hepatic vein were counted up to the sinusoids in the central and peripheral parts of the right medial lobe.

(c) Monoaminergic innervation of the hilar and peripheral liver tissues studied by the fluorescence method of FALCK et al. (1962), for the demonstration of catecholamines. The specimens were taken from the right medial lobe of 5 dogs anaesthetized with hexobarbital.

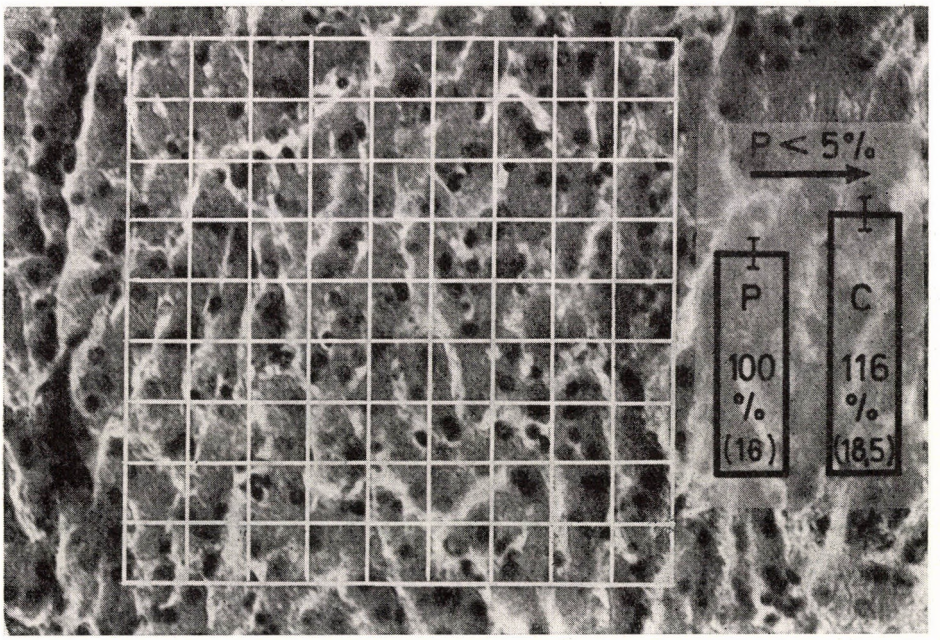


*Fig. 1.* Square grid with 10 × 10 intersections projected on liver tissue section 8 μ thick stained with HE. Column P: area of sinusoids measured in part of lobe, 16%. Column C: area of sinusoids measured in hilar (central) part of lobe, 18.5%. If the peripheral value is considered 100%, the central one amounts to 116%

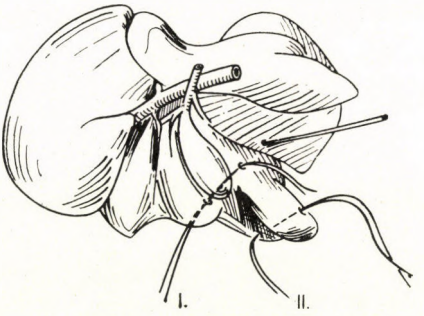


*Fig. 2.* Schematic representation of dog liver. Flexible thermoprobes introduced into the central and peripheral parts of the right medial lobe

(2) 18 dogs were anaesthetized with 0.075 g/kg chloralose +0.75 g/kg urethan intravenously. Blood flow in the hilar and peripheral areas of the right medial lobe was simultaneously and continuously recorded by the method of HENSEL et al. (1954), by means of flexible thermoelements and a two-channel Fluvograph (Fig. 2). The site of the probes was later controlled in histological sections or corrosion preparations. A Hellige-type multiscriptor was used for recording pressure in the femoral artery and in the portal vein, monitored by a Statham

pressure transducer. Pulse rate was monitored by a Hellige-type integrator and respiration rate by a thermistor. The following vasoactive agents were given to the animals. To 8 dogs each 10 μg/kg adrenaline or 10 μg/kg noradrenaline, to 7 dogs each 20 μg/kg acetylcholine or 20 μg/kg histamine, to 6 animals 2 μg/kg isoproterenol. The drugs were first administered into the portal vein then into the femoral vein. A cannula fixed into the branch of the mesenteric vein and led out through the abdominal wall was used for intraportal administration.

(3) After the effect of the last drug had subsided, 15 animals were bled. Blood pressure was stabilized at 40—45 mm Hg by the method of ENGELKING and WILLIG (1958).

Student's one sample and two sample *t*-tests were used for statistical analysis.

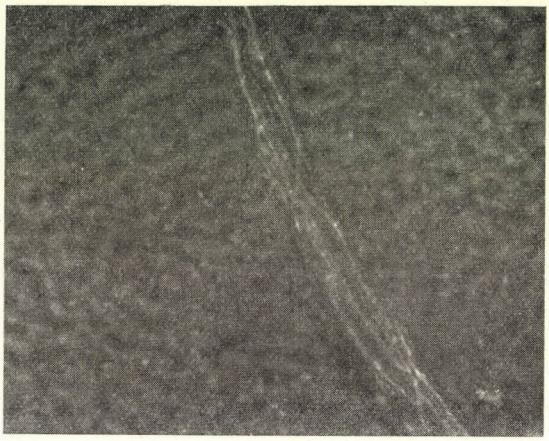

*Fig. 3.* Many monoaminergic nerve terminals in interlobular area in peripheral part of a lobe. Falck—Hillarp reaction, × 100

## Results

(1a) Quantitative analysis of the histological sections showed that the sinusoids constituted 16% of the total area in the peripheral and 18.5% in the central, part of the lobe. Taking the peripheral value as 100%, the central amounted to 116%; the difference was significant ($p < 0.05$; Fig. 1).

(b) The number of branchings were counted up to the sinusoids in the corrosion preparations made from the central and peripheral parts of the portal and hepatic vein systems. Centrally, the sinusoids corresponded to the 4th—6th branching, peripherally to the 6th—10th branching.

(c) Falck and Hillarp's method was used to demonstrate monoaminergic nerve fibres. It was found that in the interlobular area along the branches of the portal vein and the hepatic artery, monoaminergic fibres are but in the peripheral part of the lobe (Fig. 3).

(2) The intraportal administration of 10 μg/kg adrenaline significantly elevated arterial and portal pressure. Blood flow was first decreased, then increased in both the hilar and the peripheral parts of the liver lobe; there was

no difference in flow between the two areas in identical periods. Adrenaline, 10 $\mu$g/kg i.v. had practically the same effect as observed on portal administration, the only difference was a significant reduction of pulse rate (Fig. 4).

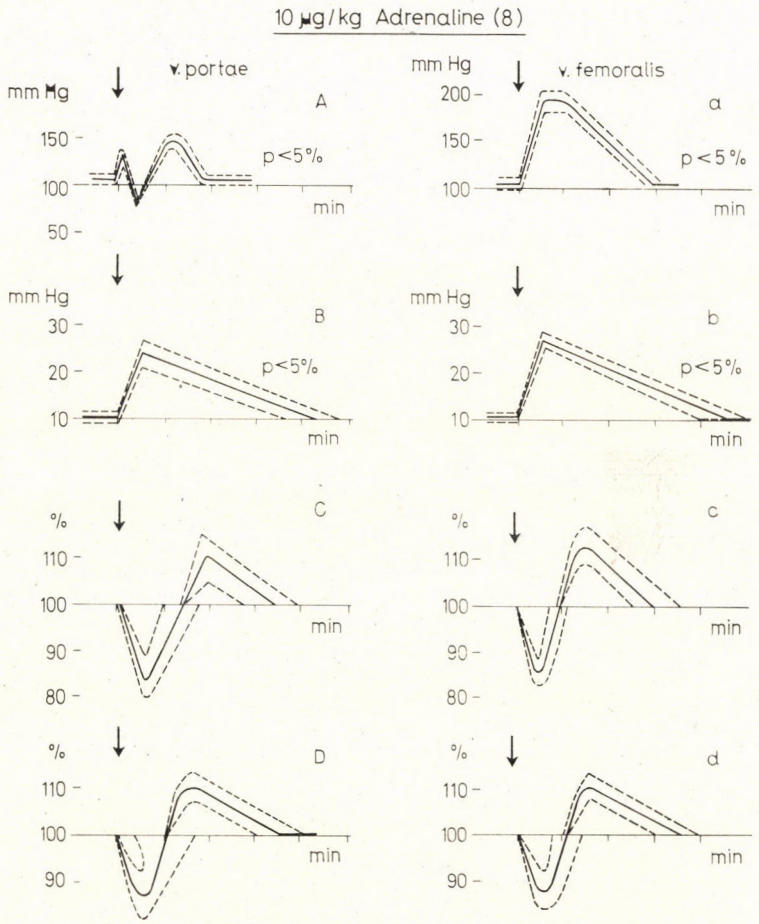


*Fig. 4.* Effect of adrenaline administered into portal vein (A, B, C, D) and into femoral vein (a, b, c, d); arterial pressure (A, a); portal pressure (B, b); flow in peripheral (C, c) and central (D, d) parts of lobe. Signs on abscissa indicate 1 minute; n = 8; ↓ adrenaline administration; ····· / ····· = mean ± SE

Noradrenaline, 10 $\mu$g/kg intraportally, elevated arterial and portal pressure ($p < 0.05$) and reduced the pulse rate ($p < 0.05$). Circulation in the central area was inconsistent. Blood flow decreased in six cases and increased in two. In two cases a short increase preceded the decrease, on two other occasions it was followed by a longer increase. Peripherally, a decrease in blood flow was invariably observed preceded in two cases by an increase, of short duration

while in two cases by a more lasting one. There were no significant differences in blood flow between the central and peripheral liver areas. Noradrenaline, 10 $\mu$g/kg intravenously induced the same changes in arterial and portal pres-

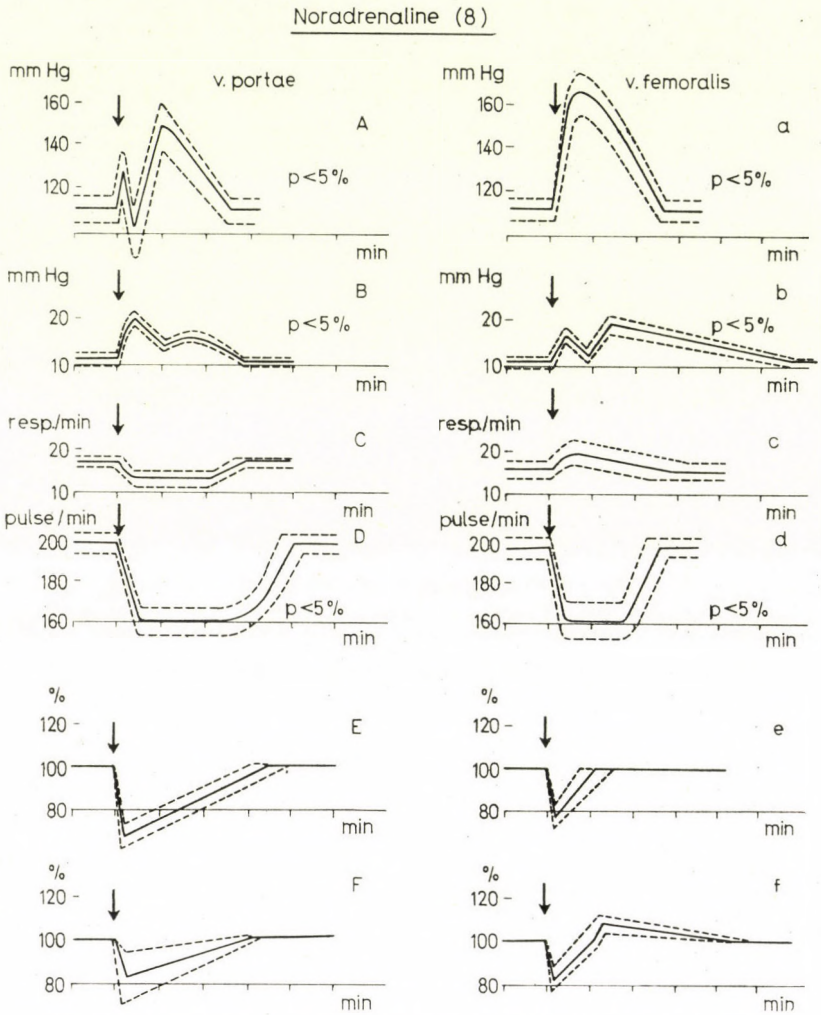


*Fig. 5.* Effect of noradrenaline administered into portal vein (A, B, C, D, E, F) and into femora vein (a, b, c, d, e, f); arterial pressure (A, a); portal pressure (B, b); respiration (C, c), pulse rate (D, d); flow in peripheral (E, e) and central (F, f) parts of lobe. Signs on abscissa indicate 1 minute; n = 8; ↓ noradrenaline administration; ····· / ····· = mean ± SE

sure, further in pulse rate as on portal administration. Blood flow in the central part showed a biphasic change. The amount of blood first decreased and then increased, both changes were significant ($p < 0.05$). The decrease was preceded in three cases by a short increase. Peripheral tissue flow decreased, followed in

four cases by a minor increase. No significant difference was found between the two areas in the changes of the same direction (Fig. 5).

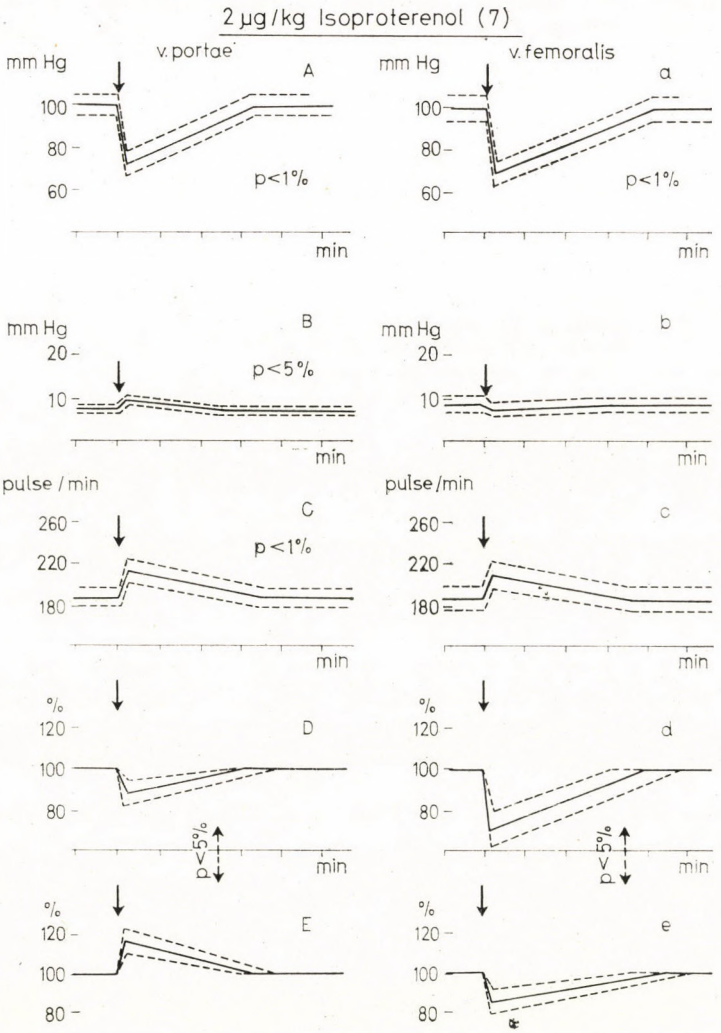


*Fig. 6.* Effect of isoproterenol administered into portal vein (A, B, C, D, E) and into femoral vein (a, b, c, d, e); arterial pressure (A, a); portal pressure (B, b); pulse rate (C, c); flow in peripheral (D, d) and central (E, e) parts of lobe. Signs on abscissa indicate 1 minute; n = 7;

↓ isoproterenol administration ····· = mean ±SE

Isopropyl noradrenaline, 2 µg/kg intraportally, reduced arterial pressure, elevated portal pressure and increased pulse rate. The difference was significant in all the three parameters ($p < 0.05$). Circulation of the central tissues increased; in two cases it was preceded by a short decrease. Mean peripheral blood flow showed a decrease, but in two cases it increased. The difference in

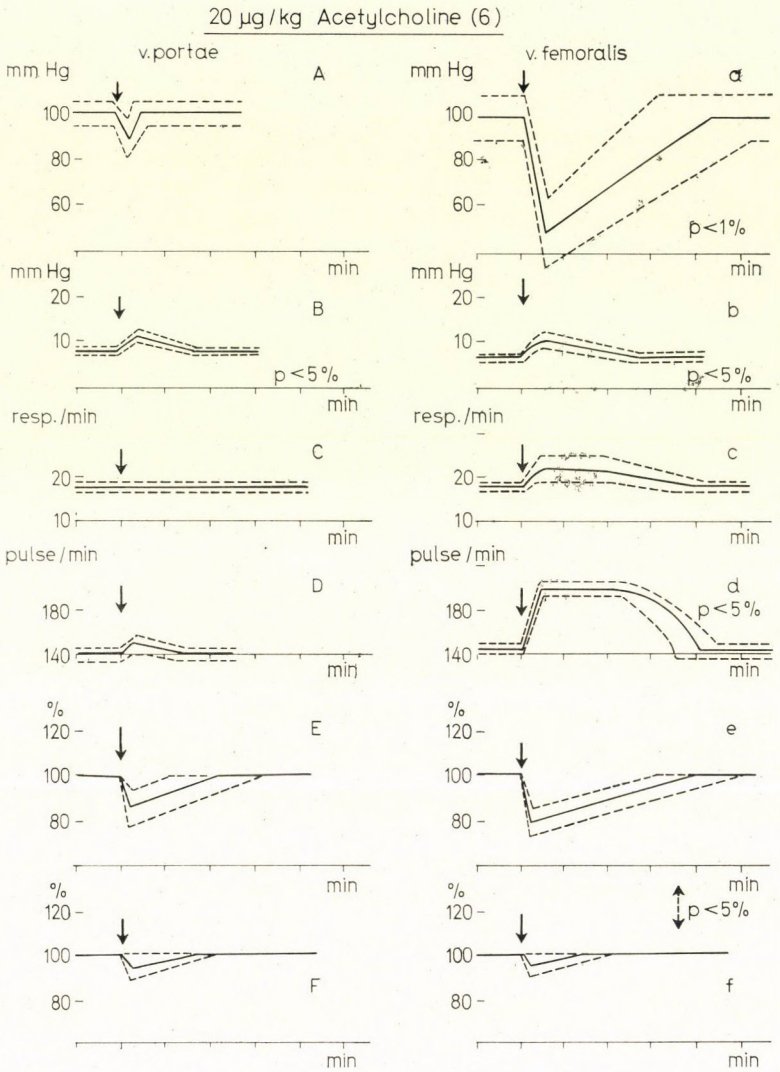


*Fig. 7.* Effect of acetylcholine given into portal vein (A B, C, D, E, F) and into femoral vein (a, b, c, d, e, f); arterial pressure (A, a); portal pressure (B, b); respiration (C, c); pulse rate (D, d); blood flow in peripheral (E, e) and central (F, f) parts of lobe. Signs on abscissa indicate 1 minute; n = 6; ↓ acetylcholine administration; $\frac{\cdots\cdots}{\cdots\cdots}$ = mean ± SE

flow between the central and peripheral parts was significant ($p < 0.05$). Isoproterenol, 2 µg/kg intravenously, reduced arterial pressure ($p < 0.01$). Portal tension was slightly elevated in four cases and slightly diminished in three. The changes in tissue flow were rather uniform, with a decrease both centrally and peripherally. Changes in flow through the central and peripheral tissues differed significantly ($p < 0.05$; Fig. 6).

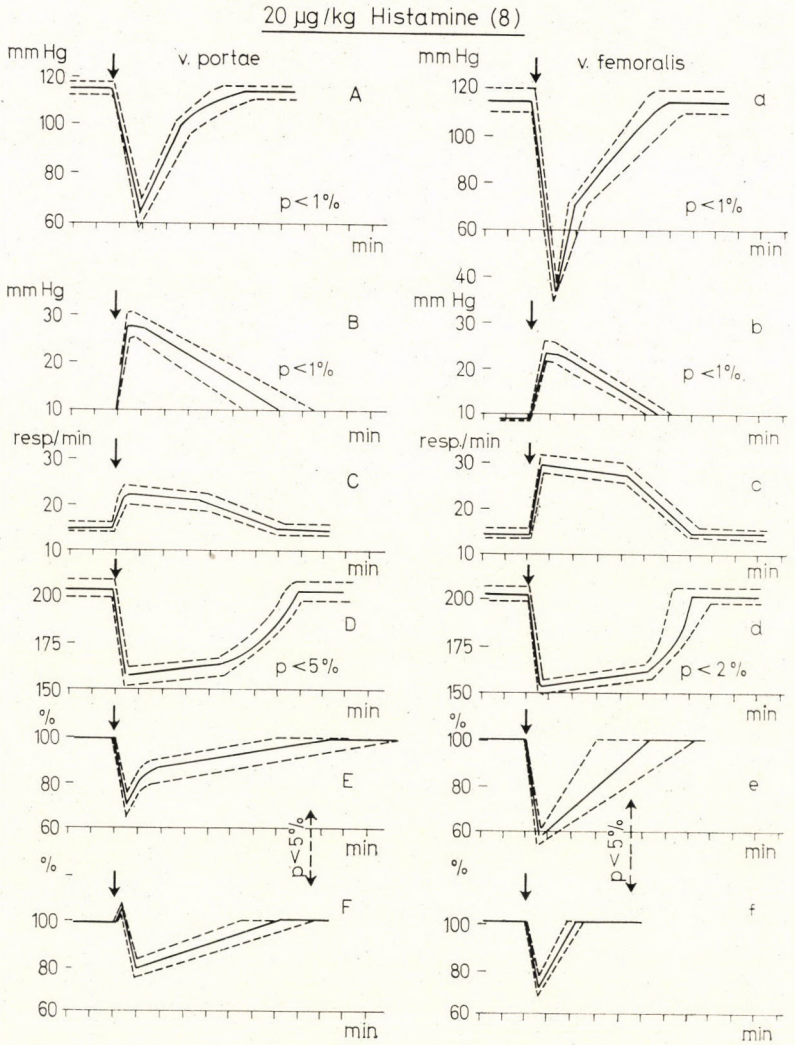


*Fig. 8.* Effect of histamine given into portal vein (A, B, C, D, E, F) and into femoral vein (a, b, c, d, e, f); arterial pressure (A, a); portal pressure (B, b); respiration (C, c); pulse rate (D, d); flow in peripheral (E, e) and central (F, f) parts of lobe. Signs on abscissa indicate 1 minute; n = 8; ↓ histamine administration; ····· / ····· = mean ± SE

Acetylcholine, 20 µg/kg intraportally, significantly elevated portal pressure ($p < 0.05$). In the central tissue flow out of six cases increased in two and decreased in four. The decrease was preceded by a short increase in two cases. Changes in the peripheral area were identical with those in the central one. Acetylcholine, 20 µg/kg intravenously lowered arterial ($p < 0.01$) and elevated portal blood pressure ($p < 0.05$) and increased pulse rate ($p < 0.05$). In the central part of the lobe, blood flow decreased slightly in four cases. A lasting

increase preceded by a short decrease occurred in two cases. In the peripheral part, blood flow decreased in all the six cases. Changes in blood flow through the central and peripheral tissues differed significantly ($p < 0.05$; Fig. 7).

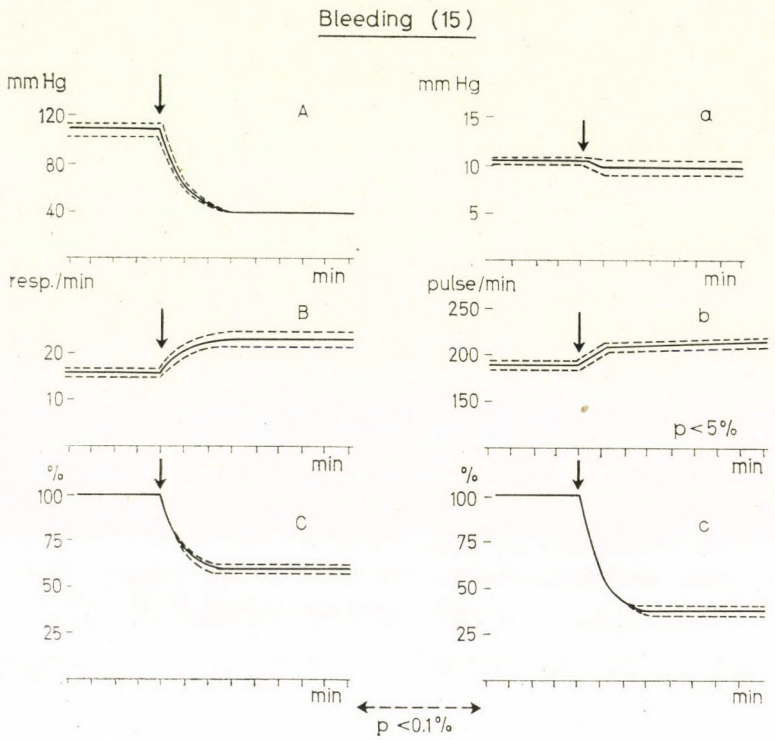


*Fig. 9.* Effect of bleeding on arterial pressure (A), portal pressure (a), respiration (B), pulse rate (b), flow in central (C) and peripheral (c) part of lobe. Signs on abscissa indicate 1 minute; n = 15; ↓ blood letting; $\frac{\cdots\cdots}{\cdots\cdots}$ = mean ± SE

Histamine, 20 μg/kg intraportally, decreased arterial blood pressure ($p < 0.01$) and pulse rate ($p < 0.05$), and elevated portal pressure ($p < 0.01$). In six dogs out of eight the short initial increase of the flow in the central area was followed by a longer marked decrease. Such a biphasic change occurred peripherally only in two cases, the other animals responded with a decrease in blood flow. The difference in flow between the central and peripheral areas was on the border of significance. Histamine, 20 μg/kg intravenously, had the same effect on arterial and portal pressure, and on pulse rate as was the case after portal administration. Blood flow decreased in both the central and the peripheral tissues but more markedly in the latter. The difference between the two values was significant ($p < 0.05$; Fig. 8).

(3) In bled dogs with 40—45 mm Hg blood pressure the pulse rate increased in both the central and the peripheral tissues. The decrease was significantly more marked in the peripheral area ($p < 0.001$). Hepatic blood flow became constant within two minutes after blood pressure had been stabilized at 40—45 mm Hg and did not change during the 30—60 minutes of examination (Fig. 9).

## Discussion

It has been shown that the course of blood in the dog liver is shorter in the area near to the hilus than in the peripheral part of the same lobe. A similar observation was made in rats by DANIEL and PRICHARD (1951b). In the resting state, sinusoids of the hilar part occupy a greater area per unit liver tissue than those of the peripheral part. As to the monoaminergic innervation of the intrahepatic vessel system, we could state that marked innervation of the perilobular space is to be found only in the peripheral parts of the lobe. Innervation of the smaller vessels in the hilar region seems to be negligible. This, of course, does not mean that the trunks of the hepatic artery and of the portal vein situated nearer to the hilus, further the trunks of the hepatic vein nearer to the caudal vena cava have not a richer innervation than the peripheral parts of the same vessels. Thus, it may be assumed that the peripheral part of the liver can respond more actively and more quickly to impacts affecting the circulation than is the case with the hilar area.

DANIEL and PRICHARD (1951a, b, c) could induce restrictive circulation more frequently by injecting 20 $\mu$g adrenaline into the superior mesenteric vein of rats. Stimulation of the hepatic plexus had the same result. As the hepatic vessels have practically only monoaminergic nerves (UNGVÁRY and DONÁTH 1969), the effect of catecholamines after intraportal and intravenous administration was studied by us first. Earlier it was suggested that only alpha receptors were present in the intrahepatic vessel system (GREEN and KEPCHAR 1959) and that adrenaline, noradrenaline and isoproterenol (the order being also the order of efficacy; AHLQUIST 1948) induced only vasoconstriction. However, FISCHER and TAKÁCS (1964) demonstrated that small doses of adrenaline injected intraarterially dilated the hepatic arteries and increased blood flow in the liver. This indicates the presence of beta receptors in the area of the hepatic artery. Therefore, we have tested the effect of isoproterenol, the most effective beta receptor stimulating agent.

Adrenaline produced a characteristic two-phasic change in blood flow in the hilar and peripheral tissue. First a decrease, then an increase was observed on both intraportal and intravenous administration. Arterial and portal pressures were significantly higher during both phases.

The effect of noradrenaline was somewhat similar to that of adrenaline,

but the second phase, the increase in blood flow developed only in part of the cases. This may correspond to the observations of GARBULINSKI et al. (1959) made on dogs. In their experiments an intravenous dose of 5 μg/kg of adrenaline first decreased blood flow in the hepatic artery and the portal vein but 40 seconds later increased it by 40% in the portal vein. The complexity of changes has been shown by GREEN and KEPCHAR (1959) who assumed that at least five different mechanisms are involved when adrenaline is given intravenously. These are a direct alpha effect, a direct beta effect, a reflex inhibition of the sympathetic vasoconstrictive tone, the release of vasodilator substances and the central activity of the gamma receptors. To these mechanisms should be added the direct constrictive effect of adrenaline and noradrenaline on the liver sinusoids and mainly on the outlet sphincter (SENEVIRATNE 1949; WAKIM 1944). Thus, the changes we have observed in response to adrenaline and noradrenaline were of a complex nature, to adrenaline they were even ambiguous, making it impossible to draw conclusions as to the development of a restrictive circulatory mechanism.

In contrast to the above catecholamines, isopropylnoradrenaline given intraportally or intravenously produced significant differences between the blood flow of the hilar and that of the peripheral liver tissue. After intraportal administration arterial pressure decreased, portal pressure increased and hilar tissue flow rose above, while peripheral flow fell below, the control values. Thus, the changes induced in the two areas were of an opposite character. On intravenous administration blood flow decreased in both parts of the lobe but the decrease was more mar ed in the peripheral one. Changes in portal pressure were not significant, arterial pressure decreased. These changes might represent a circulatory restriction and we assume that from among the actions of isoproterenol its effect of decreasing arterial pressure was the responsible one.

Acetylcholine in a dose of 10 μg/kg given intraportally elevated only portal pressure in a significant manner. Changes induced in the other parameters were negligible and often contradictory. These findings were not surprising considering the great variety of the effect of acetylcholine on hepatic circulation. WAKIM (1944) found by transillumination that acetylcholine had no effect on the circulation of sinusoids. BAUER et al. (1932) could not decide whether acetylcholine did or did not affect the perfused isolated liver of the dog, cat and goat. ANDREWS et al. (1955) injected acetylcholine into the hepatic artery of the perfused dog liver; arterial flow remained unchanged, portal and hepatic flow decreased and the volume of the liver increased. On intraportal administration 10—15-fold doses were needed to ensure the same effect; the difference was attributed to rapid portal destruction of acetylcholine. In contrast, GREEN and KEPCHAR (1959) and later FISCHER (1964) observed a marked increase in arterial flow with a reduced resistance in the hepatic artery. In the latter total hepatic blood flow was not affected.

In the present experiments, 10 μg/kg of acetylcholine given intravenously had a marked effect, apparently because it was not directly exposed to the great quantities of liver esterase. The decrease in arterial pressure and the increase in portal pressure were significant, due to the intrahepatic vasoconstriction developing in hypotension as shown by GINSBURG and GRAYSON (1954). In addition, flow in the central part of the lobe changed less than that in the peripheral one.

Histamine in a dose of 20 μg/kg given intraportally or intravenously induced a fall in arterial pressure and elevated portal pressure considerably; this was especially marked on intravenous administration. In response to intraportal administration flow in the peripheral part of the lobe decreased immediately, while in the central part this occurred after a transient increase. After intravenous administration circulation decreased in both parts of the lobe but less so in the central one. Literature abounds in data on the mechanism of action of histamine. These, however, are often contradictory. BRADLEY (1963) reviewed the relevant reports and concluded that histamine acts as a vasoconstrictor on the hepatic and splanchnic veins, whereas in other parts of the organism it dilates the arteries and arterioles. HADDY's experiments (1960) on the foreleg of the dog indicated that histamine had a marked dilatory effect on the arterioles and a constrictive one on the veins, due probably to the release of adrenal hormones. In the so-called "strangling" mechanism observed in anaphylaxis and after the intraportal administration of histamine, the musculature of the hepatic veins shows a sphincter-like contraction at the site of opening into the inferior vena cava. FISCHER and TAKÁCS (1964) did not observe this phenomenon when 1 mg/min histamine was given intravenously to dogs; in contrast, the fall in blood pressure was accompanied by an increase in total hepatic flow. In the present experiments, portal pressure showed an extreme elevation, which was attributed to a constriction of the hepatic veins. Although portal vasoconstriction during hypotension has to be reckoned with (GINSBURG and GRAYSON 1954), in itself it can hardly account for the extreme values of portal hypertension. The transillumination studies of SENEVIRATNE (1949) showed that circulation in the sinusoids was decreased on local or intraperitoneal administration of histamine, an observation also indicating a constriction of the hepatic veins. Presumably, under such conditions the central area can fully maintain blood flow.

Changes in the central and peripheral parts of the lobe were studied in haemorrhage-induced hypotension. At an arterial pressure stabilized at 40—50 mm Hg, portal pressure hardly decreased. Circulation in the central part of the lobe decreased less than in the peripheral one. According to FISCHER et al. (1960) in arterial hypoxia of dogs hepatic circulation does not change parallel with cardiac output. TAKÁCS (1963) found an increased hepatic resistance in bled rats; when the whole splanchnic fraction of cardiac output was calculated,

the decrease was at the border of statistical significance. SENEVIRATNE (1949) let 4 ml of blood from the jugular vein of rats and observed by means of transillumination a contraction of the sinusoids and of the smaller vessels; the larger vessels showed a spindle-shaped pattern, circulation was slowed down. It has to be mentioned that by the transillumination method applied he could study only the peripheral parts of the liver lobes. FRANK et al. (1962) demonstrated that in non-anaesthetized bled dogs with a blood pressure stabilized at 35 mm Hg, hepatic venous flow was hardly 10% of that of the controls. GRAYSON (1954) also observed a considerable decrease in hepatic flow below 60 mm Hg blood pressure. FISCHER (1964) concluded that as hypotension sets in, the hepatic artery supplies the greater part of total hepatic flow. If we compare the above data and the present results we may state that restrictive circulation develops when hepatic blood flow diminishes. Restriction most probably affects not only the portal system but also that of the hepatic artery.

Recently, GRIFFEN et al. (1970) perfused the dog liver in situ with blood containing isotope water through the portal vein and the hepatic artery; LIFSON et al. (1970) did the same through both vessels. The concentration of isotope water varied in different parts of the liver. Restrictive circulation as described by DANIEL and PRICHARD (1951a) was suggested as an explanation; however, the hypothesis could not be proved. This is by no means surprising as the experiments were conducted under normal perfusion pressure and, according to our results, under such conditions restrictive circulation could not have developed.

BRAUER (1963) reviewed the mechanism of restrictive circulation and stated that it was often the result of an active vasoconstriction developing in the system of the portal vein. This could account for the reduced blood supply at the periphery of the lobe, which again could be compensated by the hepatic artery, thus no real ischemia develops in the peripheral parts of the liver. In the present experiments the development of restrictive circulation was inconclusive in response to vasoactive agents (noradrenaline, adrenaline) which elevated systemic blood pressure and also portal pressure significantly and the occasional restrictive effect of which had been shown by portography. Haemorrhage and the administration of vasoactive agents (acetylcholine, histamine, isoproterenol) that caused a fall in systemic blood pressure (irrespective of the changes induced in portal pressure) produced significant differences between the same parameters of blood flow in the central and peripheral areas of the lobe. Circulation of the central part increased as compared to flow in the peripheral part. This change could correspond to the phenomenon of restriction. It seems that a redistribution of hepatic circulation invariably develops when there is a marked fall in systemic blood pressure and that simultaneous portal vasoconstriction contributes to the redistribution. The same concept was advanced by BRAUER (1963). When there is a major fall in blood pressure,

the liver receives mainly arterial blood. As also in this case, blood flow is different in the central and peripheral parts of the lobes, it can be assumed that redistribution affects the system of the hepatic artery, too, with its reflex vasoconstriction as an underlying cause. The involvement of the system of the hepatic artery is probable as nerve fibres of the portal vein joining in a common portal channel divide from the monoaminergic nerve-bundles that follow the course of the hepatic artery, thus the origin of these nerves is common (coeliac ganglia, mesenteric ganglia).

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# INTRARENAL DISTRIBUTION OF VASCULAR RESISTANCE IN THE DOG

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Renal blood flow, glomerular filtration rate and intrarenal pressure gradients have been determined in the normal anaesthetized dog.

1. Under stop flow conditions glomerular capillary pressure is considered to represent the sum of ureter occlusion pressure and of colloid osmotic pressure of the plasma proteins.

2. Under free flow conditions, deep venous pressure is considered to equal renal interstitial and proximal tubular and peritubular capillary pressures.

3. There is a good linear relationship between glomerular filtration rate and effective filtration pressure; the permeability constant amounts to about 2 ml/min/mm Hg/100 g kidney.

4. Variations in mean arterial pressure in various animals do not influence renal blood flow and glomerular filtration rate; the constancy of haemodynamics is ensured by increased total and afferent arterioral vascular resistances.

Renal vascular resistance can be considered as the sum of three series-connected resistances: (1) afferent segment, from the renal artery to the glomerular capillaries; (2) efferent segment, from the glomerular capillaries to the peritubular capillaries; and (3) venular segment, from the peritubular capillaries to the renal vein

$$R_{total} = \frac{P_{art} - P_{gl}}{RBF} + \frac{P_{gl} - P_{ir}}{RBF} + \frac{P_{ir} - P_{ven}}{RBF} = \frac{P_{art} - P_{ven}}{RBF} \qquad (1)$$

where $p_{art}$, $p_{gl}$, $p_{ir}$ and $p_{ven}$ stand for pressures in the renal artery, glomerular capillaries, peritubular capillaries and renal vein, respectively; and RBF stands for renal blood flow.

Experimental evaluation of $p_{art}$, $p_{ven}$ and RBF does not present difficulties; glomerular capillary pressure ($p_{gl}$), however, was not yet measured directly in the mammal. According to WINTON (1937, 1956), HINSHAW (1964), HINSHAW and WORTHEN (1961), HINSHAW et al. (1960) etc., in the dog $p_{gl}$ amounts to about one half to two thirds of arterial pressure. A new technique for determining glomerular capillary pressure in the rat was introduced by GERTZ et al. (1966). A proximal tubule is punctured at a site near to the glomerulus; its distal lumen is closed by a drop of viscous oil. Pressure in the

occluded tubule increases slowly, then stabilizes. It is assumed that the stabilized pressure in the occluded proximal tubule equals the difference between glomerular capillary pressure and colloid osmotic pressure. At arterial pressures below 90 mm Hg, $p_{gl}$ and $p_{art}$ are nearly equal, i.e. the pressure gradient in the afferent arteriola is negligible. At higher arterial pressures, however, $p_{gl}$ is stabilized at about 88 mm Hg, i.e. the higher the arterial pressure, the steeper the pressure gradient in the afferent arteriole.

Peritubular capillary pressure ($p_{ir}$) was measured directly in the rat by GOTTSCHALK (1964), GOTTSCHALK and MYLLE (1956), and WIRZ (1955, 1956). They found that pressure in the lumina of proximal tubuli, in the peritubular capillaries and in kidney tissue (interstitial or intrarenal pressure) is identical in a great variety of experimental situations (e.g. mannitol diuresis, ureteric occlusion, etc.). SWANN et al. (1952) showed in the dog that pressure in the deep renal veins, as measured by a wedged catheter pushed in from the renal vein, is always the same as the simultaneously determined interstitial (needle) pressure of the kidney. It is assumed that pressure in the proximal tubuli and in the peritubular capillaries has to be somewhat higher; otherwise these structures would collapse and urine and blood flow would stop.

In the present study we have attempted to evaluate the intrarenal pressure gradients in the normal dog and to determine the intrarenal resistances connected in series. Glomerular capillary and peritubular capillary pressure was inferred from ureteric occlusion and deep renal venous pressure, respectively.

## Methods

The observations were made on mongrel dogs of both sexes. Food was withheld for 16 hours prior to the experiment; water was allowed ad libitum. Under pentobarbital (0.03 g/kg) anaesthesia the left kidney was approached by laparotomy and the renal vein was connected by means of a plastic tube with the external jugular vein. A T-extension of the tube permitted the direct measurement of renal blood flow and the collection of renal venous blood samples. Suitable peripheral arteries and veins were cannulated for the measurement of arterial pressure ($p_{art}$; mercury manometer), for the withdrawal of arterial blood samples and for infusing the necessary clearance solutions. Coagulation of blood was prevented by heparin. Urine was collected through an ureter catheter.

For measuring pressure in the deep renal veins ($p_{ir}$), a French No. 5 catheter was introduced through a side-arm of the plastic tube and pushed forward into the kidney until it had wedged into one of the deep renal veins. The outer end of the catheter was connected with an Elema-Schönander electromanometer. If the catheter tip was correctly placed in the arcuate vein, the pressure exceeded renal venous pressure ($p_{ven}$) by 15 to 20 mm Hg. The latter pressure was measured by connecting another T-extension of the plastic tube with a glass tube filled with saline.

The plasma levels of PAH and creatinine were ensured by the administration of a preliminary dose and the constant infusion of a maintenance dose. PAH and creatinine content of arterial and renal-venous plasma samples was determined according to SMITH et al. (1945) and BÁLINT and VISY (1965), respectively.

Having completed the surgical procedures, urine was collected for about 10 min ("free flow" period); renal blood flow was repeatedly determined ($RBF_{total}$), and two arterial and renal-venous blood samples were taken. Arterial ($p_{art}$), deep renal ($p_{ir}$) and renal-venous ($p_{ven}$) pressures were read repeatedly.

In the next phase, the ureter was occluded by connecting the outer catheter end with the electromanometer. Ureteral pressure ($p_{ur}$) rose at first with wide oscillations; at pressures of 20 to 25 mm Hg the oscillations stopped, so that ureteral pressure stabilized at about 40 to 70 mm Hg in 10 to 20 min. After ureteral occlusion, i.e. in the stop flow period, intrarenal venous pressure ($p_{in}$) first rose together with ureteral pressure to stabilize at about 15 to 20 mm Hg lower values. RBF was determined repeatedly, blood samples were taken and pressures read.

Creatinine clearance, i.e. glomerular filtration rate (GFR) was calculated by the formula $C_{cr} = RPF \cdot E_{cr}$, where RPF stands for renal plasma flow as determined from RBF measured directly and the haematocrit value; and $E_{cr}$ is the renal extraction ratio of creatinine. Colloid osmotic pressure was inferred from plasma protein content; 4 and 5 and 6% plasma protein corresponds to 15, and 18 and 21 mm Hg colloid osmotic pressure, respectively. Considering the concentration of plasma during its passage through the glomeruli, a correction was applied by multiplying the latter values with 1.06 (when $E_{cr} = 0.1$) or 1.13 (when $E_{cr} = 0.2$), etc.

Statistical evaluation was made by the usual methods, as described by FISHER (1946).

## Results

Pressure measurements were made in 61 dogs. Some parameters of circulation and of renal function of previous and present experiments are summarized in Table I.

**Table I**

*Some parameters of circulation and of renal function in the dog*

(All renal parameters are calculated for 100 g KW)

$\bar{x} \pm s_{\bar{x}}(n)$

| | | | | | |
|---|---|---|---|---|---|
| $P_{art}$ mm Hg | 129 ± 3 | (47) | RPF ml/min | 269 ± 18 | (118) |
| CO l/min/m² BS | 3.63 ± 0.19 | (28) | GFR ml/min | 52 ± 2 | (118) |
| $TPR_{kg}$ | 50.6 ± 2.2 | (28) | $E_{cr}$ | 0.20 ± 0.01 | (118) |
| $RBF_{total}$ ml/min | 451 ± 12 | (118) | $E_{PAH}$ | 0.77 ± 0.01 | (111) |
| RF per cent | 16.5 ± 1.0 | (28) | $Tm_{PAH}$ | 12.3 ± 0.7 | (40) |
| Ht per cent | 41 ± 1 | (118) | V ml/min | 1.27 ± 0.12 | (81) |

KW = kidney weight

Means (and SE) of the various pressures, flows and resistances are presented in Table II. It was presumed that glomerular capillary pressure equals the sum of stabilized ureter occlusion pressure ($p_{ur}$) and colloid osmotic pressure of the plasma proteins ($p_{co}$), i.e. $p_{gl} = p_{ur} + p_{co}$. The validity of this supposition will be discussed later. The $p_{ir}$ values as listed in Table II corresponded to the deep venous pressures under free flow and stop flow conditions. Resistances were calculated by dividing the adequate pressure differences (mm Hg) by RBF (ml/sec/kg-kidney).

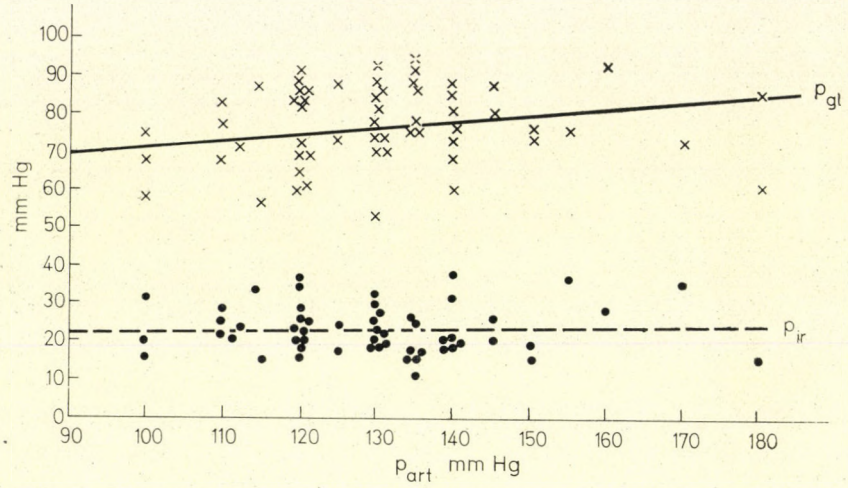


*Fig. 1.* Glomerular capillary pressure (continuous line, crosses) and intrarenal deep venous pressure (broken line, dots) vs. arterial pressure

**Table II**

*Intrarenal pressures and vascular resistances*

Pressure in mm Hg; resistance in mm Hg · sec · $cm^{-3}$ per kg KW

$\bar{x} \pm s_{\bar{x}}$ n = 61

| | Free flow | Stop flow |
|---|---|---|
| $P_{art}$ | 129 ± 2 | 127 ± 3 |
| $p_{gl} = p_{ur} + p_{co}$ | 77 ± 2 | 77 ± 2 |
| $p_{ur}$ | — | 58 ± 2 |
| $p_{ir}$ | 23 ± 1 | 40 ± 2 |
| $p_{ven}$ | 7 ± 1 | 7 ± 1 |
| $p_{co}$ | 19 ± 1 | 19 ± 1 |
| RBF ml/min/100 g KW | 464 ±16 | 464 ±16 |
| GFR ml/min/100 g KW | 56 ± 2 | 42 ± 2 |
| $R_{total}$ | 1.74± 0.08 | 1.70± 0.08 |
| $R_{aff}$ | 0.76± 0.06 | 0.76± 0.06 |
| $R_{eff}$ | 0.74± 0.03 | 0.23± 0.02 |
| $R_{ven}$ | 0.24± 0.01 | 0.71± 0.13 |
| $R_{aff}$% | 42 ± 1 | 45 ± 1 |
| $R_{eff}$% | 44 ± 1 | 14 ± 1 |
| $R_{ven}$% | 14 ± 1 | 41 ± 1 |

KW = kidney weight

In Fig. 1 the individual glomerular capillary and deep venous pressures vs. arterial pressure are presented. The increase of $p_{gl}$ with $p_{art}$ was just significant ($p < 0.05$) but $p_{ir}$ was constant over the whole range of arterial pressures examined. Individual RBF and GFR values scatter widely, being independent of arterial pressure. The corresponding regression equations are presented in Table III.

**Table III**

*Regression equations between various free flow parameters*

| Regression equation between | | n | $Y = a + b\ (\pm s_b)\,x$ | p |
|---|---|---|---|---|
| x | y | | | |
| $p_{art}$ | $p_{gl}$ | 61 | $Y = 57 + 0.152(\pm 0.073)\,x$ | + |
| $p_{art}$ | $p_{ir}$ | 61 | $Y = 21 + 0.016(\pm 0.14)\,x$ | neg |
| $p_{art}$ | RBF | 61 | $Y = 559 - 0.74(\pm 0.86)\,x$ | neg |
| $p_{art}$ | GFR | 61 | $Y = 64 - 0.062(\pm 0.11)\,x$ | neg |
| $p_{art}$ | $R_{total}$ | 61 | $Y = -0.63 + 0.0184(\pm 0.003)\,x$ | ++++ |
| $p_{art}$ | $R_{aff}$ | 61 | $Y = -1.26 + 0.0157(\pm 0.003)\,x$ | ++++ |
| $p_{art}$ | $R_{eff}$ | 61 | $Y = 0.53 + 0.0016(\pm 0.0015)\,x$ | neg |
| $R_{postglom}$ | $E_{cr}$ | 61 | $Y = 0.10 + 0.11(\pm 0.023)\,x$ | ++++ |
| BW kg | KW g | 94 | $Y = 12 + 4.33(\pm 0.25)\,x$ | ++++ |
| Stop flow parameters: | | | | |
| $p_{ur}$ | $p_{ir}$ | 61 | $Y = -3 + 0.75(\pm 0.064)\,x$ | ++++ |

neg: $p > 0.05$; +: $p < 0.05$; ++++: $p < 0.001$
BW = body weight; KW = kidney weight

It follows from the above considerations that renal vascular resistance increases with arterial pressure (Fig. 2). The increase in afferent arteriolar resistance was highly significant but the efferent arteriolar and venular resistances did not change significantly.

The creatinine extraction ratio which is equal to the filtration fraction changed significantly with the absolute value of postglomerular resistance; there was, however, no correlation between $E_{cr}$ and $R_{postglomerular}/R_{total}$.

The relation of intrarenal to ureter pressure under stop flow conditions is shown in Fig. 3 [$Y = -3 + 0.75\ (\pm 0.064)\,x$]. Mean ureteral pressure equalled exactly the sum of mean intrarenal and colloid osmotic pressures: $p_{ir} + p_{co} = 58 \pm 2$; $p_{ur} = 58 \pm 2$.

Intrarenal distribution of blood flow in the normal dog as estimated by the simultaneous determination of total (direct measurement) and nutrient ($^{86}$Rb-fractionation) blood flow was shown previously (BÁLINT et al. 1969). In

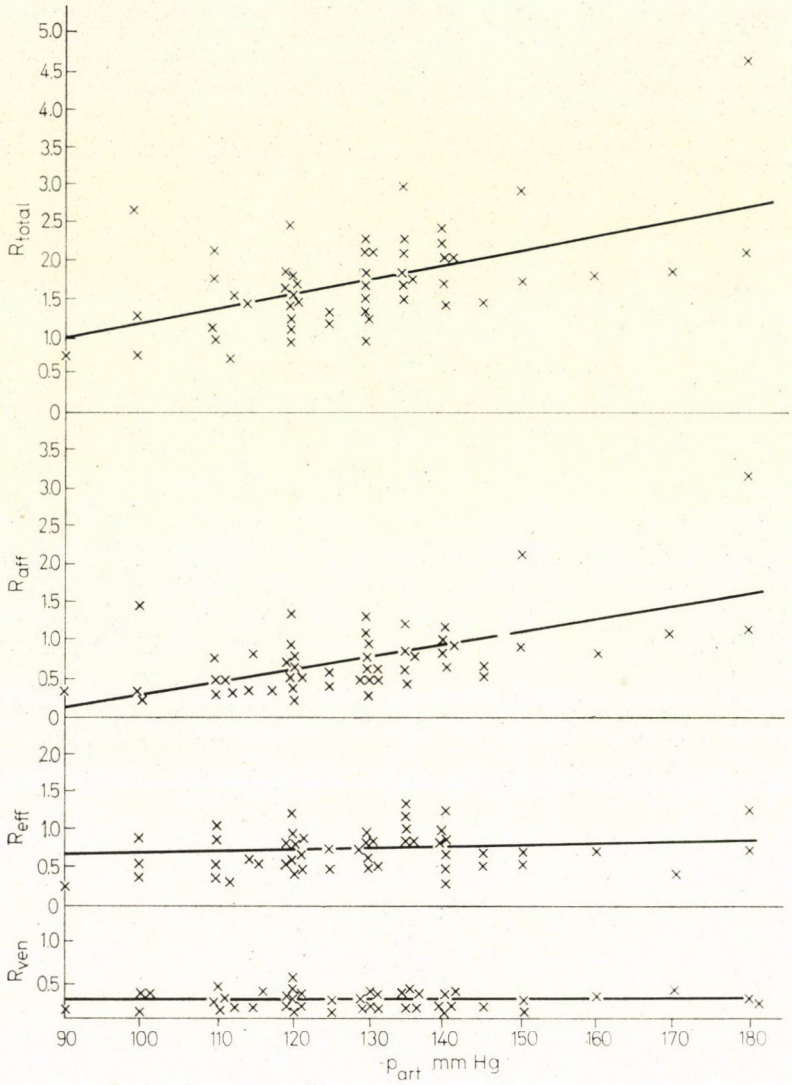


*Fig. 2.* Total ($R_{total}$), afferent ($R_{aff}$), efferent ($R_{eff}$) and venular ($R_{ven}$) vascular resistances vs. arterial pressure

Table IV the means of our completed data are presented; total, nutrient, cortical, outer and inner medullary flows are tabulated. On the basis of the Rb-fractionation values the resistances connected in parallel can be calculated. It follows from the laws of hydrodynamics that the sum of reciprocal parallel resistances equals the reciprocal of total resistance. Thus, the means for the various partial blood flows per 100 ml total blood flow correspond to the

relative conductances (= reciprocal resistance). The completed data thus have confirmed our previous conclusions in that (1) in the normal kidney of the dog, non-nutrient blood flow is negligible; and (2) cortical/total blood flow (0.77) is identical with the extraction ratio of PAH (0.77).

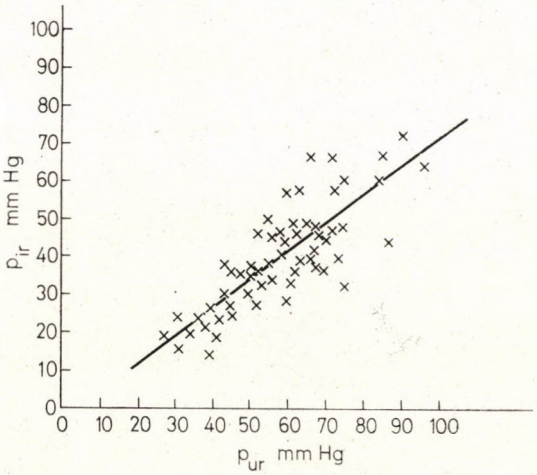


*Fig. 3.* Intrarenal deep venous pressure ($p_{in}$) vs. ureteric occlusion pressure. (Stop flow periods)

**Table IV**

*Intrarenal distribution of blood flow in the dog*

$\bar{x} \pm s_{\bar{x}}$; n = 20

| | ml/min per 100 g kidney | ml/min per 100 ml | |
|---|---|---|---|
| | | $RBF_{total}$ | $RBF_{nutr}$ |
| $RBF_{total}$ | 471±31 | *100* | |
| $RBF_{nutr}$ | 437±26 | 93.6±2.6 | *100* |
| $RBF_{cort}$ | 352±20 | — | 84.3±1.7 |
| $RBF_{om}$ | 67±6 | — | 15.2±0.7 |
| $RBF_{im}$ | 13±2 | — | 2.8±0.3 |
| $RBF_{non\text{-}nutr}$ | 30±14 | 6.4±2.6 | — |
| $\frac{RBF_{nutr}}{RBF_{total}}$ | 0.94±0.02 | — | — |
| $\frac{RBF_{cort}}{RBF_{total}}$ | 0.77±0.02 | — | — |

## Discussion

### *I. Evaluation of the various intrarenal pressures*

The relation of glomerular filtration rate (GFR) to effective filtration pressure ($p_{eff}$) can be expressed by the equation,

$$GFR = k\ (p_{gl} - p_{ir} - p_{co}) = k \cdot p_{eff}, \qquad (2)$$

where $p_{gl}$, $p_{ir}$ and $p_{co}$ stand for glomerular capillary, intrarenal (= intracapsular), and colloid osmotic pressures, respectively; k is a proportionality constant the value of which corresponds to the quantity (ml) of glomerular filtrate formed in 100 g kidney at 1 mm Hg effective filtration pressure. Constant k is related to the area and the permeability of the glomerular capillary walls.

*1. Deep venous pressure under free flow conditions.* Micropuncture experiments have shown that deep venous pressure practically equals renal interstitial and proximal tubulary and peritubular capillary pressures in many experimental situations (GOTTSCHALK 1964; GOTTSCHALK and MYLLE 1956; SWANN et al. 1952; WIRZ 1955, 1956, etc.). Our data correspond well with data in literature.

*2. Stabilized ureteral pressure under stop flow conditions.* Pressure rises are gradually following occlusion until constant values have been reached. Oscillations are due to ureteral peristalsis and cease at about 20 to 30 mm Hg (BÄCKLUND 1963). It was shown by micropuncture experiments that in the rat stabilized ureteral pressure is practically identical with the simultaneously measured proximal tubular pressure (SELKURT et al. 1965). It seems justified to suppose that pressure is equalized in the system beginning with Bowman's capsule and ending with the ureter catheter.

According to the classical concept, ureteral pressure under stop flow conditions becomes constant when pressure in Bowman's capsule equals the difference between glomerular capillary and colloid osmotic pressures, i.e. $p_{eff} = 0$. GERTZ et al. (1966) consider the difference between stop flow and free flow proximal tubular pressures identical with effective filtration pressure in the rat. In the dog, however, no direct measurements were made. Nevertheless, the sum of stabilized ureteral occlusion pressure and of colloid osmotic pressure is regarded as a measure of glomerular capillary pressure (BÄCKLUND 1963; GERTZ et al. 1966, 1969; GÓMEZ 1951; HINSHAW 1964; HINSHAW and WORTHEN 1961; HINSHAW et al. 1960; RISHOLM et al. 1959; SCHIRMEISTER et al. 1962; SELKURT et al. 1965; WINTON 1956; etc.).

This concept could not be challenged if under steady state stop flow conditions the filtration rate dropped to zero. Many authors, however, have shown that filtration continues at decreasing intensity even when ureteral

pressure is already maximum and constant (OMACHI and MACEY 1959; TAYLOR and ULLMANN 1961; SCHIRMEISTER et al. 1962; BÁLINT and FORGÁCS 1964; HARZA et al. 1967; etc.). A steady state ensues in which active tubular reabsorption is taking place and the reabsorbed fluid is being replaced by newly formed filtrate (reabsorption-replacement). This process is limited by the increased concentration of non-reabsorbable solutes in the tubular fluid.

Thus the classical concept has to be completed and/or modified. If the view is accepted that under stop flow conditions ureteral pressure equals proximal tubular pressure, the driving force of the residual filtration remains to be explained. Under free flow conditions glomerular filtration is "modellized" by ultrafiltration. Under stop flow conditions the driving force of reabsorption-replacement is active tubular reabsorption, but the factors determining the filtration equilibrium are not known. It is conceivable that under steady state stop flow conditions $p_{gl} > p_{ur} + p_{co}$, but the difference in pressure is not necessarily equal to that generally supposed to prevail under free flow conditions.

Considering the above facts we suppose that the equality $p_{gl} = p_{ur} + p_{co}$ is highly probable but in lack of direct evidence cannot be considered more than a productive working hypothesis. A second supposition concerns the presumed equality of glomerular capillary pressure under stop flow and free flow conditions. It should be stressed that all the above authors including ourselves take it for granted that glomerular capillary pressure is not changed by the occlusion.

The validity of our working hypothesis is supported by two kinds of deduction.

*a)* Equation (2) contains two data (k and $p_{gl}$) which are not accessible to direct determination. We have solved the equation by substituting $p_{ur} + p_{co}$ for $p_{gl}$ and have calculated the value of k for each experiment. We have found $k = 1.93 \pm 0.13$, i.e. 1 mm Hg effective filtration pressure leads to the formation of about 2 ml/min filtrate in 100 g kidney. Considering the structure of the glomerular membrane and taking into account Poiseuille's law, PAPPENHEIMER (1955) calculated the value of k and found 2 to 4 ml/min. The agreement between PAPPENHEIMER's calculated and our experimentally observed values seems satisfactory.

*b)* The relation of GFR to $p_{eff}$ (free flow conditions) is presented in Fig. 4. There is a very good linear relationship between the two values with a regression equation $Y = 11 + 1.44\ (\pm 0.07)\,x$. The regression line should, of course, intersect the origo but the deviation is not disturbing if we consider the possible errors of the various measurements. The linear relationship between GFR and $p_{eff}$ is most reassuring as one component of $p_{eff}$, i.e. $p_{gl}$, was calculated by determining $p_{ur}$ under stop flow conditions, whereas GFR and $p_{ir}$ represent free flow conditions.

*3. Deep venous pressure under stop flow conditions.* Average stabilized deep venous pressure following ureteric occlusion is 18 mm Hg less than ureteral pressure under the same conditions (Fig. 3). The difference can be explained by supposing that between peritubular capillaries and the renal interstitium there can exist a stop flow filtration-reabsorption equilibrium only if capillary hydrostatic pressure is equal to the sum of interstitial and colloid osmotic pressures (SMITH 1951; GÓMEZ 1951; HINSHAW et al. 1960; etc.).

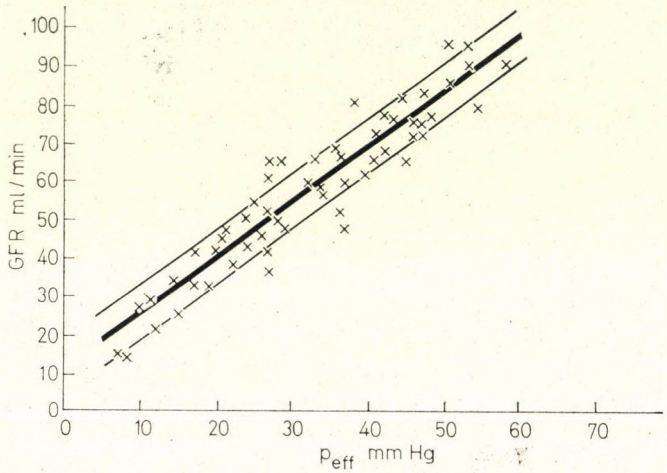


*Fig. 4.* Glomerular filtration rate vs. effective filtration pressure. Single experiments (crosses), and regression line with S. D.

As proximal tubular and peritubular capillary pressures are equal even after occlusion of the ureter (GOTTSCHALK and MYLLE 1956), the conclusion seems justified that deep venous pressure under stop flow ($p_{ir\text{-}stop}$) measures renal interstitial pressure, i.e. $p_{ur} = p_{peritub.cap} = p_{ir\text{-}stop} + p_{co}$.

## *II. Evaluation of intrarenal pressure gradients*

Mean arterial pressure of our dogs considered "normal" varied between 90 and 180 mm Hg. Glomerular capillary pressure (ureteric occlusion pressure) was slightly, while peritubular capillary pressure (free flow deep venous pressure) not at all influenced by the actual mean arterial pressure. There is no correlation between arterial pressure, on the one hand, and renal blood flow and glomerular filtration rate, on the other. The constancy of RBF with increased arterial pressure can be explained only by a compensatory increase in renal vascular resistance. The constancy of GFR speaks for an increase in afferent resistance. These suppositions have unequivocally been proved by our

experiments. Total and afferent resistances increased linearly with arterial pressure; there was, however, no significant change in efferent and venular resistances. A schematic drawing of the intrarenal pressure gradient is shown in Fig. 5; the higher the arterial pressure, the steeper the preglomerular (afferent) pressure gradient; efferent and venular gradients are not influenced by the level of arterial pressure.

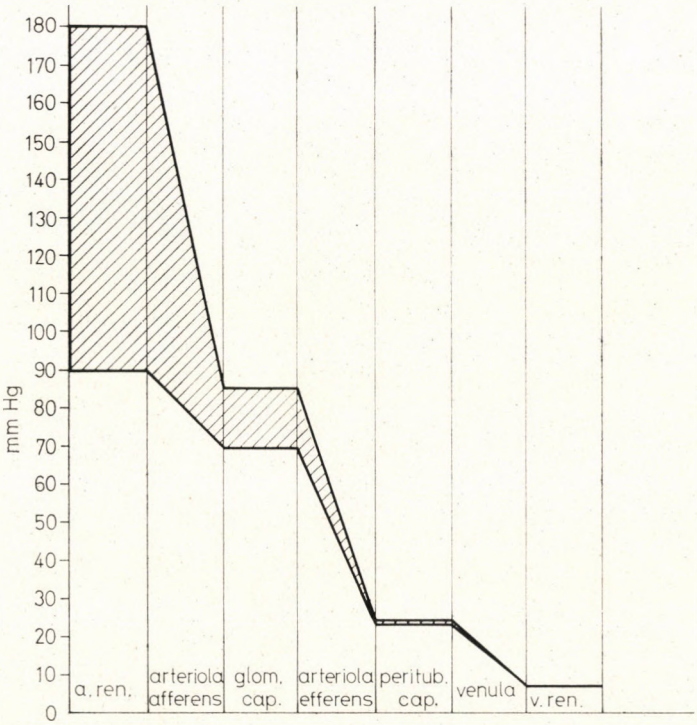


*Fig. 5.* Schematic diagram of intrarenal pressure gradients

The constancy of RBF and GFR with increasing arterial pressure in the same animal is denoted as the "autoregulation" of renal circulation. GERTZ et al. (1966) have shown in the rat that the increase in vascular resistance is localized in the afferent arterioles; in the dog, THURAU and WOBER (1962), THURAU (1964), THURAU and HENNE (1964) found that the increased resistance can be localized to the precapillary (afferent + efferent) arterioles. According to our findings, the term "autoregulation" may be extended; RBF and GFR are independent of arterial pressure even in different animals. The "myogenic" theory of autoregulation is reinforced by our data and increased intrarenal pressure cannot be considered the cause of autoregulation.

## Acknowledgement

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# DATA ON THE NEUROHUMORAL ORGANIZATION OF VASOMOTOR REFLEX MECHANISMS

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Curare sensitivity of the various vasomotor reflexes was studied in anaesthetized rabbits and cats.

It was found that 150—200 μg/kg d-tubocurarine pentahydrate given intravenously invariably inhibited or abolished the vasodepressor reflexes of somatic origin in the rabbit but did not affect or slightly enhanced those of visceral origin (vagal, depressor).

Curare reversed the vagal pressor reflexes in the rabbit.

Somatic and visceral depressor reflexes of the cat were not sensitive to curare. Pressor reflexes evoked by the stimulation of the central vagal or median stump were inhibited or even reversed by 150—300 μg/kg curare.

Curare in doses too low to induce ganglionic blockade did not inhibit the carotid sinus pressor reflex in either of the species.

It is assumed that cholinergic factors may be involved in the central mechanism of the affected reflexes.

It has been shown (SUCH 1959) that depressor reflexes evoked by acoustic and pain stimuli decreased or abolished after the intravenous administration of 200 to 400 μg/kg d-tubocurarine chloride in the urethan-anaesthetized rabbit. Under curarization, the effect of an intensive pain stimulus became first biphasic, then developed into a pressor effect. These observations could not be related to a myorelaxant action, thus it was supposed that curare abolished the inhibition of the vasomotor centre. Similar observations were made by MCLENNAN (1961) when the auricular nerve was stimulated in pentobarbital anaesthesia.

Studies on the functional organization of the vasomotor system yielded evidence suggesting that the individual vasomotor reflexes had a different neural organization (COMROE and MORTIMER 1964; REIS and CUENOD 1965; DALY and UNGAR 1966; HUMPHREY 1967; SUCH 1970). It therefore seemed obvious that these differences have to be reflected in the neurohumoral organization of the single reflex mechanisms. The following literary data support this hypothesis. WEIDMANN and CERLETTI (1958) found that LSD inhibited the carotid sinus pressor reflex and at the same time enhanced the blood pressure increase elicited from the sciatic nerve. According to SCHMITT and SCHMITT (1964) LSD affects in a different manner the pressor responses of hypothalamic and those of bulbar origin. PEISS and MANNING (1959) observed differences in

the curare sensitivity of pressor responses evoked by the stimulation of the medulla or the hypothalamus. PÓRSZÁSZ and P. GIBISZER (1969) found that inhibitions of the efferent sympathetic activity induced by the stimulation of the central stump of the sciatic or the vagus nerve were not equally sensitive to alpha adrenergic blocking agents.

Based on our earlier observations, comparative studies have been undertaken to investigate the effect of curare on certain vasomotor reflexes.

## Methods

Twenty rabbits of either sex weighing 2.5 to 3.5 kg and 15 cats of either sex weighing 2.5 to 4.5 kg were used in the experiments. Depending on the actual aim of the experiment, the animals were anaesthetized with urethan, pentobarbital, chloralose + urethan or chloralose + + pentobarbital. The doses applied are indicated in the Figures. Blood pressure was measured in the femoral artery, respiration was recorded by a Marey capsule through an intratracheal tube. Both values were recorded kymographically. The animals were vagotomized bilaterally. Vasomotor reflexes were elicited by square wave stimulation of the central stump of the femoral, vagal, depressor and median nerves for 5—10 seconds and by the occlusion of the common carotids for 10—15 seconds. The nerves attached to the electrodes were covered with paraffin oil of body temperature. Curare was administered intravenously in the form of d-tubocurarine pentahydrate. After respiratory paralysis, respiration was maintained with a Starling pump.

## Results

### *I. Curare sensitivity of vasomotor reflexes in the rabbit*

*a) Vasodepressor reflexes.* Fig. 1 shows the effect of curare on the vasodepressor reflexes of somatic and visceral origin. "A" illustrates conditions at the beginning of the experiment. Four definite somatic depressor responses are seen elicited by stimulation at 10 and 100 c/s and 1 and 3 V respectively. Vagal depressor reflexes obtained at the same stimulation parameters are shown subsequently. After the intravenous administration of 200 $\mu$g/kg d-tubocurarine pentahydrate, spontaneous respiration ceased and respiration was maintained artificially. "B" illustrates the responses recorded a few minutes after curare administration. Somatic responses elicited by a stimulation of 1 V disappeared, those of 3 V were inhibited. Visceral depressor reflexes remained practically unchanged. "C" shows the responses recorded 30 minutes after curarization. Stimulation of the femoral nerve with 3 V elicited a slight depressor response at 100 c/s; vagal responses were still expressed. Increasing the voltage of stimulation to 5 V, curare blockade was somewhat relieved.

Fig. 2 illustrates similar experiments. A marked vagal depressor response could be elicited only at 100 c/s and 3 V, at the same time the somatic depressor mechanism was very active. After the administration of 150 $\mu$g/kg curare, blood pressure increased by about 25 mm Hg and then showed a slow decreasing tendency. Stimulation of the femoral nerve at the same frequency

and voltage as was done before the administration of curare had practicaly no effect. The previously vague vagal response changed into a definite depressor response.

Similarly to the vagal depressor reflexes the blood pressure decrease induced by depressor nerve stimulation was also unresponsive to curare.

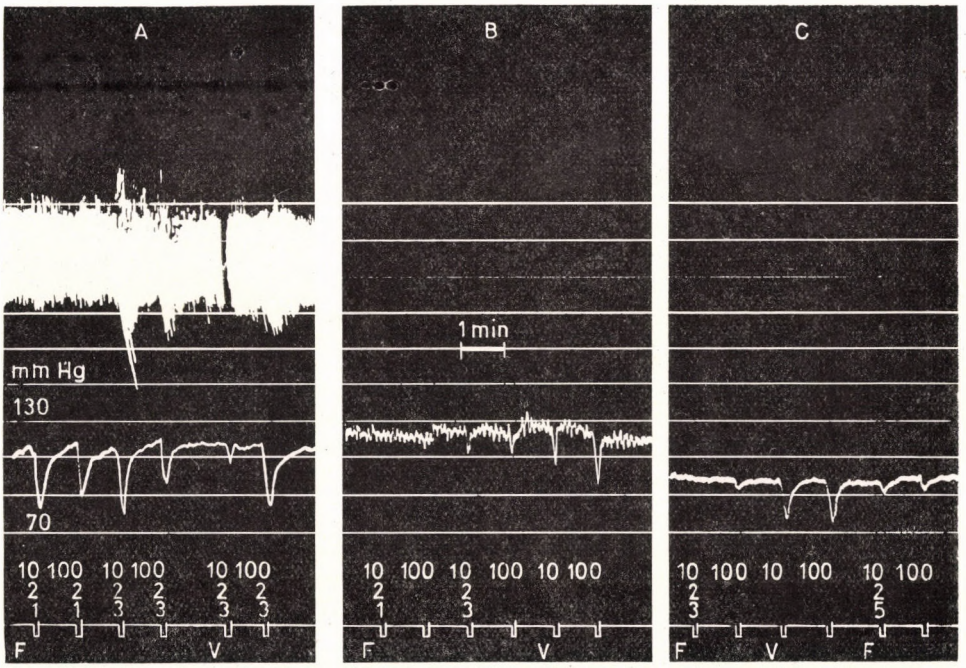


*Fig. 1.* Rabbit, 3.4 kg. Urethan 1.2 g/kg intravenously. Effect of curare on the vasodepressor reflexes elicited by the stimulation of the central femoral (F) and the vagal (V) stump. "A": basic; "B": 5 minutes after the intravenous administration of 200 $\mu$g/kg d-tubocurarine pentahydrate; "C": at 30 minutes. Above: respiration. Below: blood pressure. Bottom line: duration and stimulation parameters (c/s, msec, V). Parameters are indicated only where changed. For explanation see text

As the effect of curare subsided, the inhibited somatic depressor reflexes gradually returned parallel with the restoration of spontaneous respiration.

*b) Vasopressor reflexes.* Vagal stimulation often evoked a pressor response, especially at higher frequencies. Fig. 3 demonstrates that whereas stimulation with 10 c/s caused a blood pressure decrease, on stimulation with 100 c/s blood pressure was increased. After the administration of 150 $\mu$g/kg curare, the somatic depressor responses were inhibited, the vagal effect at 10 c/s was accentuated and the pressor reflex was reversed. In Fig. 4 the vagus also showed a pressor

tendecy. However, after the administration of curare a vagal depressor response developed. The figure also demonstrates that somatic depressor reflexes become ineffective. When stimulus intensity was increased to 10 V, the response became biphasic with a pressor excess. No such voltage was applied before the administration of curare, but a blood pressure increase can rarely be induced by somatic stimulation in anaesthetized rabbits. McLennan (1961) reported on similar observations. Our earlier studies have shown (Such 1959) that noci-

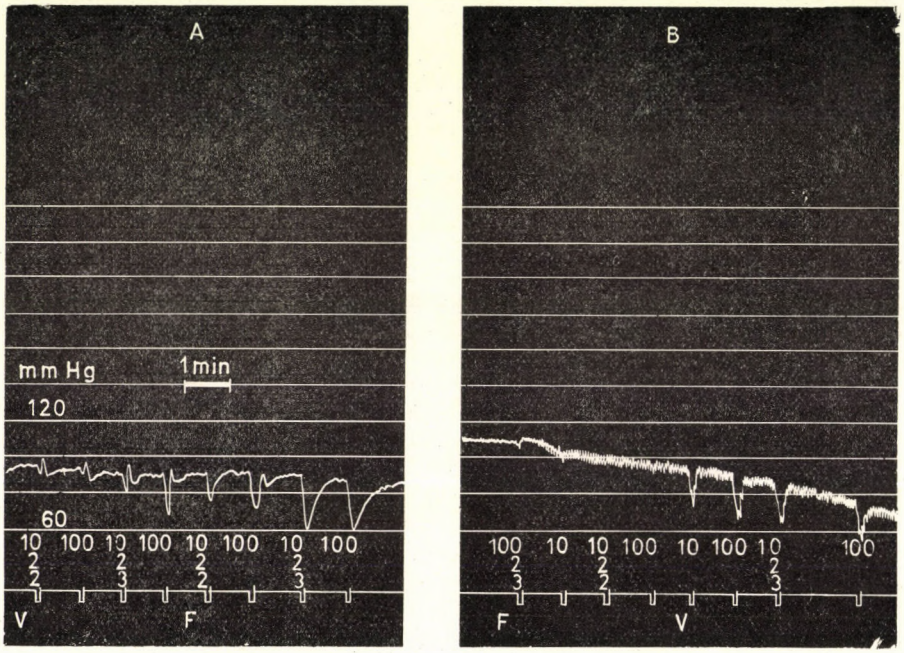


*Fig. 2.* Rabbit, 2.3 kg. Urethan 1 g/kg intravenously. Changes in the vagal and femoral vasomotor reflexes after the administration of 150 μg/kg curare. Somatic depressor reflexes are inhibited, biphasic vagal responses change into intensive depressor reflexes. Signs as in Fig. 1

ceptive stimuli inducing a marked blood pressure decrease in the anaesthetized animal gradually change into pressor reflexes under the effect of curare. Fig. 5 shows the continuation of the former experiment. After the administration of 100 μg/kg neostigmine spontaneous respiration was restored and the depressor reflexes abolished by curare appeared again. The original vagal pressor responses failed to return and the depressor ones were enhanced under the effect of neostigmine.

Whereas curare invariably reversed the vagal pressor reflex it failed to affect the carotid occlusion reflex (Fig. 6).

The above phenomena could always be observed in anaesthetized and precollicularly decerebrated rabbits but in older animals intravenous curare sometimes proved to be ineffective.

*II. Curare sensitivity of vasomotor reflexes in the cat*

In order to clarify the eventual species specificity of the different effects of curare on the different vasomotor reflexes, experiments were made on cats.

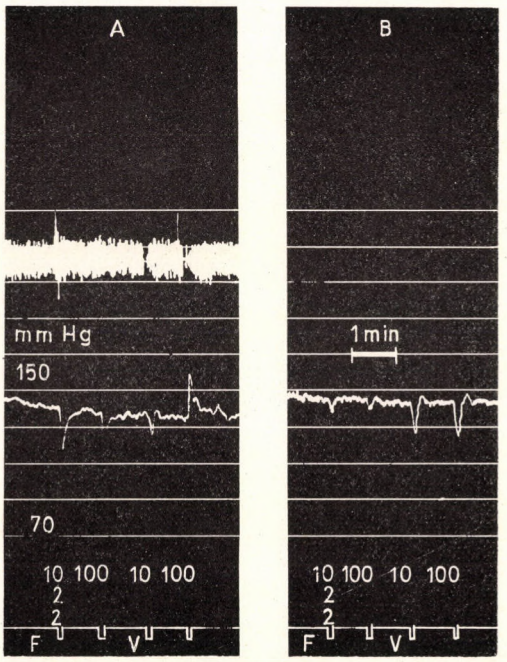


*Fig. 3.* Rabbit, 2.6 kg. Urethan 1 g/kg intravenously. Femoral and vagal vasomotor reflexes before and after the administration of 150 μg/kg curare. Beside the responses illustrated in Figs 1 and 2, it can be seen that the pressor reflex elicited by vagal stimulation at 100 c/s turns into a depressor reflex

Fig. 7 shows that $2 \times 300$ μg/kg curare given to the cat under the above experimental conditions affected the depressor responses and the carotid sinus pressor reflexes only in proportion to the intensity of ganglionic blockade, i.e. to the decrease in basic blood pressure. The elective effect recorded in the rabbit could not be observed. In contrast, certain pressor reflexes were often inhibited. In Fig. 8 it is seen that the individual pressor reflexes did not change parallel with the blood pressure level. The carotid pressor response to 150 μg/kg curare was still unchanged when the vagal and median pressor

responses were already somewhat decreased ("B"). After a second dose of 150 μg/kg curare, blood pressure was established at a low level due to ganglionic blockade ("C"). Though all the three pressor reflexes were weaker, the difference in their intensity increased further in favour of the carotid response. The carotid reflex had become practically normal 25 minutes after the second dose of curare whereas the other two reflexes still showed an about 70% inhibition ("D"). After the 35th minute ("E") when the median response was nearly normal, the vagal response was still inhibited (45%).

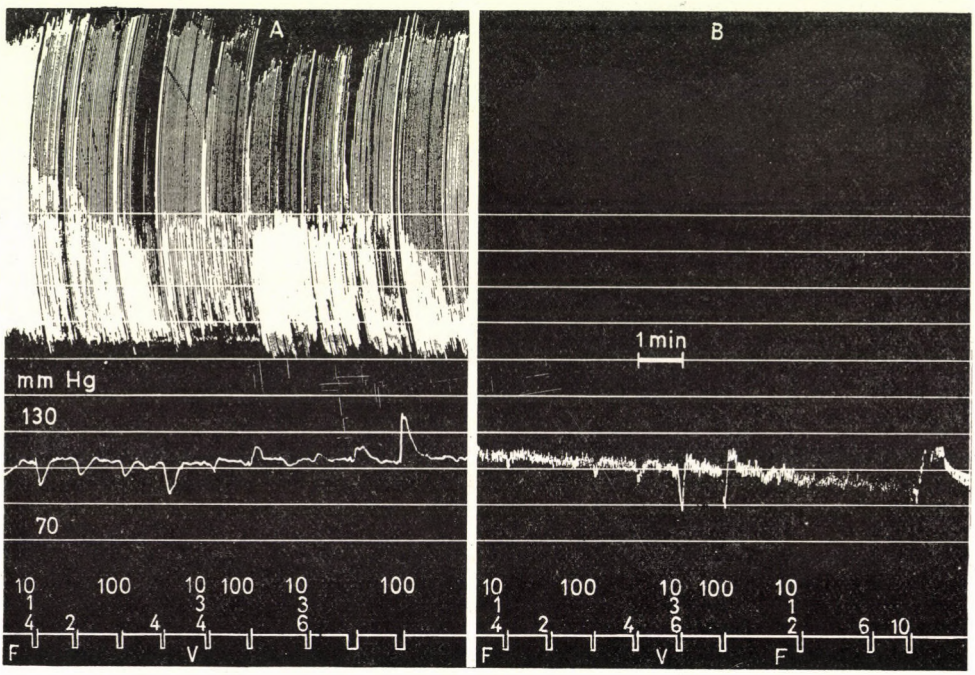


*Fig. 4.* Rabbit, 2.5 kg. Pentobarbital 45 mg/kg intraperitoneally. After 150 μg/kg curare increasing inhibition of somatic depressor reflexes and reversal of vagal pressor responses Stimulation at 10 V elicits biphasic response with pressor excess

Fig. 9 illustrates a similar experiment performed under chloralose anaesthesia. On the administration of 300 μg/kg curare the median and vagal pressor responses were reversed, the carotid pressor reflex was unaffected. After spontaneous respiration had been restored ("C") the median pressor response returned, the vagal depressor response continued to persist. It should be mentioned that PEISS and MANNING (1959) observed an inhibition or reversal of pressor responses elicited by the stimulation of the medullary area under the effect of curare.

Thus, curare has either no effect on the basic blood pressure of rabbits or else slightly increases it while in the cat, curare rather decreases basic blood pressure. The difference might be attributed to the different susceptibility of the ganglionic structures in the two species. Our observations indicate that the rabbit is rather resistant against a ganglionic blocking effect; administration of $3 \times 2$ mg/kg hexamethonium in 5-minute intervals brought about a slight decrease in blood pressure and the vasomotor reflexes studied could be evoked at almost the same intensity.

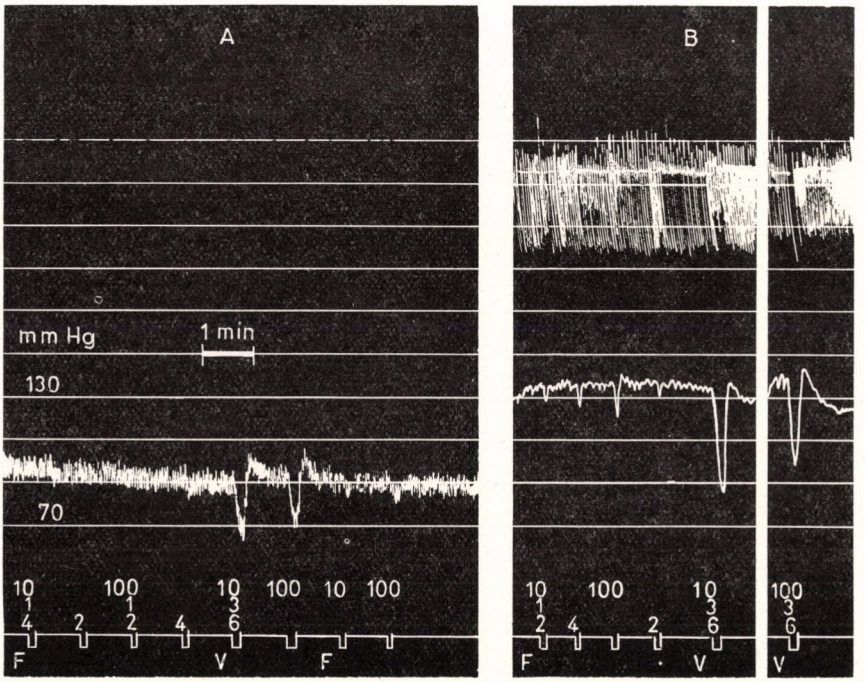


*Fig. 5.* Same rabbit as in Fig. 4. "A": Stimulation of the femoral and vagus nerves 20 minutes after the administration of curare. "B": in response to 100 $\mu$g/kg neostigmine spontaneous respiration is restored, the inhibited depresser reflexes reappear, vagal responses are enhanced

## Discussion

In the rabbit, curare invariably inhibited or abolished the vasodepressor reflexes of exteroceptive origin but had no effect on visceral responses. This elective effect could not be demonstrated in the cat.

As concerns pressor reflexes, curare in doses too low to induce ganglionic blockade had no effect on the carotid sinus pressor response in either species.

Curare always reversed the blood pressure increase of vagal origin in the rabbit while in the cat it inhibited or even reversed it (see Fig. 9). The somatic pressor reflex behaved similarly in the cat, with the difference that the effect was of shorter duration.

A somatic pressor response to femoral nerve stimulation could not be elicited in the rabbit before the administration of curare. However, after the administration of curare intensive stimuli often induced a pressor response.

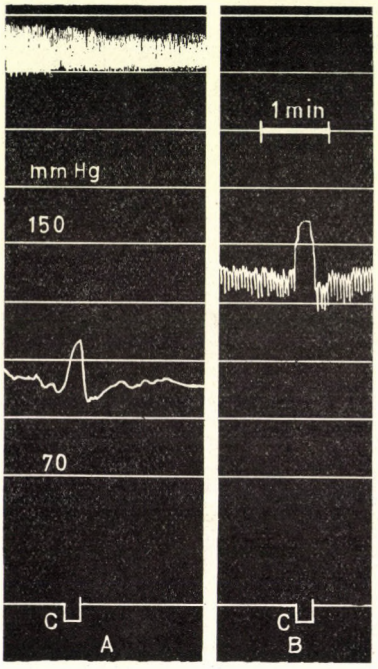


*Fig. 6.* Rabbit, 2.7 kg. Urethan 1 g/kg intravenously. Spontaneous respiration, carotid pressor reflex after 200 μg/kg intravenous curare

Consequently, our results supply but indirect evidence of the somatic pressor reflexes of the rabbit not being sensitive to curare. This view is supported by some old data showing too that stimulation of the somatic afferents induced only blood pressure increase in the unanaesthetized curarized animal, whereas in the anaesthetized one the same stimulus evoked a depressor response (CYON 1871; HUNT 1895). On the other hand, MCLENNAN (1961) could show electrophysiologically that after curarization efferent sympathetic activity was enhanced by somatic stimulation.

In the present experiments curare is assumed to have exerted a central effect, although ECCLES et al. (1956) maintain that under normal conditions

curare does not penetrate through the blood-brain barrier and COHEN (1963) also demonstrated that the drug does not pass into the CSF. In contrast, GRUNDFEST (1957), PURPURA and GRUNDFEST (1957) observed that curare given intravenously or into the carotid blocked the cortical dendritic synapses. Their experiments indicate that this effect was mainly affecting the inhibitory synapses. The findings of MCLENNAN (1961), PEISS and MANNING (1959) also suggest a central effect of curare. MURATOV (1967), further KHARKEVICH et al.

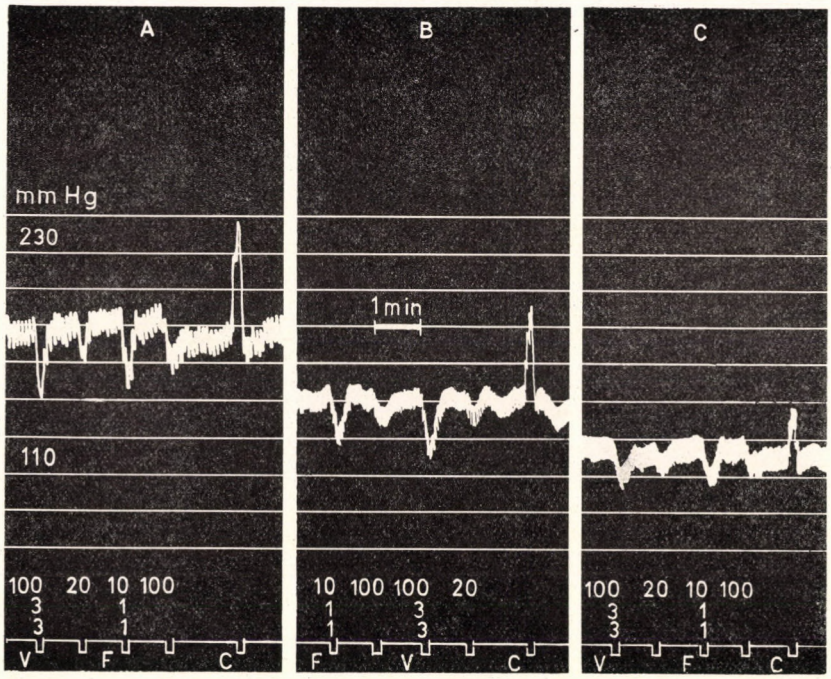


*Fig. 7.* Cat, 3 kg. Anaesthesia with 50 mg/kg chloralose +250 mg/kg urethan intraperitoneally. Vagal and femoral depressor reflexes, carotid pressor reflexes ("C") before ("A") and after the effect of $2 \times 300$ $\mu$g/kg curare ("B", "C"). Inhibition of the reflexes is proportionate to the decrease in blood pressure

(1969) assume that non-depolarizing curare-like agents inhibit the vasomotor reflexes of the cat besides by their ganglionic blocking action, also by their central point of attack.

Thus, the penetration of curare through the blood-brain barrier may be assumed. Species differences certainly exist and differences may be present within one and the same species, too. This could account for the fact that curare sometimes proved to be ineffective in older rabbits.

Our experiments indicate that curare affects the different vasomotor reflexes of the two species in a complex manner. The problem can be simplified if we assume that "nicotinic" cholinergic factors are involved in the reflex mechanisms sensitive to curare. The presence of cholinergic factors in the

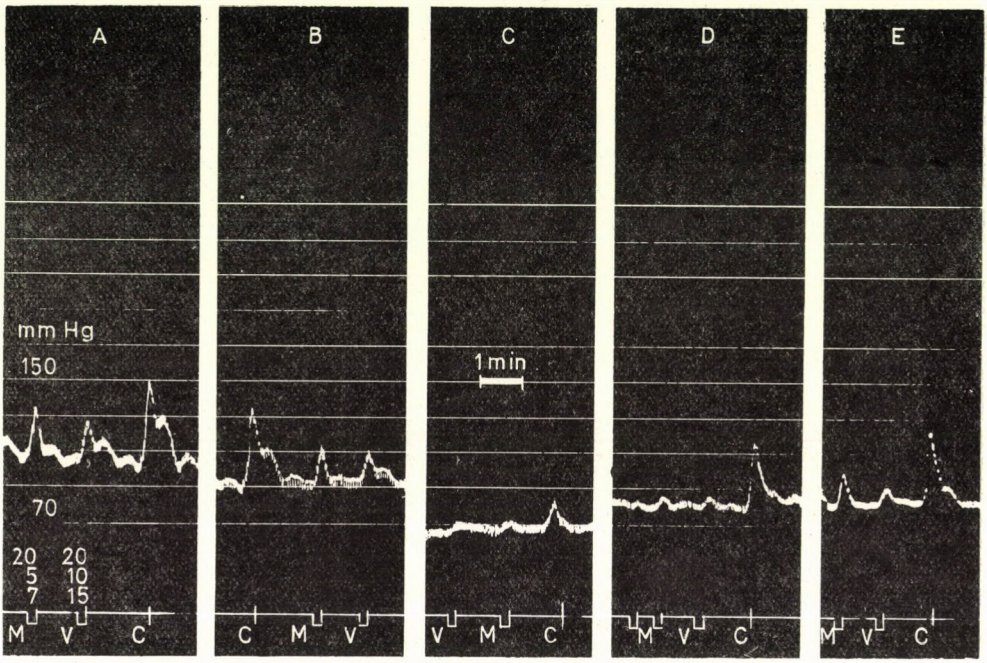


*Fig. 8.* Cat, 2.6 kg. Anaesthesia with 50 mg/kg chloralose +45 mg/kg pentobarbital intraperitoneally. Changes of the median (M), vagal (V) and carotid (C) pressor reflexes on 2×150 μg/kg intravenous curare ("B", "C"). The different sensitivity to curare of the pressor reflexes is well discernable and specially apparent after the second curare dose ("D"). Basic values: "A". For detailed evaluation, see text

central vasomotor areas is indicated also by some literary reports (SINHA et al. 1967; POVZHITKOV 1968; CHANDRA et al. 1969).

The present results further the observations on the different pharmacological sensitivity of the various vasomotor responses (WEIDMANN and CERLETTI 1958; SCHMITT and SCHMITT 1964; PEISS and MANNING 1959; PÓRSZÁSZ and P. GIBISZER 1969) supply only indirect evidence of the specific features of the central neurohumoral organization of the individual vasomotor reflex mechanisms. With systemic administration of drugs the possibility of a multiple action can never be excluded. In the case of curare its penetration into the central nervous system is especially problematic. Neither can the fact be disregarded that the different stimulations always induce an inhomogeneous (double) nerv-

vous process in the CNS, the equilibrium of which produces the actual vasomotor response (Such 1971). The different sensitivity of the individual components may equally result in an enhancement, weakening, or reversal of the response.

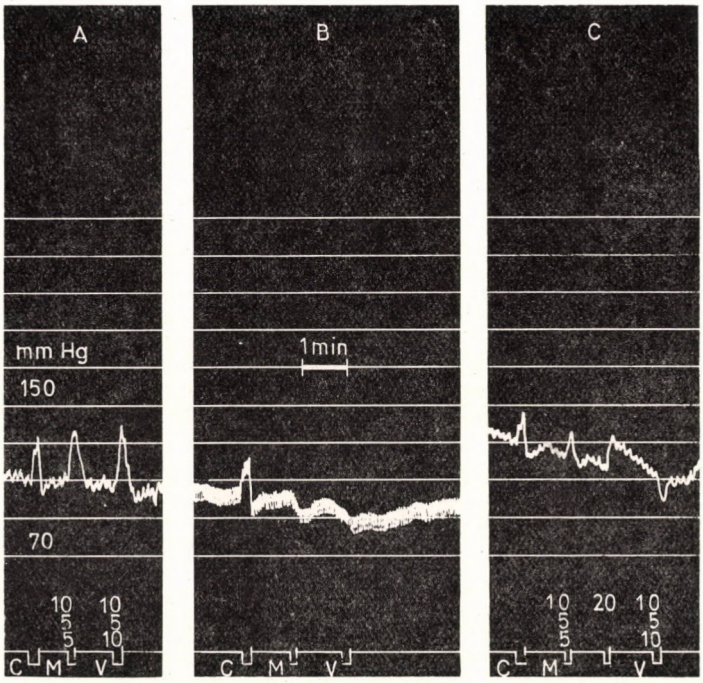


*Fig. 9.* Cat, 3.5 kg. Anaesthesia with 70 mg/kg chloralose +10 mg/kg pentobarbital intraperitoneally. "B": after 300 μg/kg curare vagal and median pressor reflexes are reversed. "C": after subsidence of curare effect median pressor response returns, vagal depressor reflex persists. "A": basic values

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# ACETYLCHOLINE AND THE EXOCRINE ACTIVITY OF THE FROG PANCREAS

By

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(Received November 20, 1970)

The effect of acetylcholine on the regulation of exocrine pancreatic function has been investigated in the frog *Rana esculenta*. The following changes were observed. (1) Acetylcholine considerably increased cellular activity as assessed by the neutral red absorption method. (2) Acetylcholine decreased granule count significantly. (3) Acetylcholine elevated the volume of pancreatic juice in starving animals and diminished it in fed frogs.

The changes observed, further the fact that the frog pancreas contains 0.001—0.1 $\mu$g acetylcholine/100 g pancreatic tissue indicate that acetylcholine has a role in the regulation of the exocrine function of the frog pancreas. Consequently, the concept that pancreatic secretion of the frog is under the exclusive control of the sympathetic nervous system has to be reconsidered.

Literary data on the regulation of exocrine pancreatic secretion are practically limited to information concerning higher vertebrates; data for amphibia thus for frogs are scarce. According to FRIEDMANN (1937), in *Rana esculenta* oesophageal and gastric secretion are under sympathetic control; in his opinion the vagus nerve has no role in the regulation of secretion in contrast with the situation in mammals.

SINGH (1963) found that the vagus nerve of the frog *Rana tigrana* contains tryptaminergic fibres and not cholinergic ones. This finding speaks against a regulatory function of the vagus nerve mediated by acetylcholine.

The important regulatory role of acetylcholine in the function of other organs of the frog, further the fact that acetylcholine affects the mechanism of exocrine function in the pancreas of other vertebrates, have induced us to re-examine the role of acetylcholine and to make a complex study of several relevant parameters in the frog *Rana esculenta*.

## Methods

*Cellular activity.* Cellular activity was estimated by ALEXANDROV—NASONOV's neutral red absorption method (1939) following the application acetylcholine at various concentrations.

*Granule count.* Formalin osmium impregnation was applied for the demonstration of secretory granules. Sections 4 $\mu$ thick were made and immersion optics focussed identically throughout to count the secretory granules in every cell. Mean granule count was obtained by counting the granules of 200 intact acinar cells in each of the experimental groups. The granule

count characteristic of the single groups was established (a) from the average of every granule count (indicated in the following SG), and (b) from the average count of granules contained in the single acinar cells (in the following ACSG).

*Volume of secretion.* A cannulation method developed in this laboratory was applied. Under ether anaesthesia an abdominal incision was made, the small intestine was opened opposite to the orifice of the pancreatic duct and a small glass capillary of calibrated lumen was inserted into the duct and fixed by a clamp.

Pancreatic juice was collected for 30 minutes and its quantity calculated from the lumen of the capillary tube and the height of the fluid column.

The acetylcholine solutions were injected into the dorsal lymph sac one hour before cannulation.

Animals with no food in the stomach and small intestine constituted the starving group. The frogs to be fed artificially were kept at 20°C for a week, there they were fed with raw frog meat 1 ml in volume, and the experiments were started 36 hours later.

Where not indicated otherwise, the changes reported in the following were significant statistically as calculated by the formula

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{(S.E._1)^2 + (S.E._2)^2}}$$

Every experimental series consisted of 10 animals.

## Results

*Starving animals* (Fig. 1). Control value for cellular activity was 9.32 arbitrary unit as assessed by the neutral red absorption method. This value increased to 12.02 U after incubation in Ringer's solution of 0.001 μg/ml acetylcholine content, amounting to a 29% activity increase. No significant changes were observed at other acetylcholine concentrations.

Control granule count was 4 SG. Incubation for 60 minutes in Ringer's solution containing 0.001 μg/ml acetylcholine diminished this value to 1 SG, i.e. by 75%. At an 0.01 μg/ml acetylcholine concentration only 60% diminution occurred. No significant differences were found between ACSG and SG values.

Normal pancreatic secretion was found to amount to 9.3 μl/30 min/100 g frog weight. After the injection of 0.001 μg/g acetylcholine, secretion increased to 20 μl/30 min/100 g frog weight, an increase of 115%. Injection of 0.01 μg/g acetylcholine caused only a 89% increase in volume.

*Fed animals* (Fig. 2). In response to feeding, the cellular activity increased to 19.6 U, more than double of that found in the starving group. It was remarkable that 0.001 μg/ml acetylcholine which was effective in the starving animals, proved ineffective in the fed ones. When 0.01 μg/ml acetylcholine was applied, the elevated activity level showed a further 37% increase, while 0.1 μg/ml acetylcholine concentration also induced a significant, 29% increase. These results indicate that the threshold concentration of acetylcholine is higher in the pancreas of fed animals.

Granule count averaged 10 SG, thus a 130% elevation was observed as compared to the same parameter of starving animals. When the pancreas of

fed animals was incubated in frog Ringer's solution containing 0.001 μg/ml acetylcholine, the granule count diminished to 4.4 SG, i.e. by 56%. Increasing the acetylcholine concentration, the decreasing tendency became more marked,

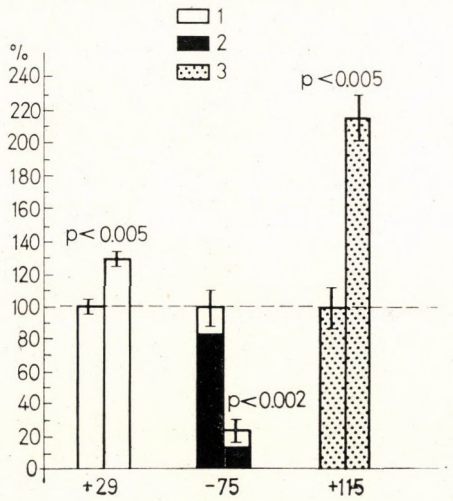


*Fig. 1.* Response to acetylcholine in starving frogs in per cent of control value

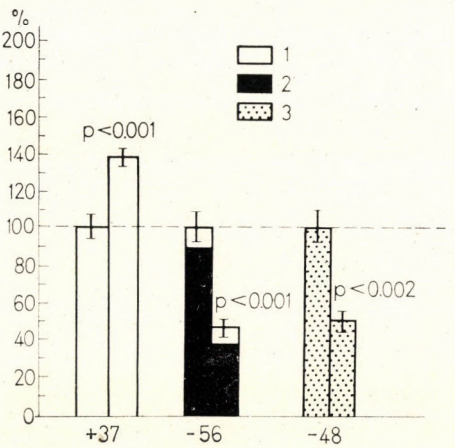


*Fig. 2.* Response to acetylcholine in artificially fed frogs in percentage of control value. 1 = Cellular activity; 2 = Granule count; 3 = Volume of pancreatic juice

at 0.01 μg/ml. The granule count averaged 3.4 SG (66% decrease) and at 1 μg/ml it averaged 1.3 SG (87% decrease). No significant difference was found between SG and ACSG values.

After artificial feeding, pancreatic secretion averaged 26 μl/30 min/100 g frog weight, representing a 178% increase in comparison to the starving con-

trols. In response to 0.01 μg/g acetylcholine injection, pancreatic secretion was reduced to 12.6 μl/30 min/100 g frog weight, amounting to a 48% decrease. This decreasing tendency was characteristic of every acetylcholine concentration and in this sense only quantitative differences were observed.

Acetylcholine produced the following cytological changes in the pancreas.

*Starving animals.* After the application of 0.001 μg/ml acetylcholine many lipid inclusions, a compact cytoplasm and active nuclei were seen. At 0.01 μg/ml acetylcholine concentration many lipid inclusions, a strongly vacuolized cytoplasm and secretory granules smaller in diameter were observed.

*Fed animals.* Applying 0.001 μg/ml acetylcholine, cytoplasmic and nuclear osmiophilia was identical, the secretory granules were well defined and of medium size. At 0.01 μg/ml acetylcholine concentration, the acinar structure appeared extremely compact, the nucleoli stained intensively, the nuclear plasm poorly. The cytoplasm was highly osmiophilic, extremely compact and small as related to the nucleus. The secretory granules were small and many lipid inclusions were seen.

## Discussion

Acetylcholine has been found to induce marked changes in the functional parameters and morphological picture of the frog pancreas. If the fact is taken into consideration that the frog pancreatic tissue contains 0.001—0.1 μg/100 g acetylcholine (PUPPI, unpublished data), the important role of acetylcholine in the regulation of the exocrine function of the frog pancreas becomes evident.

OGISO (1959) and KOMÁROMY et al. (1967) also observed that feeding induced an increase in the granule count of the frog pancreas, supporting the findings of the present study.

No differences were found between the starving and fed animals as concerns the qualitative changes in cellular activity and in granule count. Whereas cell activity was elevated in both groups, granule counts were diminished. This indicates that acetylcholine while enhancing cell activity induces an intensive extrusion of organic matter in both cases.

Paradoxical results were, however, obtained as concerns the volume of pancreatic secretion. In starving animals acetylcholine caused a marked diminution of secretion, whereas in fed frogs an inhibition of it.

Two alternatives present themselves for the explanation of this seemingly paradoxical phenomenon. It is also possible that the two mechanisms discussed below are closely interrelated.

According to EISLER and ÅGREN (1936), MAXIMOV (1939), LIN and IVY (1957) cholinomimetic substances increase the volume of pancreatic juice if small doses of secretion are applied simultaneously. However, in the presence

of high secretion amounts the volume of pancreatic secretion remains unchanged. As feeding induces secretion mobilization, such a mechanism might account for the phenomenon.

Another possibility to be reckoned with is as follows. We have observed (PUPPI, unpublished data) that changes in the redox potential of the environment may reverse the effect of acetylcholine on the frog heart and smooth muscles. We have actually found such redox potential changes when the pancreas of starving and fed frogs were compared.

We have studied the dose dependence of acetylcholine effects in a relatively small range. Thus, it may be assumed that the vagal effect at other concentrations and especially at higher ones is an inverse one, or else the mediator has no effect at all. This could account for the fact that in some cases low acetylcholine concentrations proved to be effective (e.g. on cellular activity in starving animals), whereas higher ones were ineffective. It may be assumed that the acetylcholine level representing a high concentration in our experiments falls into the neutral zone of the inversion of the effect, because FRIEDMANN (1937) applied approximately similar and also higher acetylcholine concentrations.

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# EFFECT OF LYSINE-8-VASOPRESSIN ON THIOSULPHATE AND INULIN DISTRIBUTION IN THE DOG

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The distribution spaces of inulin and thiosulphate as a function of time and the effect of synthetic lysine-8-vasopressin on the distribution of the two substances were studied in anaesthetized dogs after or without nephrectomy.

1. In nephrectomized animals, administration of vasopressin (50 mU/kg + 50 mU/kg) resulted in the reduction of the inulin space while thiosulphate space was maintained.

2. In non-nephrectomized animals the same dose of vasopressin failed to affect inulin space but increased thiosulphate space.

3. These results give support to the assumption that lysine-8-vasopressin augments the permeability of the cytoplasmic membrane in extrarenal tissues facilitating thus the diffusion of thiosulphate, the substance of lower molecular weight, across the cell membrane.

Intravenously administered polymers of high molecular weight such as inulin or substances which are not utilized in cellular metabolism (sucrose, mannitol, thiosulphate, etc.) are passing through the cell membrane with difficulty. These substances are distributed mostly in the extracellular space. However, as far as the distribution of diverse substances is concerned, the extracellular space is not homogeneous. Depending on the rate of diffusion of a substance of certain molecular weight in the extracellular compartment, spaces of rapid and slow diffusion may be distinguished. The ratio of these two spaces varies depending on the substance and time elapsing between its administration and the estimation. According to Gaudino and Levitt (1949), inulin is distributed in the extracellular space. The same conclusion was drawn by Schwartz (1950). The distribution space of inulin is increasing in the course of time, as demonstrated by White and Rolf (1956) in rats and by Bálint and Forgács (1956) in nephrectomized dogs. This points to the possibility of the passage across the cell membrane of this polymer of high molecular weight.

The thiosulphate distribution space in the dog was first estimated by Gilman et al. (1946). Raisz et al. (1953) reported that the anion thiosulphate exhibits a more rapid distribution and a larger distribution space than inulin does. A significant amount of thiosulphate traverses the cell membrane.

Vasopressin increases the water permeability of the cells of the renal tubuli and collecting ducts. The permeability increasing effect of the hormone

on the isolated frog skin was observed by HAYS and LEAF (1962) and SCHOFFENIELS and TERCAFS (1962).

There are few data concerning the extrarenal effect of vasopressin on cell permeability and on the inhomogeneity of the extracellular compartment. Such an effect might significantly influence the transport processes through the cytoplasmic membrane.

Alterations in the inulin and thiosulphate space following the administration of vasopressin may yield information on the extrarenal cell membrane permeability modifying effect of the hormone.

The effect of intravenously administered lysine-8-vasopressin on the distribution of inulin and thiosulphate was therefore examined in the present study.

## Methods

Dogs of either sex, weighing 10 to 16 kg, were anaesthetized intravenously with 25 mg/kg pentobarbital. Prior to the experiment the animals were deprived of food for 24 hours, with free access to water.

In the first experimental group, from an upper midline incision both renal hili were exposed and the renal arteries and veins were ligated (nephrectomized animals).

In the other group of animals from a lower midline incision the ureters were exposed and thin polyethylene catheters were inserted into them supravesically. The free ends of the catheters were inserted into a femoral vein, taking care for free urine flow in the catheter. Thus, the substances excreted by the kidney were led back into the circulation and with maintained renal function no material was lost from the organism (non-nephrectomized animals).

For sampling arterial blood and for intravenous administrations a femoral artery and a vein was exposed and cannulated in each animal.

Arterial blood pressure in the contralateral femoral artery was monitored by means of a mercury manometer and recorded kymographically. The animals were heparinized (0.1 ml/kg intravenously) to prevent clotting.

After the operation, 0.10 g/kg body weight of inulin and 0.50 g/kg body weight of sodium thiosulphate ($Na_2S_2O_3$) dissolved in 20 ml of physiological saline solution at 37°C was intravenously administered in a single dose.

Three to 4 ml of blood was withdrawn from the femoral artery in the 1st, 5th, 10th, 15th, 30th, 45th, 60th, 75th, 90th, 105th and 120th minutes following the injection. The blood was centrifuged and the plasma was analysed for inulin and thiosulphate, by the method of LITTLE (1949), and iodometry, respectively.

When the effect of vasopressin on the distribution of inulin and thiosulphate was to be studied, 50 mU/kg body weight of lysine-8-vasopressin (Sandoz) was added to the 20 ml of physiological saline containing inulin and thiosulphate. In the 60th minute after its first administration, 50 mU/kg of lysine-8-vasopressin dissolved in 5 ml of physiological saline solution at 37°C was injected.

Blood sampling was timed as in the control group.

Prior to the above-mentioned procedures, blood was withdrawn and the plasma blank for inulin and thiosulphate was determined. The blank values were negligible.

The distribution spaces for any given time-point were calculated by dividing the administered amount of inulin and thiosulphate with the plasma level of inulin and thiosulphate, respectively. The mean fluid space thus computed was expressed in per cents of body weight.

Statistical significance was evaluated according to Student's two sample *t*-test.

## Results

Results are summarized in Table I. In the upper part, for the nephrectomized animals the mean distribution spaces and their standard deviation in the course of time are shown. As a control, inulin and thiosulphate spaces were

**Table I**

*Effect of 100 mU/kg of lysine-8-vasopressin on inulin and thiosulphate space in per cents of body weight.* Mean ± SD

| Minutes | | 1 | 5 | 10 | 15 | 30 | 45 | 60 | 75 | 90 | 105 | 120 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NEPHRECTOMIZED DOGS | | | | | | | | | | | | |
| Control inulin space | $\bar{x}$ | 7.9 | 9.0 | 10.8 | 12.5 | 15.2 | 17.8 | 19.8 | 21.0 | 20.5 | 20.5 | 21.5 |
| n = 10 | SD | 3.8 | 4.8 | 5.7 | 5.4 | 5.4 | 6.0 | 5.5 | 4.9 | 5.5 | 5.3 | 5.3 |
| Inulin space after vasopressin | $\bar{x}$ | 5.7 | 6.1 | 7.2 | 7.6 | 9.7 | 10.9 | 12.6 | 12.7 | 13.5 | 13.3 | 14.3 |
| n = 10 | SD | 1.6 | 1.4 | 1.8 | 1.9 | 1.7 | 2.1 | 2.7 | 2.4 | 2.3 | 2.6 | 2.7 |
| Control thiosulphate space | $\bar{x}$ | 12.5 | 16.9 | 20.8 | 23.2 | 25.3 | 28.6 | 30.8 | 31.1 | 31.7 | 31.7 | 32.2 |
| n = 10 | SD | 5.7 | 5.1 | 4.4 | 5.5 | 4.1 | 5.6 | 4.6 | 7.1 | 6.3 | 5.9 | 7.0 |
| Thiosulphate space after vasopressin | $\bar{x}$ | 11.3 | 17.7 | 19.8 | 21.8 | 24.5 | 26.8 | 28.4 | 30.2 | 31.5 | 33.3 | 33.9 |
| n = 10 | SD | 3.2 | 2.5 | 2.6 | 3.3 | 3.5 | 3.2 | 4.0 | 4.3 | 6.0 | 8.2 | 9.4 |
| NON-NEPHRECTOMIZED DOGS | | | | | | | | | | | | |
| Control inulin space | $\bar{x}$ | 7.4 | 9.3 | 10.9 | 11.9 | 14.1 | 15.9 | 17.3 | 16.5 | 18.6 | 17.7 | 19.2 |
| n = 9 | SD | 1.3 | 1.7 | 2.0 | 2.4 | 3.2 | 3.7 | 3.2 | 2.3 | 3.4 | 3.0 | 4.1 |
| Inulin space after vasopressin | $\bar{x}$ | 7.6 | 9.0 | 10.0 | 11.2 | 14.2 | 16.2 | 18.0 | 18.7 | 20.8 | 20.6 | 21.2 |
| n = 8 | SD | 1.2 | 1.8 | 1.8 | 3.0 | 2.7 | 3.4 | 4.5 | 4.8 | 6.3 | 5.7 | 5.6 |
| Control thiosulphate space | $\bar{x}$ | 12.6 | 20.1 | 21.9 | 23.8 | 24.0 | 27.3 | 29.5 | 30.9 | 30.3 | 30.2 | 30.0 |
| n = 9 | SD | 4.0 | 6.4 | 6.1 | 6.2 | 7.3 | 8.5 | 10.3 | 10.9 | 11.4 | 10.0 | 10.4 |
| Thiosulphate space after vasopressin | $\bar{x}$ | 16.6 | 24.3 | 27.5 | 29.4 | 32.0 | 32.5 | 36.0 | 40.6 | 40.9 | 39.8 | 41.7 |
| n = 8 | SD | 5.5 | 3.3 | 2.1 | 3.7 | 5.2 | 6.2 | 6.1 | 7.6 | 9.5 | 12.7 | 12.2 |

estimated in 10 dogs. In the 120th min the inulin space amounted to 21.5± 5.3%, the thiosulphate space to 32.2±7.0% of body weight. The effect of lysine-8-vasopressin was studied in 10 dogs, in which inulin space was

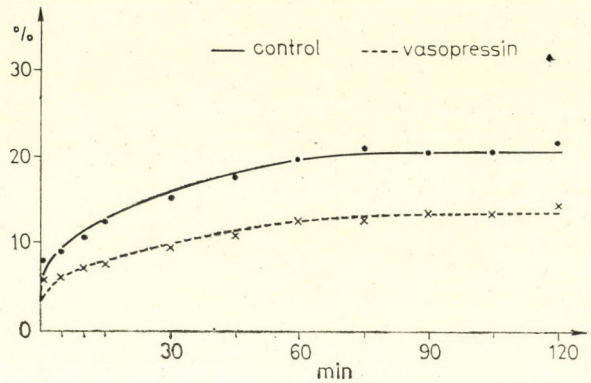


*Fig. 1.* Inulin space in nephrectomized animals as a function of time. Time is indicated on the abscissa, inulin space as per cent of body weight is indicated on the ordinate

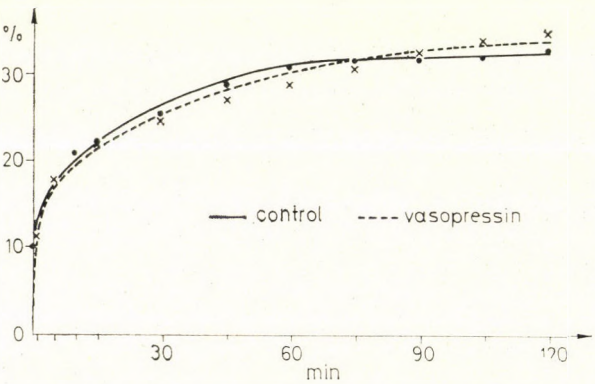


*Fig. 2.* Thiosulphate space in nephrectomized animals as a function of time. Parameters as in Fig. 1

reduced to 14.3±2.7% in the 120th min. This value differed significantly from the control ($p < 0.02$) while the thiosulphate space amounting to 33.9±9.4%, approximated the control value ($p > 0.8$).

The dynamics of the changes as a function of time is demonstrated in Figs 1 and 2. As shown in Fig. 1, the distribution of inulin was hindered by vasopressin in the first minute already and displayed values lower than the control throughout the experiment. The alterations in thiosulphate space are illustrated in Fig. 2, where the two curves are running close to each other.

The results obtained in the non-nephrectomized animals are demonstrated in the lower part of Table I. The inulin space of 19.2±4.1% of body weight, obtained in the control animals in the 120th min, did not differ sig-

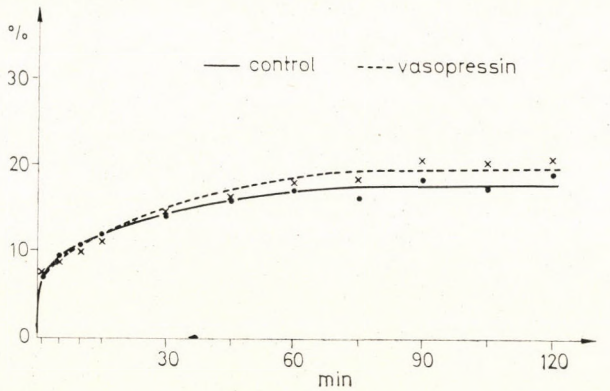


*Fig. 3.* Inulin space in non-nephrectomized animals as a function of time. Parameters as in Fig. 1

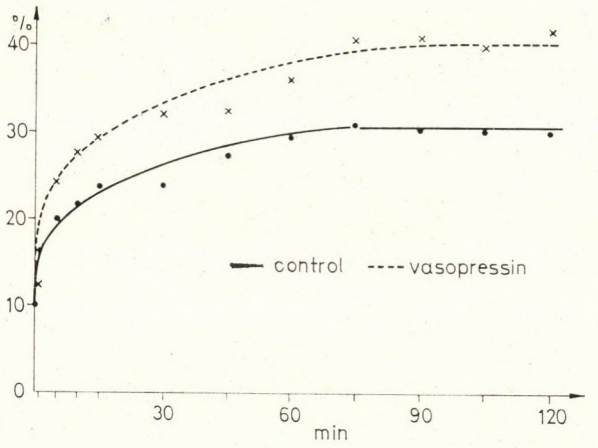


*Fig. 4.* Thiosulphate space in non-nephrectomized animals as a function of time. Parameters as in Fig. 1

nificantly from the nephrectomized controls ($p > 0.2$). Thiosulphate space amounted to 30.0±10.4% in the 120th min, approximating the values obtained in the previous control animals ($p > 0.8$).

The value of 21.2±5.6% for inulin space after the administration of vasopressin did not differ significantly from the control ($p > 0.1$) while the thiosulphate space significantly exceeded the value of 41.7±12.2% found in the controls ($p < 0.02$).

Inulin space in the non-nephrectomized animals is shown in Fig. 3. The curves, running initially together, later diverged but did not become statistically significant throughout the observed period.

The alterations in the thiosulphate space of non-nephrectomized animals are demonstrated in Fig. 4. The initial divergence of the curves grow more and more expressed with time.

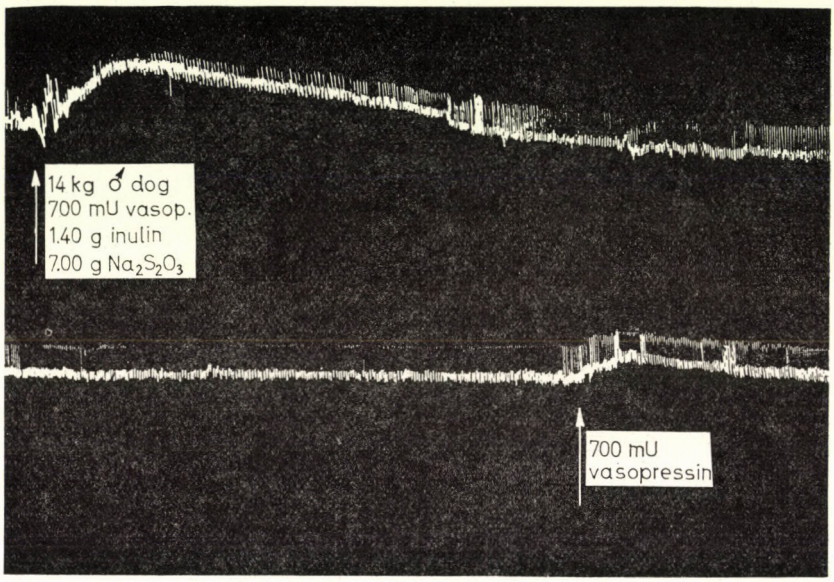


*Fig. 5.* Effect on blood pressure of two 50 mU/kg doses of lysine-8-vasopressin administered 60 min apart. Continuous record

Fig. 5 shows the changes in blood pressure after the intravenous administration of 50 mU/kg of lysine-8-vasopressin and after the second dose given 60 min later. Vasopressin caused a transient increase in blood pressure. The increase attained 20 to 25 mm Hg after the first dose and 5 to 10 mm Hg after the second one, then the blood pressure returned to normal. No significant change in blood pressure was observed in the control animals.

## Discussion

The extrarenal effect of intravenously administered pitressin was first examined by FRIEDMAN et al. (1956); they found a transitory reduction of the inulin space. According to their explanation, the elevation of blood pressure resulted in a flow of water into the intracellular space without concomitant influx of inulin, which therefore was distributed in a smaller compartment.

KÖVÉR and PÜSPÖKI (1966) reported that in rats with ureteral ligation pitressin significantly dilated the inulin space. The same phenomenon was observed also in dogs with their ureters ligated (KÖVÉR and SZŐCS 1966; SZŐCS and KÖVÉR 1968). As an explanation they suggested that the posterior pituitary hormone extrarenally increased the permeability of the cell membrane, hence inulin, a substance of high molecular weight, could easier enter the cell, i.e. was distributed in a larger compartment.

In the present experiments, lysine-8-vasopressin was shown to reduce the inulin space in nephrectomized animals. This finding agreed well with the data of FRIEDMAN et al. (1956, 1958), although in our case the reduction was more expressed and long-lasting in contrast to the transient effect described by the said authors. The difference may be accounted for by the fact that we applied a much higher dose of vasopressin which, moreover, differed also in chemical structure.

The divergence of the present results and those published previously by KÖVÉR and PÜSPÖKI (1966) may have been due to differences in experimental technique, viz. in the latter study the loss of inulin from the organism was prevented by ureteral ligation and natural hormone, pitressin was applied.

In the present study the reduction of inulin space induced by lysine-8-vasopressin may have had two causes. First, vasopressin brought about an intense and lasting vasoconstriction, practically excluding some regions from the circulation. If this were the only factor, it ought be reflected in a reduction of the thiosulphate space as well. Second, exogenous vasopressin decreases capillary permeability, hindering thereby the passage of inulin through the smaller pores, hence, its distribution space contracts. At the same time the contracted pores of the capillaries are not a hindrance to the passage of thiosulphate, a substance of low molecular weight, therefore its distribution space remains unchanged.

In the non-nephrectomized animals, lysine-8-vasopressin failed to affect the inulin space while it was increasing the thiosulphate space.

In order to compare these data with previous results, it must be assumed that vasopressin increases the permeability of extrarenal cytoplasmic membranes thus facilitating the transport processes across the cell membrane. This has been supported by the effect of vasopressin observed in nephrectomized animals where the reduction of the inulin space was not accompanied by a reduction of the thiosulphate space. We have to regard the first possibility to be more probable, i.e. the vascular effect of vasopressin is more pronounced in nephrectomized animals than in non-nephrectomized ones and therefore some vascular regions become obstructed. The maintained thiosulphate space despite its reduction by vasoconstriction is accounted for by its compensatory dilation due to the increase in cell membrane permeability.

This consideration is in agreement with our previous findings demonstrating that cell membrane permeability is extrarenally increased by vasopressin.

In the present experiments it was the thiosulphate, the substance of lower molecular weight, the transmembrane passage of which increased while that of inulin did not. However, it should be kept in mind that all this may only be valid for the synthetic hormone the effect of which might differ from that of the natural one.

In the pressor response, no difference was observed between nephrectomized and non-nephrectomized animals. Their blood pressure curve was identical, as shown in Fig. 5. This, however, does not yet mean that the distribution of blood flow and vascular reactions following vasopressin administration had been identical in the two groups of animals.

The present findings failed to shed light on the question of the organs in which lysine-8-vasopressin increases cell membrane permeability.

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# SEASONAL VARIATIONS OF ELECTROSHOCK SEIZURE SUSCEPTIBILITY IN THE RAT

By

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The seasonal variations of electroshock seizure activity and its relation to the $\gamma$-aminobutyric acid (GABA) and glutamic acid (GLU) level of the brain were investigated in male Wistar rats weighing 190 g on the average.

The 100% seizure threshold was 120 V in the summer, 70 and 60 V in the spring and autumn, respectively, and 35 V in the winter. No difference was found in the cerebral GABA level in spring and autumn, while GLU content was somewhat higher in the autumn than in the spring. GABA content in various parts of the brain was 30 to 35% lower in winter and 60 to 90% higher in summer as compared to the autumn level.

Thyroid function was inhibited by thyroidectomy, oral methyl mercaptoimidazole treatment or a warm environmental temperature (29°C). Upon the effect of these interventions the intensity of the seizures showed a diminution with a concomitant increase in cerebral GABA and GLU levels. GABA content of the brain was increased by 20 to 50% by thyroidectomy, 30 to 100% by methylmercaptoimidazole and 20 to 50% in an environment of 29°C. In the GLU level, changes of similar direction and magnitude were observed.

On the basis of these findings it is assumed that the diminution of cerebral GABA level induced by thyroid activation might be one of the possible causes of the decrease in seizure threshold in the winter.

Seasonal changes have been long known considerably to influence the functions of various organs and also the experimental results, and both the normal and pathological functions of the nervous system. The number and severity of neuroses is higher in the winter (KELLNER 1966); the peak of morbidity of mental illness occurs in winter and early spring (ZAIMOV et al. 1965; MCCARTNEY 1962). The occurrence of epileptic fits also exhibits a definite periodicity; beside the winter–summer form (BERCEL 1964) seizures are more frequent also in other types during the cold winter months (BUSCAINO 1936). A similar seasonality can be observed also in experimentally-induced convulsive states, susceptibility to metrazole and electroshock being increased in the winter and in a cold environment (WOODBURY and DAVENPORT 1952; HERR et al. 1953; SWINYARD and TOMAN 1948). Similar observations have been made in this laboratory and found electroshock susceptibility to be considerably higher in winter than in summer.

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The possibility arose that, among other factors, the $\gamma$-butyric acid level in the brain might play a part in the determination of seizure threshold (BAZEMORE et al. 1956; KILLAM 1957; EIDELBERG et al. 1959; LOVELL et al. 1963; BHATTACHARYA et al. 1964). In this case, the cerebral GABA level should markedly diminish in the winter. The present study was aimed at this question and also at clarifying the mechanism by which the seasonal changes are influencing the seizure threshold.

## Methods

Male Wistar rats weighing 190 $\pm$ 12.6 g were used. GABA and GLU content of the brain was determined by the paper chromatographic ninhydrin method of LOVELL et al. (1963).

Seizure threshold and seizure intensity were checked by electroshock. The head of the animals was epilated bitemporally and the bitemporal electrodes were applied to this area. Square wave impulses were used at 100 cps frequency, 5 msec impulse width of 1.6 sec duration; the voltage used was between 30 and 120 V depending on the season. Seizure threshold was expressed in Volts, and seizure intensity in per cent, according to the method previously described in detail (WÓRUM and PÓRSZÁSZ 1968).

Thyroid activity was inhibited by the following interventions. 1) Total thyroidectomy under ether anaesthesia, then keeping the animals at 25°C for 8 hours. 2) Oral administration of 25 mg/kg of thiamazole daily for 7 days. These two groups were kept together with the control one in a laboratory room at 19°C. 3) 29°C environmental temperature for 24 days. The necessary ventilation was ensured through a double air-lock. All animals were kept on a standard diet of identical amount and composition, with water ad libitum. Artificial illumination was ensured for 10 hours daily.

## Results

### *1. Seasonal variations in the GABA and GLU level of the rat brain*

The 100% electroshock threshold was determined in a total of 190 animals 40 autumn, 30 summer, and 60 each of winter and spring rats). The threshold was 70 V in the autumn group, 35 in the winter group, 60 V in the spring group and 120 V in the summer group. Head resistance was $3.61 \pm 0.12$ K$\Omega$ on the average without any significant difference between the individual groups. On the second day following the last electroshock administration, the cerebral GABA and GLU levels were determined in 9 animals from each group.

Table I summarizes the results concerning GABA concentration. The spring and autumn values were identical in the various parts of the brain. As compared to the autumn value, there was a significant increase in summer and a significant decrease in winter ($p < 0.01$).

Table II shows the cerebral GLU values in the same animals. As compared to the autumn level, there was a significant diminution in the winter and a significant elevation in the summer in all parts of the brain ($p < 0.01$). The

**Table I**

*Seasonal changes in GABA concentration in various parts of the rat brain*
($\mu$g/g fresh weight $\pm$ S.E.)

| Season \ Brain part | TH + HYTH | P + O | C E | C O |
|---|---|---|---|---|
| Autumn | 171.2±18.2 | 82.8± 7.2 | 97.5± 8.7 | 80.6±13.8 |
| Winter | 84.5± 9.0 | 44.1± 6.7 | 43.7± 6.2 | 49.9± 8.1 |
| t-test | $p < 0.01$ | | | |
| Spring | 189.7±20.2 | 87.4± 4.6 | 96.5± 5.0 | 77.0± 9.2 |
| t-test | N. S. | | | |
| Summer | 332.3±26.5 | 160.9±13.4 | 164.3±12.2 | 128.1±16.2 |
| t-test | $p < 0.01$ | | | |

In the t-test, the autumn concentrations served for basis of comparison.
Abbreviations: TH + HYTH = thalamus + hypothalamus; P + O = pons + oblong medulla; C E = cerebellum; C O = cerebral cortex; N. S. = not significant.

**Table II**

*Seasonal changes in GLU concentration in various parts of the rat brain*
($\mu$g/g fresh weight $\pm$ S.E.)

| Season \ Brain part | TH + HYTH | P + O | C E | C O |
|---|---|---|---|---|
| Autumn | 583.8±15.4 | 413.3±20.8 | 537.1±19.5 | 664.4±28.9 |
| Winter | 250.5±11.2 | 178.5± 8.0 | 261.9±12.6 | 444.7±19.9 |
| t-test | $p < 0.01$ | | | |
| Spring | 535.1±17.2 | 358.4±15.3 | 525.3±19.3 | 507.6±25.0 |
| t-test | $0.02 < p < 0.05$ | | N. S. | $p < 0.01$ |
| Summer | 891.0±29.0 | 655.0±18.0 | 780.4±27.2 | 845.3±32.4 |
| t-test | $p < 0.01$ | | | |

For abbrev iations, see Table I.

spring values were lower except in the cerebellum. The difference was at the border of significance in the thalamus and pons ($0.02 < p < 0.05$) and statistically significant in the cerebral cortex ($p < 0.01$).

*2. Influence of inhibition of thyroid activity on seizure susceptibility and on cerebral GABA and GLU level*

The endocrine system, especially the thyroid, responds in a sensitive way to seasonal changes and to changes in environmental temperature (MULLICK and KEHAR 1958). Thus the question arose whether there was any relationship between the seasonal rhythm of thyroid activity and the susceptibility to seizures.

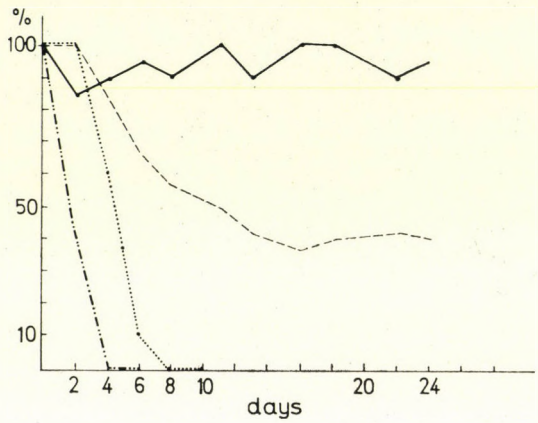


*Fig. 1.* Effect of inhibition of thyroid activity on the electroconvulsion intensity in the rat. Stimulus parameters: 30 V, 100 cps, 5 msec, 1.6 sec. Abscissa: time (days). Ordinate: seizure intensity (per cent). ------: control; — — — : 29°C environmental temperature; - · - · - · -: total thyroidectomy; . . . . .: thiamazole

To check this question, we studied the alterations in electroconvulsions during the inhibition of thyroid activity (Fig. 1). It is clearly seen that seizure intensity in the control animals (15 rats) was practically identical during the 24-day observation period and lay around 95%. The seizure susceptibility of animals kept at 29°C (10 rats) showed a gradual diminution in 16 days to 40% at which level it then persisted. Under the effect of thiamazole (8 rats) the intensity of convulsions began to decrease on the 3rd day to reach zero on the 8th day. Thyroidectomy was performed in 8 animals. Seizure intensity in these rats underwent a rapid diminution to zero by the 4th postoperative day. On the second day following the last electroshock, cerebral GABA and GLU concentrations were determined. The results of these investigations are summarized in Tables III and IV.

It can be seen that, upon the effect of the above-mentioned thyroid-inhibitory influences, GABA and GLU levels underwent a significant elevation in all brain parts investigated. An exception was the cortical GLU level in the warm environment group which failed to show any alteration. It was remark-

able that in the cerebral GABA and GLU levels thiamazole treatment caused a higher elevation than did thyroidectomy.

**Table III**

*Effect of inhibition of thyroid activity on the GABA concentrations in various parts of the rat brain* ($\mu$g/g fresh weight $\pm$ S. E.)

| Intervention \ Brain part | TH + HYTH | P + O | C E | C O |
|---|---|---|---|---|
| Control | 89.7± 7.0 | 42.4± 6.4 | 40.7± 5.7 | 54.8± 6.2 |
| Thyroidectomy | 127.6±16.2 | 56.3± 6.2 | 58.9± 8.6 | 82.7± 7.1 |
| t-test | $p < 0.01$ | $0.02 < p < 0.05$ | | $p < 0.01$ |
| Thiamazole | 187.6±26.2 | 59.4± 4.4 | 69.0±10.9 | 112.3±14.2 |
| t-test | $p < 0.01$ | $0.01 < p < 0.02$ | $p < 0.01$ | |
| 29 °C | 110.8± 9.5 | 60.1± 5.4 | 66.3± 8.3 | 61.3± 5.8 |
| t-test | $p < 0.01$ | $0.01 < p < 0.02$ | | |

This series of experiments was performed in the winter. The control values are practically identical with the winter values shown in Table I.

For abbreviations, see Table I.

**Table IV**

*Effect of inhibition of thyroid activity on the GLU concentration in various parts of the rat brain* ($\mu$g/g fresh weight $\pm$ S. E.)

| Intervention \ Brain part | TH + HYTH | P + O | C E | C O |
|---|---|---|---|---|
| Control | 260.1±15.4 | 181.4±10.0 | 264.8±13.2 | 437.2±16.8 |
| Thyroidectomy | 445.4±26.3 | 282.6±27.4 | 394.9±15.5 | 607.0±27.2 |
| t-test | $p < 0.01$ | | | |
| Thiamazole | 553.6±32.8 | 332.8±31.9 | 406.3±20.0 | 659.9±25.0 |
| t-test | $p < 0.01$ | | | |
| 29 °C | 328.7±20.5 | 234.6±16.3 | 374.9±17.0 | 439.5±20.2 |
| t-test | $p < 0.01$ | $0.01 < p < 0.02$ | $p < 0.01$ | N. S. |

For explanation and abbreviations, see Tables I and III.

## Discussion

According to our observations, susceptibility of the rat to electroshock is increased in the winter and decreased in the summer as compared to the spring and autumn values. The experimental results led to the conclusion that changes

in the environmental temperature might be one of the important factors influencing seizure susceptibility. This conclusion is in accordance with the results of WOODBURY and DAVENPORT (1952), HERR et al. (1953) as well as of SWINYARD and TOMAN (1948), since the elevation of environmental temperature led to an increase of the seizure threshold in our experiments, too. An important role has been ascribed to certain chemical agents in the determination of seizure susceptibility. One of these agents is GABA. Whenever the seizure threshold decreases, the GABA level also decreases while, in the case of an increased seizure threshold, there usually occurs an increase in the GABA content (EIDELBERG et al. 1959; BHATTACHARYA et al. 1964; WÓRUM and PÓRSZÁSZ 1968). The role ascribed to GABA in influencing seizure susceptibility has been supported by our own results; the changes in the GABA concentration of the rat brain found by us were well compatible with the seasonal changes of the seizure threshold, the GABA level being decreased in winter and increased in summer.

The effect on thyroid activity of seasonal changes and of changes in environmental temperature is a well-known fact (MCGAVACK 1951; BAUMAN and TURNER 1967). In the regulation of the thyroid response to cold and warm the nucleus habenulae may play an important part as pointed out by DONHOFFER et al. (1959) as well as by MESS (1964). At the same time, thyroid activity has been demonstrated to influence the excitability of the nervous system and also the seizure threshold (SÓS 1939; TIMIRAS and WOODBURY 1956; PFEIFER et al. 1962).

On the basis of these data, seasonal changes seem to alter thyroid activity through the environmental temperature which, in return, affects neural excitability through the GABA system. This assumption is corroborated by the findings according to which thyroidectomy, thiamazole treatment and a high environmental temperature all diminish seizure susceptibility with a concomitant increase in the cerebral GABA and GLU levels.

In our experiments, cerebral GABA content was found to increase also after thyroidectomy, in contrast to the finding of ROBERTS et al. (1959) who failed to observe any change in the GABA level after hypophysectomy, adrenalectomy or thyroidectomy.

On the basis of the data in the literature one can fairly well interpret the effect of thyroid activity on the GABA and GLU levels. Thyroxin increases oxygen consumption of the brain (TROJANOVÁ and MOUREK 1966; PUT and HOGENHUIS 1962) which leads, by the inhibition of glutamic acid decarboxylase, to a diminution in the GABA level (WOOD et al. 1966), a finding explaining the decreased seizure threshold during increased thyroid activity. However, it must not be left out of account that thyroxin may alter directly or indirectly the activity of some other enzymes such as acetylphosphatase, dehydrogenase, monoamino oxidase, altering thereby also the cerebral concentration of other

biologically active substances involved in the determination of seizure susceptibility. For instance, the level of monoamines also changes (PUT and HOGENHUIS 1962), and alterations in cerebral noradrenaline concentration especially its abrupt fall in the winter have been reported by BEAUVALLET et al. (1962).

Seasonal changes elicit more marked quantitative changes in the GABA and GLU levels than do any of the antithyroid interventions. Taking all these findings into consideration we have to suppose that in addition to the above-outlined thyroid gland—GABA mechanism, other mechanisms and substances are also involved in the seasonal changes of the seizure threshold.

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# *Pathophysiologia*

## EXPERIMENTAL PULMONARY EMBOLISM AFTER PHARMACOLOGICAL HEART DENERVATION IN THE RAT

By

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In 36 experiments the course of major pulmonary embolism has been observed in rats denervated by cholinolytic and sympatholytic drugs.

Continuous ECG tracings were recorded in 20 experiments and changes in respiration and carotid pressure were monitored in 16 cases.

Out of the 7 rats with regular ECG records, one died in five minutes. In four cases only minor T lability and some supranodal extrasystoles were found. One rat with experimentally induced embolism survived the critical period of 5 minutes despite multiform extrasystole and low voltage. Eight rats succumbed in 2 to 3 minutes with ECG signs of vagal excitation. Out of the 16 cases with controlled respiration and pressure, 8 did and 8 did not survive the massive pulmonary embolism.

The outcome of the pharmacological inhibition of the vegetative nervous system depends on the degree of the sympathotonia induced by cholinolytics in the course of pulmonary embolism. In the case of a sympathotonic domination, the animal survives the acute phase of pulmonary embolism, similarly as after atropine pretreatment.

In previous experiments a model of massive pulmonary embolism has been developed in the rat (KENEDI et al. 1969) and the dose of atropine and propranolol necessary for the complete blocking of the cholinergic and the beta receptor systems, respectively, and the latency between administration and effect have been determined (KENEDI and DÁVID 1969).

In the present experiments the reaction to massive pulmonary embolism has been studied in the rat deprived pharmacologically of its vegetative regulations (JOSE 1966).

### Methods

To 36 albino rats with an average weight of 200 to 250 g, 4 mg/kg atropine and 2 mg/kg propranolol were administered intraperitoneally and intravenously, respectively and eight and two minutes later pulmonary embolism was induced by the rapid injection into the femoral vein of 0.15 ml of an 1 : 2 mixture of Lipiodol and adeps pro supp.

In 20 experiments, control ECG tracings by a one channel direct recording ECG apparatus preceded the control of drug effects in the $V_1$ lead then the reaction to pulmonary embolism of the pharmacologically denervated heart was continuously observed.

In 16 experiments changes in respiration and carotid pressure following the same pretreatment were registered continuously by a respiration recording apparatus devised by DÁVID for use in small animals.

Animals surviving the 5 min period were killed by the tear of the spinal cord.

Considering the conflicting results of previous histological examinations, such studies were performed in 5 cases only and X-ray controls were completely abandoned.

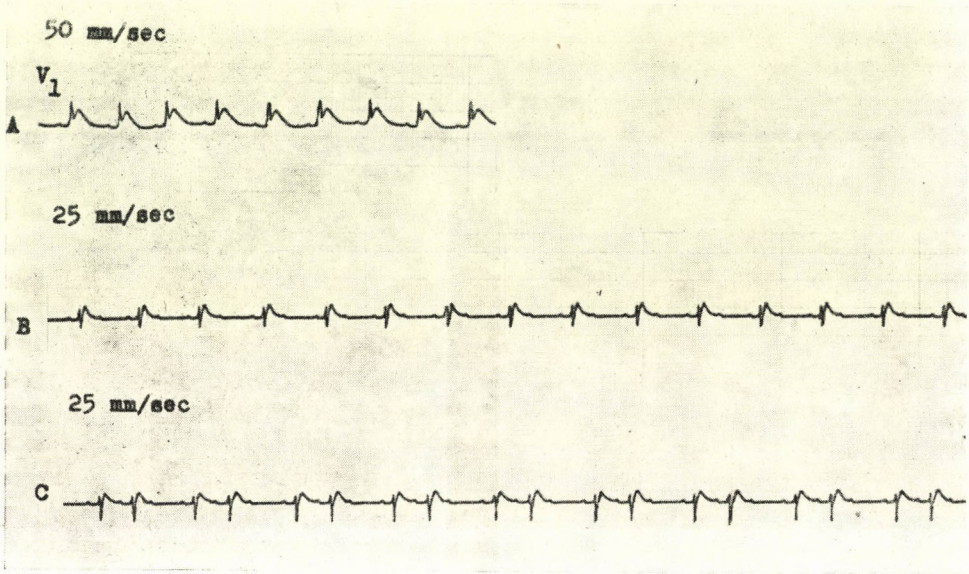


*Fig. 1.* Record A: $V_1$ lead before pulmonary embolism. 330/min heart rate, high ST segment. Record B: After embolism, 2 : 1 block. QRS is changed, deep S wave, ST is not elevated. Record C: 3 : 2 Wenckebach periods

## Results

During the 5 min observation period following pulmonary embolism no ECG changes were noted in 7 cases. One animal, however, died suddenly in 5 minutes despite normal ECG tracings throughout.

In 3 surviving animals, a minor periodical lability of the T wave and in 1 case some supranodal extrasystole were observed. Another rat survived the acute phase of pulmonary embolism in spite of multifocal ventricular extrasystole and low voltage.

Eight animals succumbed rapidly with signs of partial atrio-ventricular block turning into a complete one. Two ECG records of these lethal cases are shown in Figs 1 and 2.

Record A of Fig. 1 was taken in the $V_1$ lead at a paper velocity of 50 mm/sec before injection of the oil. The highly elevated ST segment on the tracing with a 330/min sinus rate is a sign of marked sympathotonia. Record B demonstrates a 250/min sinus activity and a 2 : 1 atrio-ventricular block follow-

ing embolism, which soon transformed into a regularly recurring 3 : 2 Wenckebach periodicity (Record C). Meanwhile, the ventricular complex turned into

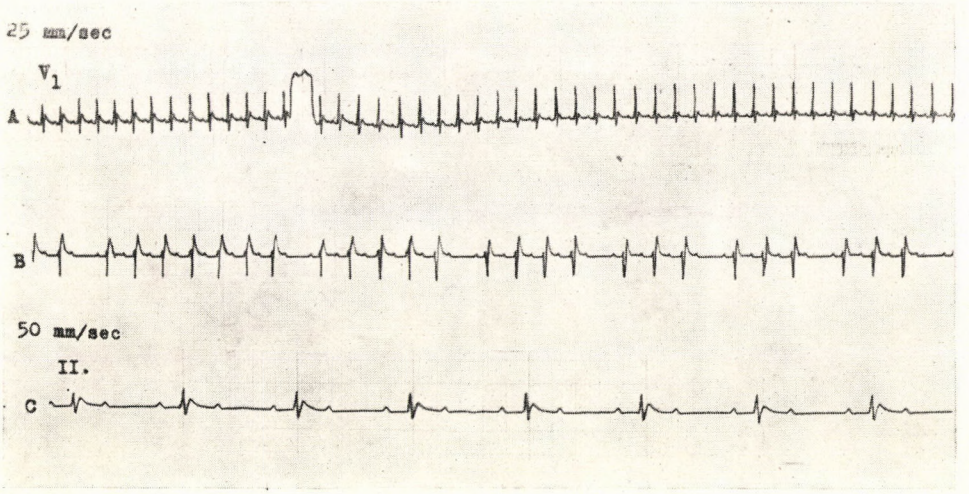


*Fig. 2.* Record A: After pulmonary embolism (sign), flattened T wave. Record B: Wenckebach periods with varying coupling. Record C: Terminal 2 : 1 block leading to death

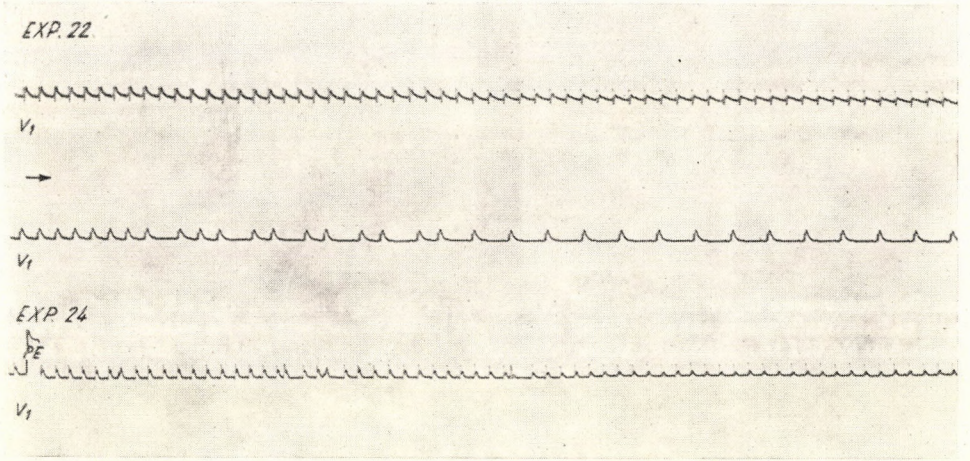


*Fig. 3.* In experiment No. 22, massive pulmonary embolism (upper tracing) was promptly followed by a progressive depression of the T wave. The 2 : 1 and 3 : 1 atrio-ventricular blocks preceding the death of the animal are accompanied by high T waves (asphyxia; middle tracing). In the similarly lethal experiment No. 24, the high origin of the gradually decreasing T wave is increasingly depressed. Supraventricular extrasystole (25 mm/sec paper velocity)

an S wave indicating the activation of the right ventricle. By this time the sympathotonic ST elevation had been overcome and the record showed only a flattened T wave.

After pulmonary embolism (PE) on Record A of Fig. 2, the ST elevation ceased, then conduction disturbances and Wenckebach periods of varying duration appeared (Record B). Meanwhile the QRS complex underwent the same changes as before (deep S wave and a very high T wave). The animal died eventually after a 2 : 1 block with a low T wave (Record C). The phenomenon

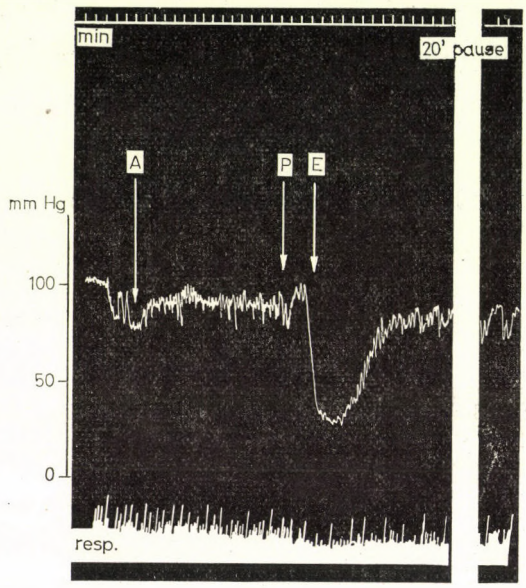


*Fig. 4.* Rat with pharmacologically denervated heart (A = atropine, P = propranolol) survives the considerable fall in arterial pressure induced by pulmonary embolism (E)

demonstrated in Figs 1 and 2, i.e. the decrease and low origin of the T wave, has been observed several times in the lethal cases as the first alarm signal of imminent vagal excitation. A flattened T wave after pulmonary embolism in 2 further experiments is shown in Fig. 3.

Out of the 16 cases pressure and respiration records, 8 rats succumbed to while 8 animals survived the pulmonary embolism. The kymograms of one surviving and one lethal case are presented.

In Fig. 4, injection of the pulmonary embolus at sign E was preceded by atropine (A) and propranolol (P) treatment. Pulmonary embolism induced an approximatively 70 mm Hg fall in arterial pressure and a shock which was gradually compensated in about 2 minutes, then respiration and arterial pressure persisted at the normal level even after ten minutes.

Fig. 5 shows that a prolongation of the interval between atropine pretreatment and pulmonary embolism resulted in a domination of the sympatholytic effect of propranolol leading to death. In this case atropine administration

preceded the embolism by 30 minutes and according to the kymogram, the embolism (E) promptly suspended respiration and heart action, and arterial pressure fell to zero.

Histological results in 5 animals agreed with those observed previously, even the individual cases indicated but quantitative differences.

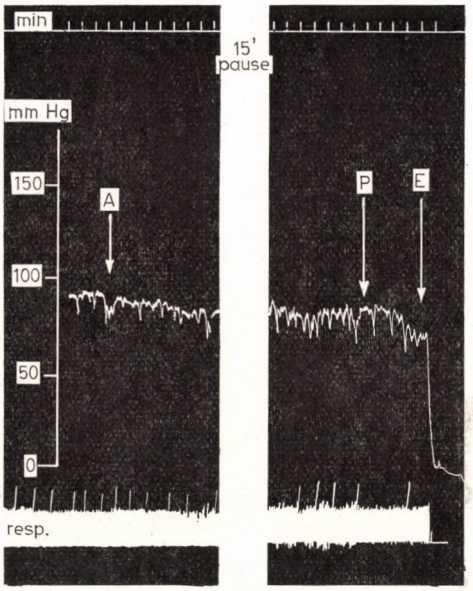


*Fig. 5.* Atropine pretreatment followed in about 30 minutes by propranolol (P) and pulmonary embolism (E). Immediate death

## Discussion

Experimental massive pulmonary embolism in the pharmacologically denervated rat proved that (i) there are considerable differences in the individual vegetative tone; and (ii) the vegetative tone determines that the same drug dose is followed by a domination of the stimulatory or of the inhibitory cardiac innervation.

If by atropine administration an intense sympathotonia is brought about, the animal survives the acute phase of pulmonary embolism despite the deleterious effect of propranolol.

If, however, there is a 30 min delay during which the atropine is metabolized, then the animal, deprived of its sympathetic defense, is promptly killed by the shock.

Thus, complete pharmacological denervation cannot be effected according to the same schedule even by high doses. The dynamic balance of the vegetative

nervous system is altered. The direction of this change determines the capacity or incapacity to compensate the acute reflectory action of pulmonary embolism. Further studies will clarify the rat's reaction to massive experimental pulmonary embolism after cutting the cardiac fibres of the sympathetic and the vagus nerves.

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# *Pharmacologia*

## ACUTE TOLERANCE TO SOME EFFECTS OF OXOTREMORINE AND ITS MODIFICATION BY COMPOUNDS WITH A CENTRAL EFFECT

By

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1. The analgesic effect of oxotremorine was inhibited by tremorine given 24 hours earlier. Tremorine pre-treatment for 1—4 days had no effect on the pentobarbital-potentiating activity of oxotremorine. Acute tolerance developed to its motility-decreasing effect.

2. Repeated oxotremorine treatment had no effect on carbachol and neostigmine lethality.

3. The tolerance developing to the disturbing effect of oxotremorine on motor co-ordination was inhibited by phenobarbital and antagonized by haloperidol.

Oxotremorine tolerance developed in the rat (rotating rod) but failed to come about in the cat (aggressive behavior).

DECSI et al. (1961a, b) reported that tremorine lost much of its analgesic, ether narcosis-potentiating and tremorigenic properties when given to mice for 10—16 days. In our experiments, tolerance to tremorine and oxotremorine took as little as a few hours to develop and 1—3 doses sufficed to produce this effect (GYÖRGY et al. 1970). Neither of the compounds induced tremor or disturbed motor co-ordination when given 16 hours after a single tremorine injection (the animals did not drop off the rotating rod). When to mice three doses of oxotremorine were given in 120—150 minutes, the last dose was practically ineffective. This schedule of administration also reduced nicotine and physostigmine lethality.

This paper presents some data to the following issues. a) Is there an acute tolerance to the other effects of oxotremorine? b) Do repeated doses of oxotremorine affect the lethality caused by cholinergic stimulants with an exclusively peripheral effect? c) Assuming that oxotremorine tolerance is of central origin, how do compounds acting on the CNS (hypnotics, neuroleptics, etc.) affect its development? d) Is a tolerance to oxotremorine developing in species other than the mouse?

## Methods

OELSSNER (1961) reported that mice cannot cling to the rotating rod when treated with tremorigenic compounds. In the present experiments tremorine dichlorhydrate and oxotremorine oxalate were applied and the doses indicated refer to these compounds. The co-ordination disturbance was estimated by using a rod 13 mm in diameter operated at 7 r.p.m. Trained untreated mice are able to cling to the rod for at least 180 seconds. After the intraperitoneal administration of 0.5 mg/kg oxotremorine every animal drops off the rod within 10 seconds. The maximum effect is generally reached in the 20th minute, thus measurements were made at that point of time. The effect wears off completely in 60—90 minutes.

The motility-reducing activity of oxotremorine was assessed by a motimeter (KNOLL and VAJNOVSZKY 1961). The apparatus records motility of mice by closing a circuit when the animals pass through given points.

The analgesic effect of oxotremorine was estimated in mice by using a hot plate of 52°C (WOOLFE and MCDONALD 1944; PÓRSZÁSZ and HERR 1950) and reaction time was evaluated by the method of Mann-Whitney (SOKAL and ROHLF 1969). The narcosis-potentiating effect of oxotremorine was studied in mice under the effect of 40 mg/kg intraperitoneal pentobarbital.

In rats, the muscular weakness developing on the intraperitoneal administration of 1 mg/kg oxotremorine was studied by using a rotating rod 40 mm in diameter.

In experiments on cats, the behavioral changes induced by oxotremorine treatment were recorded (anxiety, fear, spontaneous or provoked aggressivity).

## Results

### *a) 1. Tolerance to the analgesic effect of oxotremorine*

The analgesic potency of $3\times0.1$ or $3\times0.2$ mg/kg oxotremorine given intraperitoneally within 1.5 hours showed no decline or else an insignificant one. The effect of 0.5 mg/kg lasts for 3—4 hours, thus the examination cannot be carried out in one day. A second dose of oxotremorine given 24 hours after the first one was already effective.

Oxotremorine in a dose of 0.1 mg/kg was ineffective when given 24 hours after the intraperitoneal administration of 20 mg/kg tremorine, a compound of more prolonged action. Reaction time in the NaCl pre-treated group showed a twofold increase (Table I).

### *a) 2. Narcosis-potentiating effect of oxotremorine*

Tremorine pre-treatment was applied in these experiments. A single dose of tremorine left oxotremorine activity unaffected. Tremorine pre-treatment for two days (3 doses in all) even without oxotremorine was potentiating narcosis. Tremorine pre-treatment for three days followed by one day interval showed that oxotremorine had the same activity as in the untreated controls (Table II). Thus, under these conditions no acute tolerance to oxotremorine has been observed.

**Table I**

*Changes in the analgesic effect of oxotremorine after tremorine pre-treatment. Median and extreme values of increase in reaction time. Pre-treatment: 20 mg/kg tremorine or 0.9% NaCl intraperitoneally, 24 hours before oxotremorine. n = 12/group*

| Pre-treatment | Reaction time before OT treatment, sec | Oxotremorine | Increase in reaction time, sec |
|---|---|---|---|
| NaCl | 14.5 (7—27) | 0.05 mg/kg | 5.5 (—7—41) |
| Tremorine | 11.5 (7—19) | 0.05 mg/kg | —1.5 (—13— 9) |
| NaCl | 23 (13—40) | 0.10 mg/kg | 32.5 (20—47)* |
| Tremorine | 24.5 (13—44) | 0.10 mg/kg | 0 (—24—22)** |

Between * and ** $p < 0.01$ in Mann-Whitney test
OT = oxotremorine

**Table II**

*Changes induced by tremorine pre-treatment in the pentobarbital narcosis-potentiating effect of oxotremorine. Mean sleeping time in minutes ± S.E., after 40 mg/kg pentobarbital intraperitoneally. Pre-treatment doses of tremorine (at 9 and 16 hours)*

| | Chronic pre-treatment (days) | | | | Acute pre-treatment 10 minutes before pentobarbital | |
|---|---|---|---|---|---|---|
| | —4 | —3 | —2 | —1 | NaCl | 0.1 mg/kg OT |
| 1 | — | — | — | NaCl | 42.3± 9.1<br>n = 10 | 79.5± 8.6<br>n = 10 |
| | — | — | — | 20 mg/kg T | 39.0± 3.4<br>n = 8 | 91.0±10.5<br>n = 9 |
| 2 | — | — | NaCl | 2×NaCl | 52.7± 7.9<br>n = 10 | 86.9± 5.0<br>n = 9 |
| | — | — | 20 mg/kg T | 2×20 mg/kg T | 93.0±15.5<br>n = 10 | 99.4±16.3<br>n = 9 |
| 3 | 2×NaCl | 2×NaCl | 2×NaCl | — | 26.6±13.4<br>n = 10 | 55.6±20.3<br>n = 10 |
| | 2×10 mg/kg T | 2×10 mg/kg T | 2×20 mg/kg T | — | 38.0±11.1<br>n = 10 | 59.9±19.0<br>n = 10 |

T = tremorine
OT = oxotremorine

*a) 3. Motility-decreasing effect of oxotremorine after repeated pre-treatment*

LÉVY et al. (1967) reported that 0.05—0.15 mg/kg oxotremorine given intraperitoneally reduced spontaneous motility of mice.

Table III demonstrates the results of our experiments made on randomly treated mice. A strong motility-decreasing effect was evident after administra-

**Table III**

*Motility-decreasing effect of oxotremorine in the mouse. Motility count 0—30 minutes after oxotremorine administration. n = 24/group*

| | Compound and dose *i. p.* | Mean motility count ±S.E. | In per cent of control |
|---|---|---|---|
| 1 | 0.9% NaCl | 187±22 | 100 |
| 2 | 0.02 mg/kg OT | 101±12 | 54* |
| 3 | 0.05 mg/kg OT | 43± 8 | 23** |
| 4 | 0.10 mg/kg OT | 45±16 | 24** |
| 5 | 0.20 mg/kg OT | 64±17 | 34** |
| 6 | 0.50 mg/kg OT | 116±22 | 62— |

Significant difference as compared to the control in Mann-Whitney test *: under 0.05 **: under 0.01.

tion of 0.02—0.2 mg/kg oxotremorine; 0.5 mg/kg caused no significant decrease in motility, whereas in other experiment the latter dose proved to be effective. To clarify the discrepancy, the effect of 1.0 mg/kg oxotremorine was tested separately; it was found that the tail of the mice exhibiting a strong tremor often closed the circuit although the animals did not pass the border-line between the two fields. Consequently, the effect of such oxotremorine doses cannot be investigated by the motimetric method.

In experiments aimed at investigating the acute tolerance to the spontaneous motility-decreasing effect of oxotremorine, the compound was administered to mice at 90-minute intervals and motility was recorded for 30 minutes after each treatment. Five times 0.5 mg/kg oxotremorine was given to the animals as the tremor which produced the disturbance was insignificant after the 2nd 0.5 mg/kg and previous experiments had shown that tolerance developing to smaller doses was limited. Results are shown in Table IV and Fig. 1. It can be seen that in the control mice spontaneous motility was considerably decreased, whereas in the oxotremorine-treated animals it remained practically unchanged. If the motility counts of oxotremorine-treated animals are expressed in the percentage of those of the controls measured at the same

points of time, the former showed a marked increase. (It should be mentioned that the tremor could have interfered with the results only at the first treatment and without this interfering factor the percentual difference would have been but greater.)

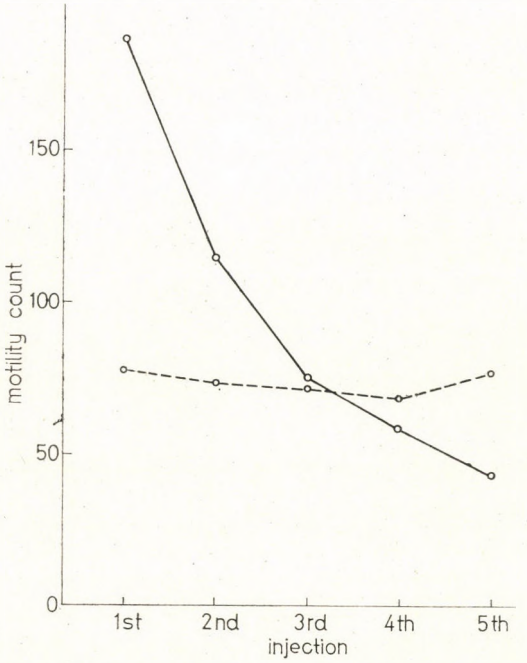


*Fig. 1.* Abscissa: number of injections. Ordinate: motility count. ——: NaCl; — — —: 0.5 mg/kg oxotremorine

**Table IV**

*Changes in the motility-decreasing effect of OT as compared to the control values. Five intraperitoneal doses of 0.5 mg/kg oxotremorine at 0, 90, 180, 270 and 360 min. Recording for 30 min after each treatment*

| | Mean motility count ±S.E. | | Motility of experimental animals in per cent of the controls |
|---|---|---|---|
| | NaCl-treated mice (0 9%) n = 22 | OT-treated mice (0 5 mg/kg) n = 21 | |
| 1st 30 min | 187±23.9 | 77±15.1 | 41 |
| 2nd 30 min | 115±17 | 73±13.5 | 63 |
| 3rd 30 min | 75±14.4 | 70±15.3 | 93 |
| 4th 30 min | 58±16.5 | 68±13.0 | 117 |
| 5th 30 min | 37±11.6 | 76±14.2 | 205 |

*b) Effect of repeated oxotremorine treatment on carbachol and neostigmine lethality in the mouse*

It has been shown earlier (GYÖRGY et al. 1970) that repeated oxotremorine treatment reduced physostigmine and nicotine lethality. In the present experiments, $3 \times 1.0$ mg/kg oxotremorine had no effect on carbachol lethality (Table V), nor did it affect neostigmine lethality. $LD_{50}$ was practically the same

**Table V**

*Effect of oxotremorine on carbachol lethality. Pre-treatment $3 \times 1.0$ mg/kg oxotremorine intraperitoneally, or NaCl 120, 90 and 60 min before carbachol intravenously*

| Pre-treatment | n | Carbachol dose | Lethality |
|---|---|---|---|
| $3 \times 0.9\%$ NaCl | 12 | 0.4 mg/kg | 7 |
| $3 \times 1.0$ mg/kg OT | 12 | 0.4 mg/kg | 8 |
| $3 \times 0.9\%$ NaCl | 12 | 0.5 mg/kg | 11 |
| $3 \times 1.0$ mg/kg OT | 12 | 0.5 mg/kg | 11 |

OT = oxotremorine

when intravenous neostigmine was given 60 minutes after two NaCl treatments applied at an interval of 60 min or after $2 \times 1.0$ mg oxotremorine administered in the same way. $LD_{50}$ after NaCl treatment was 0.376 (0.311—0.455) mg/kg, after oxotremorine treatment 0.42 (0.341—0.517) mg/kg as observed on 60 mice each. Relative activity amounted to 1.12 (0.85—1.45), a value not reaching statistical significance. (In brackets the 95% confidence limits.)

*c) 1. Effect of phenobarbital on acute tolerance to oxotremorine*

PFEIFER et al. (1953) reported that acute tolerance to some effects of peripherally acting compounds (e.g. adrenaline) can be prevented in the rat by decortication, phenobarbital, morphine, scopolamine, etc. Since these observations pointed to the role of the CNS in the development of acute tolerance, we have investigated whether the CNS was instrumental in inducing tolerance to oxotremorine and whether this tolerance was influenced by phenobarbital.

Muscular weakness was induced in mice by the administration of 1.0 mg/kg oxotremorine and to the experimental group 40 mg/kg phenobarbital-Na was given subcutaneously. The effect of oxotremorine in the experimental group of 49 mice disappeared in 50% of the animals in 128 minutes, while in 86 minutes in 50% of the 46 controls.

Two methods were applied in studying the tolerance to oxotremorine. Phenobarbital or NaCl was always given 30 minutes before the first 0.5 mg/kg dose of oxotremorine. In one experimental series both pretreated groups

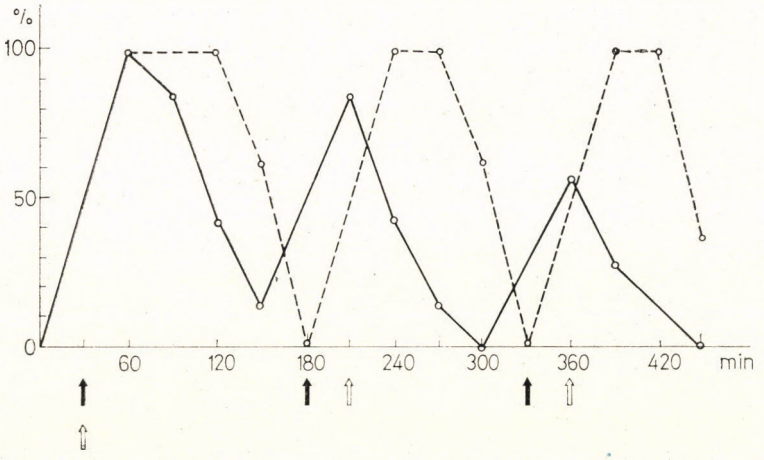


*Fig. 2.* Abscissa: time, ordinate: per cent of mice dropped off the rotating rod (8 mice each in both groups). ——: NaCl pre-treatment; — — — 40 mg/kg phenobarbital pre-treatment; ↑ oxotremorine administered to the controls; ⇧ oxotremorine administered to the phenobarbital treated mice

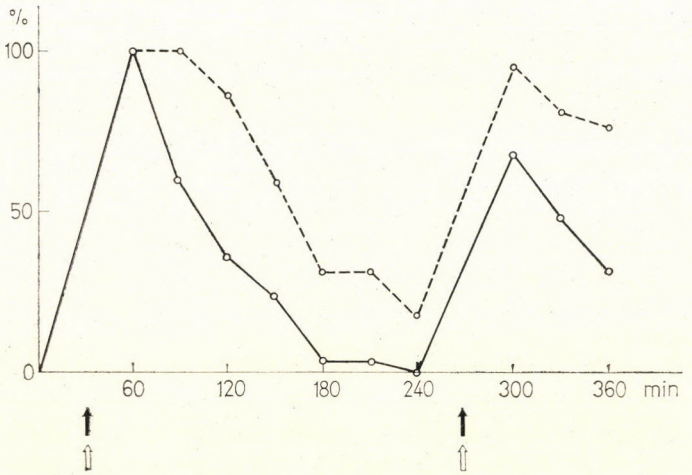


*Fig. 3.* Signs as in Fig. 2. Control group, 25 mice; phenobarbital-treated groups, 22 mice

received the second dose of oxotremorine when the effect of the first one had already subsided. This, of course, occurred at different points of time in the two groups. Such an experiment is shown in Fig. 2. In another experiment both groups received the second dose of oxotremorine at the 270th minute,

when in the phenobarbital treated animals the oxotremorine effect had subsided (Fig. 3). It was evident in both experiments that phenobarbital prolonged the effect of, and inhibited the acute tolerance to, oxotremorine.

*c) 2. Re-establishment of oxotremorine susceptibility in oxotremorine tolerance*

From the above results it seemed that central effects had a role in abolishing the effect of oxotremorine in the course of acute tolerance, which was not

**Table VI**

*Restitution of sensitivity in acute tolerance to oxotremorine by large intraperitoneal doses of oxotremorine. Number of mice dropping off the rotating rod after oxotremorine treatment at 90 minute intervals*

| Dose | | | 4th OT dose | Effect after the 4th OT dose |
|---|---|---|---|---|
| 1st | 2nd | 3rd | | |
| 500 μg/kg OT dose | | | | |
| 10/10 | 4/10 | 2/8 | 500 μg/kg | 1/8 |
| 10/10 | 3/10 | 1/8 | 1000 μg/kg | 9/10 |
| 10/10 | 5/10 | 0/10 | 2000 μg/kg | 10/10 |

OT = oxotremorine

**Table VII**

*Effect of haloperidol on acute tolerance to oxotremorine as observed on rotating rod. The mice were given 0.5 mg/kg oxotremorine 240, 150 and 60 min before the administration of haloperidol (NaCl) and 40 min after haloperidol. Effects observed after the first three doses are not shown*

| Number of mice dropped off the rod after NaCl or haloperidol | | | Number of mice dropped off the rod after 4th dose of oxotremorine | |
|---|---|---|---|---|
| NaCl | 1/46 | 2.2% | 6/46 | 13 % |
| 0.05 mg/kg haloperidol | 2/46 | 4.4% | 26/46 | 56.5% |
| 0.10 mg/kg haloperidol | 2/38 | 5.3% | 30/37 | 81.0% |

an absolute one since high doses were still affecting the animals' behaviour on the rotating rod (Table VI). Thus, we examined whether the tolerance could be suspended by haloperidol, the effect of which was studied on the tolerance developing after the third dose of oxotremorine. Table VII shows that haloperidol in itself was ineffective, but in doses of 0.05—0.1 mg/kg it rendered effective the otherwise inactive fourth oxotremorine dose. These doses of haloperidol abolished the tolerance to oxotremorine but did not render effective 0.1—0.2 mg/kg of oxotremorine which in itself was only slightly or not at all effective.

*d) 1. Oxotremorine tolerance in the rat*

Only a small per cent of rats respond with expressed tremor or muscular weakness to 0.5 mg/kg oxotremorine, while an intraperitoneal dose of 1.0 mg/kg was usually 100% active on the rotating rod. The effect of repeated injections showed a decline but not such a rapid one as in the case of mice. (Oxotremorine activity of $3 \times 1$ mg/kg daily for three days changed as follows: 100—50—40, 50—40—30 and 50—30—10% of the animals, respectively, dropped off the rod.)

*d) 2. Effect of repeated oxotremorine treatment in the wakeful cat*

The wakeful cat responds to the intraperitoneal administration of 0.1—0.2 (0.5) mg/kg oxotremorine in a characteristic manner. In case of a minor response the animals crouch, show fear, in a more expressed one they bite and behave aggressively when irritated. The strongest response is a spontaneous spitting, snarling. Four cats were given repeated doses of oxotremorine daily for several days. Before the first dose they received 0.5 mg/kg atropine methyl bromide. The single doses of oxotremorine ranged from 0.2 to 0.5 mg/kg, the daily dose from 1.0 to 2.0 mg/kg. Under these conditions the effect of the individual doses showed no decline on the second, third or fourth day of the experiment.

## Discussion

It has been reported previously that tolerance developed to the tremor-producing and co-ordination-disturbing effect of oxotremorine. The present experiments have shown that tolerance to the analgesic effect of oxotremorine can be observed 24 hours after a single injection of tremorine. The finding that the motility-decreasing effect of five consecutive oxotremorine treatments showed a gradual decline, seems to be a phenomenon analogous to tolerance. In contrast, no tolerance developed to the narcosis-potentiating effect of oxotremorine even after 3 days tremorine pretreatment. DECSI et al. (1961a, b) studied the ether narcosis potentiating effect of tremorine after a pretreatment for 8 days, thus there is no contradiction between their findings and the present results.

It can be stated that whereas repeated oxotremorine treatments inhibit the lethality of central cholinergic stimulants, the drug is ineffective against the peripherally acting neostigmine and carbachol. This points to a CNS origin of the phenomenon. (Tachyphylaxis has not been observed to develop against the peripheral effect of oxotremorine).

The fact that phenobarbital inhibited and haloperidol abolished the tolerance to oxotremorine indicates that the effect is of central origin. We assume that it did not subside because of its complete elimination. HAMMER et al. (1968) found that the time needed for the elimination of 0.5 mg/kg oxotremorine is more than 180 minutes in the mouse. The finding that narcosis-potentiating effect of oxotremorine persisted, thus not all of its central effects are involved in the tolerance, speaks against the elimination being enhanced in the state of acute tolerance. We presume that oxotremorine initiates a counter-regulation, or else its stimulating activity otherwise not observed in the mouse comes to the fore. This stimulating activity is the most marked in the cat but also occurs in the rat. In addition to the convulsions observed in this species by COX and POTKONJAK (1970) agressivity and biting may occur. The fact that no tolerance develops in the cat seems to contribute to the above concept.

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We are indebted to Mrs. M. HALMOS and Mrs. O. SZABÓ for skilful technical assistance.

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# *Recensiones*

J. H. K. VOGEL (Ed.)

## Hypoxia, High Altitude and the Heart

Advances in Cardiology. Vol. 5. S. Karger, Basel, München, Paris, New York 1970. X + 196 pages, with 77 figures and 30 tables. Price: US $ 14.14; DM 59.—

The volume presents proceedings of the First Conference on Cardiovascular Diseases held in Snowmass at Aspen, Aspen, Colorado, in January, 1970. The four main chapters of the book are Physiological Changes at Altitude; The Coronary Circulation; Respiratory Control; The Pulmonary Circulation. They contain 21 papers with references. Unfortunately, the discussions have not been included.

The Editor comes to the point in the introduction: "The information obtained from high altitude studies has provided a better understanding of the effects of hypoxia on cardiovascular performance, and integrating these observations with those obtained in patients with various diseased states, it should be possible to develop a more sophisticated appreciation of the effects of hypoxia". Some of the papers in the chapter of Physiological Changes at Altitude are very interesting from the point of view of sportsphysiology, reporting on important experience made at the Mexico City Olympic games, and in general concerning human adaptation to high altitudes.

The book is recommendable to physiologists, sports-physiologists and to everybody interested in this field of medicine.

K. LISSÁK

O. GIEBEL

## Ventilation, Gasaustausch und Kreislauf unter künstlicher Totraumvergrößerung

Anaesthesiology and Resuscitation. Vol. 41. Springer Verlag, Berlin, Heidelberg, New York 1969. 74 pages, with 38 figures. Price: DM 18.—

The author constructed a relatively simple apparatus by which dead space can be increased. The effect of the apparatus on ventilation, gas exchange and circulation as assessed in healthy subjects and in patients with respiratory failure is described.

The apparatus, actually a tube with variable volume, increases minute ventilation and decreases alveoloarterial $O_2$ difference. Arterial $CO_2$ tension does not increase significantly when the dead space of an adequate extent is increased. Blood pressure and heart rate remain uninfluenced.

The artificial increase of the dead space prevents atelectasis and partial respiratory failure developing after surgery. The book offers useful information on the physiology of respiration and will be of interest to surgeons and anaesthesiologists.

I. ANDIK

A. B. DUBOIS and K. P. VAN DE WOESTIJNE (Eds)

## Body Plethysmography

Progress in Respiration Research. Vol. 4. International Symposium on Body Plethysmography, Nijmegen, September 17—18, 1968. S. Karger, Basel, New York 1969. XII + 260 pages, with 144 figures and 19 tables. Price: US $ 18.—; DM 75.—; 150s.

The volume comprises 24 papers read at the Symposium. In the first part six papers are discussing the principles of construction and the technical problems. In the second section six papers report on the measurement of ventilation parameters. The third part contains three papers treating questions of pulmonary blood flow. In the fourth part seven papers give a detailed discussion on the application and value of body plethysmography. The reports of two Japanese authors on the present state of body plethysmography in their country make up the last part of the volume.

The papers survey the important literary data on body plethysmography. The volume will be a valuable piece of reading for all who are engaged in the theory or practice of body plethysmography.

I. ANDIK

O. EICHLER, A. FARAH, H. HERKEN and A. D. WELCH (Eds)

## Erzeugung von Krankheitszuständen durch das Experiment

Teil 2: Atemwege. Handbuch der experimentellen Pharmakologie. Bd. 16. Bearb. von J. E. ALBERTY, O. EICHLER, H. FRIEBEL, K. KARZEL, J. LULLING and J. PRIGNOT. Springer Verlag, Berlin, Heidelberg, New York 1969. XI + 308 pages, 59 figures. Price: DM 124.—; US $ 34.10

The second part of the 16th volume of the handbook deals with the experimentally induced pathological states of the respiratory passages.

The chapter "Experimental Chronic Aspecific Respiratory Affections", written by PRIGNOT and LULLING, is an excellent survey of the modern methods applied for the production of abnormal states in the respiratory passages. The authors concisely summarize and evaluate the experimental results obtained by means of these methods. FRIEBEL gives a fascinating summary and critical evaluation of the experimental methods provoking coughing. The chapter written by ALBERTY provides a view on the methods applied for producing experimental asthmatic reactions. It appreciates the experimental results and deals in detail with the problems of the diverse techniques. KARZEL offers an excellent summary and critical assessments of the methods used for inducing pulmonary oedema. The volume contains almost the complete literature concerning the subject. The index is very reliable, facilitating orientation in the book. The handbook will be most useful for both specialists and theoreticians.

I. ANDIK

F. S. MEERSON

## Hyperfunktion, Hypertrophie und Insuffizienz des Herzens

VIII + 447 pages, 77 figures and 46 tables. VEB Verlag Volk und Gesundheit, Berlin 1969. Price: M 65.50

The author is a distinguished authority in the field of experimental cardiology. His monograph has now been published simultaneously in Russian and in German. The introduction gives a detailed discussion on the five basic processes of cardiac function: stimulus, transmission, contraction, energy supply and synthesis of structural proteins. After presenting a molecular biological interpretation of these issues, MEERSON sets up his working hypothesis suggesting that an increased load leads to cardiac insufficiency through hyperfunction, hypertrophy and exhaustion. An experimental model of aortic stenosis in animals was used in the biochemical studies. The monograph is divided into 7 chapters.

*1) Contraction of the heart in cardiac hyperfunction, hypertrophy and insufficiency.* An experimental model of isometric cardiac insufficiency was developed. In the course of adaptation there is a K efflux and a Na influx in the myocardium, bound Ca is released, thus a natural inotropic effect is induced. On constriction of the aorta the heart is dilated, its efficiency diminished, tension being proportionate to radius and pressure, work only to pressure. Initially contractile force and velocity decrease, then they become re-established and stabilized followed by a decrease in exhaustion. The noradrenaline content of the hypertrophic heart diminishes and thus its inotropic effect is also decreased.

*2) Transformation of myocardial energy in cardiac hyperfunction, hypertrophy and insufficiency.* First, energy formation per mass unit increases, to become normal in hypertrophy followed later by a decrease in the concentration of high energy phosphates. The hypertrophy itself can be inhibited by actinomycin but then the above energetical changes also become inhibited.

*3) Nucleic acid and protein synthesis in the myocardium.* After constriction of the aorta the mass of the heart increases, due first to water uptake then to an increase in dry matter content. RNA and DNA synthesis is intensified followed by enhanced protein synthesis. When the constriction is released, catabolism increases and many lysosomes can be observed electron microscopically.

*4) Processes of replication, transcription and translation.* Sarcolysine, aminopterin and actinomycin D inhibit hypertrophy and incorporation of $^3$H-thymidine. In spite of this, dividing cells can only be observed in the end-phase of hypertrophy when polyploidy is found. The diameter of the muscle fibre increases more rapidly, so there is a relative decrease in the DNA level. At the same time, DNA synthesis in the connective tissue is more expressed than in the myocardium. Every sign indicates that in hypertrophy connective tissue accumulates more rapidly than does muscle tissue. Data on the number of nucleoli and on myocardial protein synthesis are reported.

*5) Structural proteins.* The known structural proteins are discussed in detail. Morphological evidence is presented that hypertrophy is followed by cardiac sclerosis, a lysis of fibres and vacuolization. Subendothelial, epicardial and papillary muscle atrophy develop later.

*6) The role of regulation.* Catecholamine precursors accumulate, the quantity of catecholamines increases owing to the inhibition of tyrosine hydroxylase activity. At the same time, acetylcholine and vagal function are undisturbed. The effect of the different hormones and BEZNÁK's observations concerning the role of growth hormone are discussed in detail.

*7) Effect of the co-factors of nucleic acid synthesis.* From the results discussed in this chapter some data deserve special attention, especially that folic acid and orotic acid significantly improve myocardial efficiency in experimental myocardial infarction and the catecholamine content also becomes normal by their favourable influence.

The material presented is comprehensive and well documented by figures and tables. The author reviews a great number of literary data but supports his concept mainly by the results of his own experiments. The book is written in an easy and enjoyable style. It provides both the theoretician and the practitioner with new ideas that may reshape his attitude to cardiology. It also presents a good example how one comes to reconsider cardiological notions showing by a biochemical approach that the heart must not be considered a mere mechanical pump.

T. JÁVOR

K. THURAU and H. JAHRMÄRKER (Eds)

## Renal Transport and Diuretics

International Symposium, Feldafing, June 21—23, 1968. Springer Verlag, Berlin, Heidelberg New York 1969. XV + 487 pages, 235 figures, 62 tables. Price: DM 68.00

The volume contains all the papers and discussions of the meeting. The introductory remarks of the Chairman, K. KRAMER and H. SCHWIEGK emphasize that symposia are one of the best and quickest means of communicating scientific information. Surveying the contents, of the volume from the nephrologist's point of view, one fully agrees with the Chairman's view.

The papers are mostly in English, some in German. They cover problems of interest to nephrologists engaged in research and to those working in clinical practice. The volume is divided into three parts. The shortest first part treats the metabolic processes of the kidney and includes papers by KREBS, PITTS, OELERT et al. and KRAMER et al. The second part deals with renal transport processes based primarily on the results obtained by micropuncture. Several papers report on the tubular transport of electrolytes and on factors affecting the transport, such as angiotensin, steroid hormones, diuretics. The third and most comprehensive part discusses the clinical application of diuretics.

It has to be emphasized that the volume was published in a remarkably short time; it was available within one year of the Symposium.

The book will be indispensable to all those engaged in research in any of the broad topics of the Symposium and will be a useful tool for every physician who wants to deepen his knowledge in the theoretical implications and practical aspects of diuretic therapy.

P. BÁLINT

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# PROCEEDINGS OF THE THIRD INTERNATIONAL MEETING OF THE INTERNATIONAL SOCIETY FOR NEUROCHEMISTRY

Budapest 5—9 July, 1971

Edited by J. DOMONKOS, A. FONYÓ, I. HUSZÁK and J. SZENTÁGOTHAI

The volume contains the abstracts of the papers read during the sessions of the Meeting. This collection is of interest to physicians and research workers employed in institutes and departments investigating neurology and psychiatry, biochemistry, or in clinical laboratories, neurological and biological research institutes; and of course it is furthermore useful for all those involved in the study of anatomy, histology and electron microscopy.

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ACTA PHYSIOLOGICA

ТОМ 40—ВЫП. 1

Резюме

## ВРЕМЯ КАК ФАКТОР В ИЗУЧЕНИИ МЕТАБОЛИЗМА Peromyscus

А. А. ХЕЙЗНЕР, Й. Ц. РОБЕРТС и Р. Э. СМИС

1. Потребление кислорода мышами было измерено в каждый час в течение 50-часового периода в закрытой системе, которая содержалась в темноте при нейтральной температуре.

2. Потребление кислорода показало выраженный дневной ритм, который отразил тот цикл темнота-свет, которому животные были подвергнуты перед опытом.

3. Обращение с животными (handling) и новая обстановка метаболической камеры независимо от времени начала опыта вызывали высокий начальный метаболизм у животных в течение 4—5 часов, но на их дневные часы не влияли.

4. Данные показывают, что период голодания, длительность опыта и фаза дневного ритма являются важными факторами, которые нужно стандартизировать, чтобы получить повторяющиеся величины потребления кислорода. Так как голодание, комбинированное с введением в новое окружение, повышает активность, поэтому нужно комбинировать период приручения к камере и период голодания с помещением голодной мыши в камеру в $\varrho$-время, и величину метаболизма, измеренную в последующее $\varrho$-время, считать как референтной или стандартной величиной метаболизма. У мышей такие $\varrho$-величины сходятся с величинами базального метаболизма, опубликованными раннее.

## ДЕЙСТВИЕ ФИЗОСТИГМИНА НА ВЫХОД $^{24}Na$ ИЗ ПОРТНЯЖНОЙ МЫШЦЫ ЛЯГУШКИ

Т. КОВАЧ и Б. САБО

1. В нормальном растворе Ringer физостигмин задерживает только 25% выхода меченого натрия из мышцы, в которой внутриклеточное Na-содержание было высоким.

2. Чувствительная к физостигмину часть выхода натрия значительно повышается, если заменить натрий во внешнем растворе отчасти или в целом литием или холином.

3. В растворе Ringer, содержащем 55 миллимол Na, чувствительная к физостигмину часть выхода натрия составляет 45% полного Na-выхода; а в растворе без натрия, 60% полного выхода оказался чувствительным к физостигмину.

3. Установлено, что тормозное влияние физостигмина на выход натрия зависит от концентрации натрия в мышечном волокне. Если внутренняя концентрация натрия в мышце меньше, чем 15—18 миллимол, тогда полный выход $^{24}Na$ оказывается нечувствительным к физостигмину.

4. Тормозное влияние физостигмина на выход натрия во многих отношениях напоминает влияние оуабаина. Физостигмин задерживает только чувствительный к оуабаину нетто-транспорт, а не оказывает влияние на несенситивный к оуабаину компонент диффузии натрия.

## ВЛИЯНИЕ ИОНОВ НА ПОТЕНЦИАЛЫ ПОКОЯ И ДЕЙСТВИЯ СЕРДЕЧНЫХ МЫШЕЧНЫХ ВОЛОКОН УЛИШКИ (Helix pomatia)

Т. КИШШ и К. Ш.-РОЖА

Авторы исследовали ионные механизмы мембранных потенциалов и потенциалов действия на сердечных мышечных волокон у Helix pomatia с помощью микроэлектродных отведений. Установили, что величина мембранного потенциала 50,6 $\pm$ 3,4 mV. Величина

потенциалов действия составляла 18—40 mV, а длительность 300—500 мсек. На контрольных сердцах можно регистрировать потенциалы действия в виде растянутых и острых спайков. Под влиянием снижения внешней концентрации $Na^+$, мембрана гиперполяризуется, и при полном отсутствии $K^+$ и $Ca^{++}$, деполаризуется. Избыток $Na^+$ не вызывает значительного изменения мембранного потенциала. При 50%-ном уменьшении $Na^+$ потенциал действия изменяется и принимает вид острых спайков, при полном отсутствии $Na^+$ образование потенциала прекращается. Образование потенциала в среде, свободной от $Ca^{++}$ в начале учащается, а после длительного влияния тормозится. Восходящая часть потенциала действия на сердце у Helix pomatia зависит от $Na^+$, и по ионному механизму обнаруживает те же закономерности, что и сердца млекопитающих, но не других безпозвоночных или раков.

## ВНУТРИЛОБАРНОЕ РАСПРЕДЕЛЕНИЕ ТКАНЕВОГО КРОВООБРАЩЕНИЯ В ПЕЧЕНИ СОБАК, I. ДЕЙСТВИЕ НЕКОТОРЫХ ВАЗОАКТИВНЫХ ВЕЩЕСТВ И ГЕМОРРАГИЧЕСКОЙ ГИПОТОНИИ

Г. УНГВАРИ и Б. ВАРГА

У старых собак обоих полов процентное отношение объёма синусоидов и клеток в единице печеночной ткани было высчитано в тех частях долей печени, которые лежат близко к гилюсу (центральной) и на расстоянии от гилюса (периферической). Установлено, что тотальный объём синусоидов в единице ткани центрально больше, чем периферически.

С помощью коррозионных препаратов было установлено, что синусоиды являются 4—6-ыми ветвями стволов воротной вены или печеночной вены в центральных, и 6—10-ыми ветвями в периферических частях. Из-за этого, путь кровотока короче центрально, чем периферически.

Моноаминергические нервы наблюдались авторами только в периферической части доли с флюоресцентным гистохимическим методом Falck—Hillarp.

Изменение тканевого кровотока под влиянием адреналина, норадреналина, изопротеренола, ацетилхолина и гистамина, которые были введены внутриворотно или внутривенно, и после геморрагии (кровяное давление составляло 40—50 мм рт. ст.) было измерено гибкими термоэлементами Hensel в центральной и периферической частях печеночной доли. При увеличении портального давления на адреналин или норадреналин не было отмечено разницы между тканевыми кровотоками центральной и периферической частей доли. Изопротеренол повышал тканевый кровоток в центральной, и снижал в периферической части доли ($p < 0.05$). Ацетилхолин, введенный внутриворотно, казался неэффективным, а введенный внутривенно, увеличивал воротное давление и снижал тканевый кровоток, менее в центральной и более выраженно в периферической части доли ($p < 0.05$). Гистамин, введенный внутриворотно или внутривенно, повышал воротное давление и уменьшал тканевый кровоток в центральной и периферической частях доли ($p < 0.05$). При геморрагической гипотонии тканевый кровоток тоже снижался в центральной и периферической частях доли: периферически снижение было более выраженное ($p < 0.001$).

Эти наблюдения позволяют сделать вывод, что вмешательства, вызывающие заметное снижение системного кровяного давления в нормоволемии, и гиповолемическая гипотония вызывают т. н. ограничивающую (restrictive) циркулярную дистрибуцию в печени собак.

## ВНУТРИПОЧЕЧНОЕ РАСПРЕДЕЛЕНИЕ ВАСКУЛЯРНОГО СОПРОТИВЛЕНИЯ У СОБАК

П. БАЛИНТ, А. ФЕКЕТЕ, Л. МОЛНАР и Е. СЁЧ

Кровообращение почек (RBF), клубочковая фильтрация (GFR) и градиент внутрипочечного давления изучались у собак под наркозом.

1. При «stop flow» условиях численная величина коллоид-осмотического давления и давления в мочеточнике соответствует величине давления в клубочковой капилляре.

2. При «free flow» условиях давление в внутрипочечных глубоких венах соответствует интерстициальному давлению почки, и давлению в проксимальном канальце и перриканальцевых капиллярах.

3. Линейная зависимость была отмечена между клубочково-фильтрационным и эффективно-фильтрационным давлениями: величина константы пермеабильности — около 2 мл/мин/мм рт. ст. /100 г веса почки.

4. Физиологические изменения артериального среднего давления не оказывают влияния на кровоток и клубочковую фильтрацию у разных животных: константная гемодинамика обеспечивается изменениями по соответствующему направлению полного и афферентного сопротивлений.

## ДАННЫЕ К ВОПРОСУ НЕЙРОГУМОРАЛЬНОЙ ОРГАНИЗАЦИИ ВАЗОМОТОРНЫХ РЕФЛЕКТОРНЫХ МЕХАНИЗМОВ

Г. ШУХ и А. ВЕТРО

Авторы исследовали чувствительность к кураре различных вазомоторных рефлексов на кроликах и кошках под наркозом, и установили, что после внутривенного введения 150—200 $\mu$г/кг d-тубокурарин-пентагидрата у кроликов тормозятся или исчезают соматические вазодепрессорные рефлексы, но не изменяются или несколько увеличиваются висцеральные рефлексы (vagus, depressor). Кураре изменяет на противоположный вагусный прессорный рефлекс у кролика; у кошек соматические и висцеральные депрессорные рефлексы не чувствительны к кураре.

При раздражении центрального конца вагусного и срединного нервов, полученные прессорные рефлексы можно тормозить или изменять на проитвоположные введением 150—300 $\mu$г кураре на кг веса тела.

Кураре в дозе, которая не парализует ганглии, не тормозит прессорный рефлекс каротидного синуса ни у кроликов, ни у кошек.

На основании результатов авторы предполагают, что холинергические элементы принимают участие в центральном механизме указанных рефлексов.

## ВЛИЯНИЕ АЦЕТИЛХОЛИНА НА ВНЕШНЕСЕКРЕТОРНУЮ ДЕЯТЕЛЬНОСТЬ ПОДЖЕЛУДОЧНОЙ ЖЕЛЕЗЫ ЛЯГУШЕК

А. ПУППИ, Т. МОНЧКО, Л. КОМАРОМИ и А. ТИДЬИ

Исследовалась роль ацетилхолина в регуляции внешнесекреторной функции поджелудочной железы лягушки (Rana esculenta). Установлено, что:

1. под влиянием ацетилхолина значительно увеличивалась активность клеток, измеренная по методу всасывания нейтрального-красного;
2. количество гранул значительно уменьшалось;
3. количество выделенного поджелудочного сока увеличивалось у голодных, а уменьшалось у сытых животных.

На основании вышеизложенного и того факта, что поджелудочная железа лягушек содержит 0,1—0,001 $\mu$г ацетилхолина на 100 мг веса панкреаса, сделано заключение, что ацетилхолин может играть значительную роль в регуляции внешнесекреторной деятельности поджелудочной железы лягушки. Кажется спорной существующая в настоящее время концепция, что у лягушек секреция регулируется только симпатической нервной системой.

## ДЕЙСТВИЕ ЛИЗИН-8-ВАЗОПРЕССИНА НА РАСПРЕДЕЛЕНИЕ ТИОСУЛЬФАТА И ИНУЛИНА У СОБАКИ

Х. ТОСТ, Г. КЁВЕР и Й. САБОЛЧ

Область распределения инулина и тиосульфата в зависимости от времени, и действие синтетического лизин-8-вазопрессина на распределение этих веществ исследовались у собак под наркозом, до и после нефрэктомии.

1. Вазопрессин (50 миллиед/кг + 50 миллиед/кг) снижал область распределения инулина у животных с нефректомией по сравнению с контрольными, а тиосульфат-область идентична с контрольными.

2. У животных без нефректомии инулин-область не изменялась на действие вазопрессина, но тиосульфат-область увеличивалась по сравнению с контрольными.

3. Эти результаты согласуются с предположениями авторов, что лизин-8-вазопрессин внепочечно повышает проницаемость клеточной мембраны, и таким образом через мембрану клетки легче проникают преимущественно молекулы тиосульфата меньшего веса.

## СЕЗОННЫЕ ИЗМЕНЕНИЯ ЭЛЕКТРОСУДОРОЖНОЙ ЧУВСТВИТЕЛЬНОСТИ У КРЫС

Й. ПОРСАС и И. ВОРУМ

На крысах-самцах исследовалось изменение электросудорожной чувствительности в различные сезоны года и её связь с уровнем гамма-аминомасляной (GABA) и глютаминовой кислот (GLU) в мозге.

Установлено, что порог 100% судорожной активности летом составляет 120 V, осенью и весной 70 и 60 V, а зимой 35 V. Уровень GABA в мозге осенью и весной существенно не различается, содержание GLU осенью несколько выше, чем весной. Зимой содержание GABA в некоторых отделах мозга на 30—50% ниже, а летом на 60—90% выше, чем осенью.

При торможении функции щитовидной железы путем скармливания метил-меркаптоимидазола, содержания крыс в тепле (+29°C), или тиреоидэктомии интенсивность судорог снижалась, в то же время содержание GABA и GLU в мозге повышалось. Это повышение концентрации GABA в различных отделах мозга после тиреоидэктомии составляло 20—50%, после введения метилмеркаптоимидазола 30—100%, а при содержании крыс в температуре окружающей среды 29°C 20—50%. Концентрация GLU изменялась подобным образом.

На основании полученных результатов и литературных данных, авторы приходят к заключению, что снижение GABA в мозге, вызванное активацией щитовидной железы, является одной из возможных причин снижения судорожного порога в зимний период года.

## ЭКСПЕРИМЕНТАЛЬНАЯ ЛЕГОЧНАЯ ЭМБОЛИЯ У КРЫС ПОСЛЕ МЕДИКАМЕНТОЗНОЙ ДЕНЕРВАЦИИ СЕРДЦА

Г. ДАВИД и И. КЕНЕДИ

Течение массивной легочной эмболии исследовалось у 36 крыс, денервированных холинолитическими и симпатиколитическими лекарствами. В 20 опытах постоянно регистрировалась ЭКГ; у 7 крых с легочной эмболией ЭКГ осталась регулярной, однако одна из них в 5 минут погибла. В 4 опытах наблюдались только небольшая лабильность Т волны и отдельные супранодальные экстрасистолы. Одна крыса выжила критические 5 минут после эмболизации, хотя многообразные экстрасистолы и «low voltage» наблюдались у нее. Восемь крыс погибли через 2—3 минуты при симптомах тяжелого вагусного возбуждения на ЭКГ.

У 16 крыс проверялись дыхание и кровяное давление в общей сонной артерии. Из них 8 выжили массивную легочную эмболию, 8 погибли.

Результат медикаментозного торможения вегетативной нервной системы во время легочной эмболии зависит от симпатикотонии, вызванной холинолитиками. Если симпатикотония преобладает, животные выживут острую фазу легочной эмболии, так же, как и после введения атропина, как было показано в ранних опытах авторов.

## ТОЛЕРАНТНОСТЬ К РАЗЛИЧНЫМ ВЛИЯНИЯМ ОКСОТРЕМОРИНА И ВОЗДЕЙСТВИЕ НА НЕЕ СОЕДИНЕНИЙ, ДЕЙСТВУЮЩИХ НА ЦЕНТРАЛЬНУЮ НЕРВНУЮ СИСТЕМУ

Л. ДЬЕРДЬ, Б. ГЕЛЛЕН, М. ДОДА, К. ПФЕЙФЕР и А. БИТЕ

1. Противоболевой эффект оксотреморина (ОТ) уменьшается треморином, введенным за 24 часа. 1—4-дневное предварительное лечение треморином не оказывает влияния на способность ОТ-а потенцировать пентобарбиталовый наркоз, но быстрая толерантность развивается к способности ОТ-а уменьшать движение животных.

2. Повторные дозы ОТ-а не влияют на смертность от карбахола и неостигмина.

3. Усойчивость к влиянию ОТ-а на координацию движения (измеренную на вращательном пруте) тормозится фенобарбиталом и снимается галоперидолом. Явление устойчивости можно наблюдать у крыс, но не у кошек.

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# *Physiologia*

## ARTERIO-VENOUS ANASTOMOSES IN THE KIDNEY, IX

### INTRARENAL CIRCULATION DURING TOURNIQUET SHOCK

By

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(Received January 20, 1971)

The circulatory effects of tourniquet shock have been investigated in dogs. According to the phase of the shock, blood pressure decreased, cardiac output diminished even more significantly and total peripheral resistance increased. Shifting of the systemic circulation was confirmed by the considerable reduction of the renal fraction of cardiac output. Total renal blood flow measured by electromagnetic flowmeter dropped to one-third of the normal value. Nutrient renal blood flow estimated by $^{86}Rb$ uptake pointed to a pronounced ischaemia of the renal parenchyma. The 0.649 quotient of $RBF_{nutr}/RBF_{air}$ versus the normal 0.97, suggests that in tourniquet shock a significant portion of the total renal blood flow returns into the systemic circulation through arterio-venous anastomoses thus bypassing the renal parenchyma and aggravating the renal ischaemia.

In previous papers (BENCSÁTH et al. 1969; NAGY et al. 1971) data were provided concerning the effects on renal circulation of some hypotonic states of shock characterized by a low cardiac output. It has been pointed out that both in dehydration and in haemorrhagic hypotension a peculiar form of intrarenal circulation characterized by an increase in the so-called inert flow takes place, constituting the difference between total and nutrient renal blood flow. GÖMÖRI et al. (1964) employing the PVC corrosion method in the kidneys of dogs subjected to tourniquet shock found signs pointing to arterial spasms, incomplete filling of glomeruli, and filling up of the stellate veins through arterio-venous anastomoses. The corrosion technique is well-suited for investigating the problem from the anatomical point of view, but as far as renal and intrarenal circulation are concerned, the method fails to offer numerical data. The well-known circulatory characteristics of dehydration as well as of haemorrhagic hypotension, i.e. hypovolaemia, a decrease in cardiac output, an increase in the arterio-venous oxygen-difference and increased TPR may be observed in tourniquet shock, too. This has prompted us to study intrarenal circulation in this type of shock.

## Methods

The experiments were performed on eleven mongrel dogs of both sexes, weighing 12—16 kg each and anaesthetized with 0.10 g/kg body weight of chloralose. After anaesthesia on both hind limbs tourniquets were applied in the usual manner to achieve complete ischaemia for a period of four hours. Meanwhile the necessary preparations were carried out: arterial blood pressure in the common carotid artery was measured by an Elema-Schönander type EMT-35 electromanometer and continuously registered on an Elema-Schönander Mingograf 81. Total renal blood flow (in the following, $RBF_{dir}$) was continuously measured by a Nycotron electromagnetic flowmeter the probe of which was placed on the main trunk of the renal artery. The left renal hilum was approached retroperitoneally through a left flank incision and the renal artery was carefully exposed leaving the renal nerves intact. Occasionally, if the left kidney was supplied with two arteries or the trunk of the artery was too short ruling out the possibility of flow-measuring, the site of the operation was closed and the same procedure was carried out on the right side. The dogs lay on their side opposite the one at which renal blood flow was measured. To obtain zero flow reference the renal artery was occluded with pincers for a few seconds between the probe and the aorta. Arterial blood pressure and renal blood flow were monitored at least two hours prior to the removal of the tourniquets and registration was performed continuously throughout the experiment. To estimate nutrient renal blood flow (in the following, $RBF_{nutr}$) the method described in a previous paper was used (BENCSÁTH et al. 1969). A bolus containing about 100 $\mu$Ci $^{86}Rb$ was injected through a Cournand catheter into the right atrium; 240 seconds later the animals were killed with intravenously administered chloroform and this marked the end of the experiment. The course of each experiment was as follows: tourniquets placed into position soon after anaesthesia, necessary preparations, tourniquets off, observation period until blood pressure decreased to about 70 mm Hg. This level was selected in view of the fact that in some experiments (which have not been included in the series discussed here) blood pressure below 60 mm Hg seemed critical and on subsequent samplings its further drop made it impossible to evaluate the dilution curve. After blood pressure had decreased to about 70 mm Hg, 100 $\mu$Ci $^{86}Rb$ was administered and the animals were sacrificed 240 seconds later. To evaluate mean values and standard deviations the usual calculations were used, and Student's one sample and paired sample *t*-tests were applied for evaluating statistical significance.

## Results

Tables I, II and III contain the results obtained. Table I provides data concerning systemic circulation. In two experiments shock occurred 10 and 22 minutes after removal of the tourniquets, while in the remaining 9 experiments its development took from 80 to 480 minutes. In the 11 experiments mean blood pressure was $71.4 \pm 13.8$ mm Hg when the $^{86}Rb$ was administered. In one experiment (No. 123) the $^{86}Rb$ was injected at a blood pressure of 105 mm Hg, but the presence of shock was obvious as revealed by a cardiac index of 911 ml/min/m$^2$. The marked decrease in cardiac output to $838.0 \pm 283.9$ ml/min, or $1308.0 \pm 333.9$ ml/min/m$^2$ by calculating the cardiac index, showed a serious deterioration of systemic circulation. At the same time, TPR presented an elevation of $4798.6 \pm 1967.8$ dyne·sec·cm$^{-5}$, as calculated from the cardiac index. Thus the values for $RBF_{dir}$ calculated per 100 g kidney weight, and $RBF_{nutr}$, furthermore the quotient $RBF_{nutr}/RBF_{dir}$, and the renal fraction of cardiac output are presented in Table II for each of the eleven experiments. Among these, experiments No. 120 and 128 have not been evaluated, in consideration of the low blood flow values, although they did not contradict the general trend. The marked decrease in $RBF_{dir}$ to

110.0 ± 47.6 ml/min/100 g kidney weight was interpreted as the effect of the tourniquet shock. Renal ischaemia brought about by this type of shock was characterized by the fact that $RBF_{nutr}$ decreased even more markedly, to 68.8 ± 27.3 ml/min/100 g kidney. The difference between $RBF_{dir}$ and $RBF_{nutr}$ was significant statistically ($p < 0.01$). The quotient $RBF_{nutr}/RBF_{dir}$ was

**Table I**

*Effect of tourniquet shock on systemic circulation*

| Experiment | T | BP | CO | CI | TPR |
|---|---|---|---|---|---|
| 103 | 10 | 70 | 1047 | 1689 | 3316 |
| 108 | 115 | 60 | 1059 | 1708 | 2810 |
| 113 | 22 | 60 | 725 | 1169 | 4106 |
| 114 | 82 | 90 | 1352 | 1802 | 3996 |
| 118 | 263 | 65 | 937 | 1442 | 3606 |
| 120 | 90 | 70 | 479 | 812 | 6897 |
| 123 | 80 | 105 | 565 | 911 | 9220 |
| 125 | 140 | 65 | 1087 | 1672 | 3110 |
| 128 | 80 | 65 | 565 | 831 | 6258 |
| 130 | 480 | 70 | 841 | 1401 | 3997 |
| 131 | 270 | 65 | 561 | 951 | 5468 |
| n = 11 | | | | | |
| $\bar{x}$ | 148 | 71.4 | 838.0 | 1308.0 | 4798.6 |
| S.D. | ±138 | ±13.8 | ±283.9 | ±333.9 | ±1967.8 |
| Normal | | 139.0 | | 3250.0 | 3672 |
| values* | | ±27.6 | | ±1010.0 | ±1335 |

T minutes elapsed between release of tourniquet and end of the experiment
BP arterial blood pressure, mm Hg
CO cardiac output, ml/min
CI cardiac index, ml/min/$m^2$
TPR total peripheral vascular resistance, dyne · sec · $cm^{-5}$
* Normal values as published by NAGY et al. 1967

0.649, highly significantly lower than the normal 0.97 ($p < 0.001$). As a sign characteristic of the circulatory shifting, RBF diminished more considerably than did cardiac output. The renal fraction was 8.25 ± 2.04% against the normal 15—20%. The decrease was even more marked when calculated on the basis of the equation, $RBF_{nutr} \cdot 100/CO$.

Table III illustrates the parameters of intrarenal circulation. Both cortical and medullary flow decreased markedly, but the absolute value for the cortical blood flow was even more affected, as shown also by the $RBF_{cort}/RBF_{dir}$ coefficient.

**Table II**

*Data for renal circulation*

| Experiment | $RBF_{dir}$ | $RBF_{nutr}$ | Q | RF | |
|---|---|---|---|---|---|
| | | | | dir | nutr |
| 103 | 81 | 50.0 | 0.617 | 6.49 | 4.01 |
| 108 | 166 | 101.9 | 0.722 | 7.55 | 5.46 |
| 113 | 139 | 96.2 | 0.692 | 11.03 | 7.64 |
| 114 | 184 | 86.6 | 0.470 | 8.87 | 4.17 |
| 118 | 118 | 91.8 | 0.770 | 8.54 | 6.57 |
| 120x | 10 | 3.0 | 0.250 | — | — |
| 123 | 66 | 41.1 | 0.621 | 9.91 | 6.57 |
| 125 | 46 | 33.4 | 0.727 | 4.05 | 2.94 |
| 128x | 36 | 9.7 | 0.270 | — | — |
| 130 | 120 | 80.7 | 0.672 | 8.56 | 5.75 |
| 131 | 70 | 37.4 | 0.553 | 9.26 | 5.13 |
| n = 9 | | | | | |
| x | 110.0 | 68.8 ·· | 0.649+ | 8.25 | 5.31·· |
| S.D. | ±47.6 | ±27.3 | ±0.095 | ±2.04 | ±1.44 |
| Normal | 331 | 322 | 0.97 | | |
| values* | ±105 | ± 98 | ±0.03 | | |

$RBF_{dir}$ total renal blood flow, ml/min/100 g kidney weight, measured in the renal artery
$RBF_{nutr}$ nutrient renal blood flow, ml/min/100 g kidney weight, as estimated by $^{86}Rb$ fractionation
Q $RBF_{nutr}/RBF_{dir}$
RF renal fraction of cardiac output for two kidneys
·· $p < 0.01$ by one sample *t*-test
\+ $p < 0.001$ by paired *t*-test
* Normal values from BENCSÁTH et al. 1969

Summarizing the results, in tourniquet shock RBF decreased more markedly than it would follow from the deterioration of systemic circulation.

## Discussion

Circulation in shock is characterized by a low blood pressure, a decrease of cardiac output, hypoxia of the stagnant type and by the shifting phenomenon. TAKÁCS et al. (1961) have shown in the dog that in 3 hours' hind limb ischaemia cardiac output diminishes, TPR increases and the arterio-venous oxygen difference increases in a manner characteristic of stagnant type

hypoxia. Besides, an increase in pulmonary vascular resistance has also been found. Blood pressure determination alone may supply incorrect information as to the severity of shock since TPR, probably owing to the increased catecholamine output, is usually elevated. Thus, a slightly reduced blood pressure does not yet exclude the presence of shock; e.g. in experiment No. 123 cardiac index was low and blood pressure normal. Still, the instability of circulation

**Table III**

*Data for intrarenal circulation*

| Experiment | Cort | OM | IM | Q |
|---|---|---|---|---|
| 103 | 64.0 | 25.2 | 5.4 | 0.50 |
| 108 | 148.0 | 38.0 | — | 0.57 |
| 113 | 106.4 | 80.6 | 33.7 | 0.49 |
| 114 | 124.1 | 13.5 | 3.6 | 0.43 |
| 118 | 106.5 | 51.2 | 28.0 | 0.58 |
| 123 | 70.6 | 6.6 | 1.0 | 0.68 |
| 125 | 40.1 | 25.2 | 8.8 | 0.59 |
| 130 | 103.5 | 49.5 | 10.2 | 0.55 |
| 131 | 45.9 | 20.0 | 9.6 | 0.42 |
| n = 9 | | | | |
| $\bar{x}$ | 89.9 | 34.4 | 12.5 | 0.534 |
| S.D. | ± 36.7 | ±23.0 | ±11.1 | ±0.082 |
| Normal values* | 366 | 163 | 78 | 0.71 |
| | ± 91 | ±72 | ±29 | ±0.08 |

Cort renal cortical blood flow, ml/min/100 g cortical tissue
OM outer medulla, blood flow, ml/min/100 g
IM inner medulla, blood flow, ml/min/100 g
Q $RBF_{cort}/RBF_{dir}$ from blood flow values calculated for 100 g kidney weight
* Normal values from BENCSÁTH et al. 1969

was shown by the fact that in this situation an insignificant blood loss was capable of eliciting a rapid collapse of the circulation. The decrease in cardiac output was of a higher degree than in dehydration (NAGY et al. 1968), but less serious than in haemorrhagic hypotension (NAGY et al. 1971). Catecholamines are supposed to be responsible for the increase in TPR; for the circulatory shifting, too, catecholamines and other factors mediated by the sympathetic nervous system may be responsible. The low renal fraction is a characteristic sign of the shifting in shock, and the phenomenon was elicited in every experiment in this series. TAKÁCS et al. (1962) using the $^{86}Rb$ method found

a decreased renal fraction in rats with tourniquet shock. They reported a renal fraction of 9% under the effect of 3-hour tourniquet. However, there was an increase in the coronary fraction besides the reduced renal fraction. In our experiments the effect of 4-hour tourniquet was investigated and the renal fraction decreased to 8.25% as calculated from $RBF_{dir}$; meanwhile, if $RBF_{nutr}$ served as the basis, a much more striking decrease to $5.31 \pm 1.44\%$ was observed. The difference in the renal fraction values (9% of TAKÁCS et al. and 5.31% in the present series) is attributable to the fact that we employed a 4-hour while TAKÁCS et al. (1962) a 3-hour hind limb ischaemia and race differences might also have had a role. The decreased cardiac output and the increased TPR combined with a low renal fraction pointed to renal vasoconstriction. A similarly decreased renal fraction was observed in haemorrhagic hypotension by NAGY et al. (1971), while BÁLINT et al. (1969a) failed to find this. GÖMÖRI et al. (1964) injected a 5% PVC solution into the renal artery of dogs subjected to tourniquet shock; in the corrosion preparations there were arterial spasms, filling defects of the glomeruli and filling up of the stellate veins through arterio-venous shunts. The present experiments have confirmed these results as regards the renal ischaemia and the functionally active arterio-venous shunts. There can be no doubt concerning the renal ischaemia in view of the low $RBF_{dir}$ and the decreased renal fraction, especially if $RBF_{nutr}$ is regarded as a useful amount of blood from the functional point of view. As has been pointed out, there was roughly a 40 ml difference between $RBF_{dir}$ and $RBF_{nutr}$. This means that about one-third of the RBF failed to reach the renal parenchyma, the tissue capable of extracting the Rb. On the other hand, the difference between $RBF_{dir}$ and $RBF_{nutr}$ means that *one-third of the RBF has by-passed the renal parenchyma through arterio-venous shunts*. The results supported the opinion that all the tests estimating only the total RBF, whether by a flowmeter or by the venous outflow method, are unreliable for drawing appropriate conclusions as to the degree of renal ischaemia. Obviously, there was a statistically significant difference between the $RBF_{dir}$ and $RBF_{nutr}$ as well as between the renal fractions calculated from these RBF values.

The difference observed between $RBF_{dir}$ and $RBF_{nutr}$ strongly suggests that in tourniquet shock, like after human serum albumin infusion in dogs (GÖMÖRI et al. 1969), in dogs dehydrated by pyloric ligation (BENCSÁTH et al. 1969; BÁLINT et al. 1969b), in haemorrhagic hypotension (BÁLINT et al. 1969a; NAGY et al. 1971), a significant amount of the total RBF bypasses the renal parenchyma. Ischaemia of the renal tissue can be explained by at least two factors. Firstly, $RBF_{dir}$ decreases owing to the deterioration of systemic circulation and in view of the shifting; secondly, the ischaemia is aggravated by the intrarenal shifting. In tourniquet shock this, according to the present experiments, affects above all the renal cortex as proved by

the low $RBF_{cort}/RBF_{dir}$ quotient. This observation may explain also the low PAH extraction found in traumatic shock (GÖMÖRI et al. 1965).

In our opinion, this intrarenal shifting is a consequence of the opening up of the arterio-venous shunts.

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# ARTERIO-VENOUS ANASTOMOSES IN THE KIDNEY, X

## EFFECT OF PHENOXYBENZAMINE ON INTRARENAL CIRCULATION OF DOGS IN TOURNIQUET SHOCK

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The effect of phenoxybenzamine injected into the renal artery in a dose of 1.5 mg/kg body weight has been studied in dogs subjected to tourniquet shock. Intrarenal circulation was unaffected although total renal blood flow decreased and cardiac output, blood pressure and the renal fraction of cardiac output diminished. Phenoxybenzamine prevented the intrarenal shifting as well as the ischaemia of renal parenchyma due to the constriction of renal vessels and the subsequent opening up of arterio-venous shunts.

In a previous paper (NAGY et al. 1971) the effect of tourniquet shock on the different parameters of systemic circulation and of intrarenal shifting has been discussed. It was shown that in this type of shock a change of intrarenal circulation takes place, characterized by the fact that more than one-third of the total renal blood flow ($RBF_{dir}$) bypasses the renal parenchyma through arterio-venous shunts. The role of the sympathetic nervous system and/or of some sympathetic mediators was suggested to be responsible for the alteration. Examining the effect of unilateral kidney denervation, GÖMÖRI et al. (1965) observed in PVC corrosion preparations of dogs subjected to traumatic shock, marked changes on the innervated side, such as an incomplete filling of the arterial tree and of the glomeruli, and the PAH extraction was low; in contrast, the corrosion preparation of the denervated side showed normal condition and an almost normal PAH extraction, in spite of the fact that the systemic circulation ought to have produced identical changes on both sides. Isolated head hypoxia elicited the well-known renal circulatory changes (GÖMÖRI et al. 1960) but unilateral renal denervation by cutting the major splanchnic nerve or sympatholythine (Dibenamine) treatment prevented the hypoxic effects. GUMP et al. (1968) reported about the effect of phenoxybenzamine on renal circulation. They found that this agent if injected into the renal artery inhibited the effect of norepinephrine and the influence of haemorrhage on the constriction of the renal artery. According to TRUNIGER and GRANDCHAMP (1969), intrarenal shifting, due to haemorrhage is mediated mainly through the alpha stimulating effect of the circulating catecholamines. Using the Xenon washout method, they found a marked

renal cortical ischaemia after haemorrhage which disappeared following the injection of phenoxybenzamine into the renal artery. The present study was devoted to the question of whether or not phenoxybenzamine influenced the intrarenal shifting.

## Methods

The experiments were carried out on ten normally hydrated mongrel dogs of both sexes, weighing 12 to 15 kg, anaesthetized with 0.10 g/kg of chloralose. The methods used were the same as in previous experiments (NAGY et al. 1971), with the only exception that soon after anaesthesia and prior to any other manipulations, 1.5 mg/kg of phenoxybenzamine dissolved in about 3 ml physiologic saline solution was administered into the renal artery. This procedure took about 2 minutes. To prevent an injury of the renal artery, the abdominal aorta was punctured with a specially curved needle and this was introduced into the renal artery through its aortic opening. According to our observation, the 1.5 mg/kg body weight dose of phenoxybenzamine did not affect systemic circulation since blood pressure failed to show a decrease, and the test-injection of 30 $\mu$g of adrenaline induced an increase of blood pressure. Phenoxybenzamine was administered usually into the left renal artery where $RBF_{dir}$ was measured. In exceptional cases when the left kidney was supplied with two or more arteries, the right kidney had to be used. After the phenoxybenzamine treatment on both hind limbs tourniquets were applied in the usual manner to achieve complete ischaemia for a period of 4 hours. Arterial blood pressure was recorded in the common carotid with an Elema-Schönander electromanometer, and registered over the whole period of the experiment. $RBF_{dir}$ was measured by a Nycotron electromagnetic flowmeter the probe of which was placed around the main trunk of the renal artery and it was registered continuously together with the blood pressure by the direct-writing Mingograf 81 of Elema-Schönander. Zero reference was achieved by occluding the renal artery between the aorta and the probe for a few seconds. After removal of the tourniquets an observation period followed until blood pressure decreased to about 70 mm Hg. Nutrient renal blood flow ($RBF_{nutr}$) was estimated by SAPIRSTEIN's $^{86}Rb$ method (1956) as modified by HÁRSING and PELLEY (1965). A bolus of about 100 $\mu$Ci $^{86}Rb$ was injected into the right atrium through a Cournand catheter inserted into the jugular vein and washed through with 5 ml physiologic saline. Blood samples for plotting the dilution curve and the estimation of cardiac output, were obtained from the carotid artery. The animals were sacrificed 240 seconds after the administration of $^{86}Rb$.

The course of each experiment was as follows: anaesthesia, phenoxybenzamine administration into the renal artery, tourniquet application on both hind limbs, necessary preparations, tourniquets off, observation period until blood pressure decreased to about 70 mm Hg. After blood pressure had decreased to 70 mm Hg, $^{86}Rb$ was administered and the animals were killed with intravenously administered chloroform 240 seconds later. Estimation of $^{86}Rb$ in the renal cortex and medulla was performed as described by GÖMÖRI et al. (1969).

To test mean values and standard deviations the usual methods were employed and statistical analysis was carried out according to Student's one sample and paired sample *t*-tests. Since the method adopted provided no opportunity for comparison with self-control values, the values reported by NAGY et al. (1967) and BENCSÁTH et al. (1969) were accepted as normal.

## Results

Results are shown in Tables I, II and III. Table I contains the results concerning systemic circulation. Mean blood pressure at the time of $^{86}Rb$ administration was 70.5 mm Hg; cardiac output decreased to a value characteristic of shock; cardiac index was as low as $1356 \pm 379$ ml/min/m$^2$; TPR as calculated from the cardiac index increased to $4354 \pm 980$ dyne·sec·cm$^{-5}$. For the sake of comparison, Tables I, II and III contain the normal values together with those observed in untreated tourniquet shock.

Table II summarizes data concerning renal circulation. Mean $RBF_{dir}$ was $116.3 \pm 29.6$ ml/min/100 g kidney, indicating a renal ischaemia. $RBF_{nutr}$

estimated on the basis of $^{86}Rb$ accumulation was 111.1 ± 32.5 ml/min/100 g kidney, almost the same as the value for $RBF_{dir}$. From these values it follows that the $RBF_{nutr}/RBF_{dir}$ was 0.950, almost normal. The renal fraction of cardiac output was 8.50 ± 1.85%, lower than normal. Obviously, no difference could be detected in the renal fractions calculated from the values for $RBF_{dir}$ and $RBF_{nutr}$.

**Table I**

*Effect of tourniquet shock on systemic circulation in phenoxybenzamine treated dogs*

| Experiment | T | BP | CO | CI | TPR |
|---|---|---|---|---|---|
| 114/a | 82 | 90 | 1352 | 1802 | 3996 |
| 115 | 440 | 70 | 1136 | 1600 | 3500 |
| 116 | 65 | 90 | 1120 | 2000 | 3600 |
| 118/a | 263 | 65 | 937 | 1442 | 3606 |
| 121 | 240 | 65 | 810 | 1285 | 4047 |
| 122 | 240 | 80 | 865 | 1331 | 4808 |
| 134 | 137 | 60 | 734 | 1244 | 3858 |
| 135 | 120 | 60 | 750 | 1210 | 3966 |
| 136 | 300 | 65 | 531 | 857 | 6068 |
| 137 | 360 | 60 | 465 | 788 | 6091 |
| n = 10 | | | | | |
| $\bar{x}$ | 225 | 70.5 | 870 | 1356 | 4354 |
| S.D. | ±123 | ± 11.9 | ±276 | ± 379 | ± 980 |
| Tourniquet shock | 148 | 71.4 | 838 | 1308 | 4798 |
| | ±138 | ± 13.8 | ±284 | ± 334 | ±1967 |
| Normal | | 139.0 | | 3250.0 | 3672 |
| | | ± 27.6 | | ±1010.0 | ±1335 |

T minutes elapsed after release of tourniquets
BP mean arterial blood pressure, mm Hg
CO cardiac output, ml/min
CI cardiac index, ml/min/$m^2$
TPR total peripheral vascular resistance, dyne · sec · $cm^{-5}$
Tourniquet shock values, see NAGY et al. (1971)
Normal values from NAGY et al. (1967)

Table III provides data concerning intrarenal circulation. It can be seen that, as compared to the normal, flow values were reduced in the cortex as well as in the medulla. It was interesting, however, that the quotient cort/dir which shows the relative blood supply of the cortex, gave a normal value. According to GÖMÖRI et al. (1969) the cort/dir ratio approximates PAH extraction, so a normal PAH extraction would be expectable in the

phenoxybenzamine treated dogs despite the shock. The same follows from the 142.5 ± 36.1 ml/min/100 g cortex flow value, which was significantly ($p < 0.001$) higher than in the untreated dogs with tourniquet shock.

**Table II**

*Effect of Dibenzyline on renal circulation in tourniquet shock*

| Experiments | $RBF_{dir}$ | $RBF_{nutr}$ | Q | RF | |
|---|---|---|---|---|---|
| | | | | dir | nutr |
| 114/a | 132 | 123.7 | 0.936 | 6.51 | 6.09 |
| 115 | 81 | 75.2 | 0.925 | 5.63 | 5.21 |
| 116 | 144 | 170.9 | 1.184 | 6.79 | 8.03 |
| 118/a | 124 | 103.5 | 0.836 | 9.39 | 7.85 |
| 121 | 134 | 129.4 | 0.964 | 9.13 | 8.81 |
| 122 | 103 | 95.5 | 0.941 | 7.16 | 6.76 |
| 134 | 134 | 124.4 | 0.927 | 9.80 | 9.10 |
| 135 | 156 | 138.3 | 0.883 | 11.20 | 9.89 |
| 136 | 87 | 89.2 | 1.005 | 9.03 | 9.07 |
| 137 | 68 | 61.1 | 0.904 | 10.32 | 9.33 |
| n = 10 | | | | | |
| $\bar{x}$ | 116.3 | 111.1 | 0.950 | 8.50 | 8.01 |
| S.D. | ± 29.6 | ± 32.5 | ±0.094 | ± 1.85 | ±1.54 |
| Tourniquet shock | 110.0 | 68.8 | 0.649 | 8.25 | 5.31 |
| | ± 47.6 | ± 27.3 | ±0.095 | ± 2.04 | ± 1.44 |
| Normal | 331 | 332 | 0.97 | | |
| | ±105 | ± 98 | ±0.03 | | |

$RBF_{dir}$ total renal blood flow, ml/min/100 g kidney weight, as measured in renal artery by electromagnetic flowmeter

$RBF_{nutr}$ nutrient renal blood flow, ml/min/100 kidney weight, as estimated from $^{86}Rb$ accumulation

Q $RBF_{nutr}/RBF_{dir}$

Tourniquet shock values, see NAGY et al. (1971)

Normal values from BENCSÁTH et al. (1969)

## Discussion

The present results have confirmed our earlier data concerning systemic circulation and circulatory changes in hind limb ischaemia in that cardiac output and blood pressure decreased to the shock level and the TPR increased. The changes were identical with those obtained in previous tourniquet shock experiments without phenoxybenzamine and in this sense they can be regarded as control experiments to determine whether alpha blockade is capable of influencing the circulatory changes caused by tourniquet shock. Table IV

**Table III**

*Intrarenal circulation in tourniquet shock in phenoxybenzamine treated dogs*

| Experiments | Cort | OM | IM | Cort/dir |
|---|---|---|---|---|
| 114/a | 176.7 | 27.4 | 7.5 | 0.85 |
| 115 | 154.9 | 63.7 | 31.8 | 1.22 |
| 116 | 203.0 | 48.6 | 9.5 | 0.90 |
| 118/a | 111.8 | 62.5 | 30.7 | 0.53 |
| 121 | 177.1 | 50.7 | 19.7 | 0.85 |
| 122 | 152.2 | 47.1 | 11.3 | 0.95 |
| 134 | 115.4 | 67.6 | 36.8 | 0.55 |
| 135 | 130.8 | 87.6 | 47.8 | 0.54 |
| 136 | 114.1 | 26.5 | 5.4 | 0.84 |
| 137 | 88.9 | 22.5 | 4.9 | 0.84 |
| n = 10 | | | | |
| $\bar{x}$ | 142.5 | 50.4 | 20.5 | 0.807 |
| S.D. | ± 36.1 | ± 20.7 | ±15.2 | ±0.215 |
| Tourniquet shock | 89.9 | 34.4 | 12.5 | 0.534 |
| | ± 36.7 | ± 23.0 | ±11.1 | ±0.082 |
| Normal | 366 | 163 | 78 | 0.71 |
| | ± 91 | ± 72 | ±29 | ±0.08 |

Cort cortical renal blood flow, ml/min/100 g cortex
OM outer medullary flow, ml/min/100 g
IM inner medullary flow, ml/min/100 g
Cort/dir calculated for 100 g kidney weight
Tourniquet shock values, see NAGY et al. (1971)
Normal values from BENCSÁTH et al. (1969)

contains the mean values and standard deviations of different parameters of the untreated as well as of the phenoxybenzamine treated groups. There were no significant differences in the parameters concerning systemic circulation: mean blood pressure, cardiac index and TPR were identical in both groups. On this basis it seems justified to assume that phenoxybenzamine in the dose applied and administered in the said manner brought about no systemic circulatory effect. According to GUMP et al. (1968) the drug is deposited in and bound to renal tissue and this may have been responsible the failure of exerting a circulatory effect. Another interesting problem in connection with the lack of a systemic effect was that phenoxybenzamine failed to influence the shifting phenomena, as shown by the fact that the renal fraction of the cardiac output was the same in the untreated and in the phenoxybenzamine treated animals. Consequently, the treatment appeared to be ineffective not only in inhibiting the decrease of cardiac output but also

**Table IV**

*Differences between parameters of untreated and phenoxybenzamine treated groups, as calculated by Student's one sample and paired t-tests*

| | T | BP | CI | TPR | $RF_{dir}$ | $RF_{nutr}$ |
|---|---|---|---|---|---|---|
| Tourniquet shock | 148 | 71.4 | 1308 | 4798 | 8.25 | 5.31·· |
| | ±138 | ± 13.8 | ± 334 | ±1967 | ± 2.04 | ± 1.44 |
| Phenoxybenzamine treated | 225* | 70.5* | 1356* | 4354* | 8.50* | 8.01+ |
| | ±123 | ± 11.9 | ± 379 | ± 979 | ± 1.85 | ± 1.54 |

| | RBF | | Q | Cort | OM | IM | Cort/dir |
|---|---|---|---|---|---|---|---|
| | dir | nutr | | | | | |
| Tourniquet shock | 110.0 | 68.8·· | 0.6493 | 89.9 | 34.4 | 12.5 | 0.534 |
| | ± 47.6 | ± 27.3 | ±0.0945 | ± 36.7 | ±23.1 | ±11.1 | ±0.082 |
| Phenoxybenzamine treated | 116.3* | 111.1+ | 0.9505++ | 142.5++ | 50.4* | 20.5* | 0.807+ |
| | ± 29.6 | ± 32.6 | ±0.0937 | ± 36.1 | ±20.7 | ±15.2 | ±0.215 |

·· one sample $p < 0.001$
\+ paired *t*-test $p < 0.01$
++ paired *t*-test $p < 0.001$
* not significant by paired *t*-test

in preventing the shifting, i.e. in normalizing the renal fraction which was 8.25% in the untreated group, and 8.50% in the phenoxybenzamine treated group. These facts might mean that the alpha-receptor blockade was incapable of increasing $RBF_{dir}$. In fact, this was 110.0 ml in the untreated tourniquet-shock series and 116.3 ml/min/100 g kidney in the treated group.

In spite of the fact that phenoxybenzamine in the given dose did not influence systemic circulation, there occurred significant changes in intrarenal circulation. In the treated animals, phenoxybenzamine succeeded in favourably influencing $RBF_{nutr}$. As can be seen in Table IV, in the untreated group $RBF_{nutr}$ was 40 ml less than $RBF_{dir}$, and this could be ascribed to intrarenal vasospasm and the arterio-venous shunts consequently opened up. In contrast, $RBF_{dir}$ and $RBF_{nutr}$ showed practically no difference in the animals treated with phenoxybenzamine. These observations prompted the conclusion that the drug prevented the intrarenal shifting associated with tourniquet shock. Our results are in complete accord with the observation (GUMP et al. 1968) in that exogenous nor-epinephrine or haemorrhage failed to cause vasoconstriction in the kidney into the renal artery of which phenoxybenzamine had been injected. While studying the effect of severe haemorrhage MCGIFF (1964) pointed out that after unilateral renal denervation blood loss does not increase renal vascular resistance, at the same time, however, renal vasoconstriction was detectable on the innervated side. In animals treated with guanethidine, the innervated and denervated kidney showed no difference. The importance of renal nerves in eliciting changes in RBF under the effect of acute arterial hypoxia has been shown by GÖMÖRI et al. (1960) who found a significant decrease in PAH clearance caused by acute hypoxia in the innervated kidney and only minor changes were observed on the denervated side; however, PAH clearance increased to the value for the denervated kidney after the animals had been given 5 or 10 mg/kg of Dibenamine. The neural regulation of intrarenal circulation was studied by POMERANZ et al. (1968) who by stimulating the renal nerves and using the $^{85}Kr$ washout method, found a decreased perfusion in the peritubular capillaries and an increase in the blood flow supplying the outer medulla. These changes were observed in the presence of an unchanged total renal blood flow. The neural control of intrarenal circulation has been shown by GÖMÖRI et al. (1965) who pointed out that in traumatic shock severe ischaemia developed in the innervated kidney, while the corrosion pattern and PAH extraction were normal in the denervated kidney. TRUNIGER and GRANDCHAMP (1969) using the $^{133}Xe$ washout technique detected a remarkable renal cortical ischaemia in haemorrhagic hypotension, but they succeeded in preventing this intrarenal shifting by injecting phenoxybenzamine into the renal artery and therefore attributed an important role in intrarenal shifting to the circulating catecholamines. In a previous paper (NAGY et al. 1971) we have shown that on the basis of

the difference found between the $RBF_{dir}$ and $RBF_{nutr}$ values, in tourniquet shock approximately one-third of the $RBF_{dir}$ does not reach the renal parenchyma, since this amount of blood bypasses the capillary network through arterio-venous anastomoses. This amount of blood was called inert flow. These alterations in intrarenal circulation may be regarded as a different type of intrarenal shifting and the present experiments, however, suggest that phenoxybenzamine was effective in preventing this kind of change (Fig. 1). In our opinion, under the effect of the alpha blockade caused by phenoxybenzamine

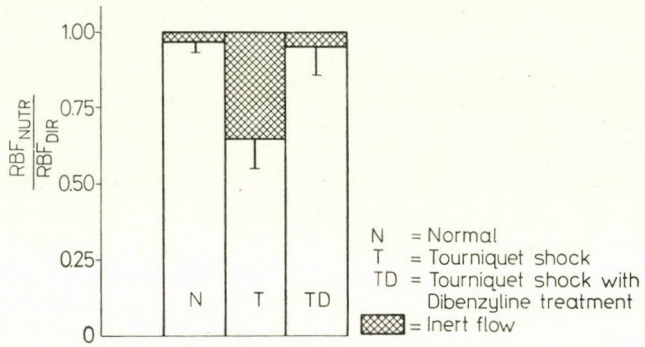


*Fig. 1* Ratio of nutr/dir RBF in control experiments, in untreated tourniquet shock and in dibenzyline treated groups. Shaded area represents the inert (shunt) flow

the tourniquet shock failed to cause vasoconstriction in the small intrarenal arteries and free passage was allowed to the flow towards the capillaries supplying the renal parenchyma instead of taking other routes of lesser resistance through the arterio-venous shunts. The increased nutrient blood flow ensures a better blood supply to the renal cortex. In tourniquet shock, cortical blood flow was 89.9 ml/min/100 g cortical tissue, while in the phenoxybenzamine treated group it was 142.5 ml/min/100 g in spite of there having been no difference in $RBF_{dir}$ (Fig. 2). These results have confirmed the data published by TRUNIGER and GRANDCHAMP (1969), who in haemorrhagic hypotension found normal cortical flow after phenoxybenzamine. The improved perfusion of the renal cortex can be documented not only in absolute terms, but it follows also from the ratio cort/dir, demonstrating a relatively normal distribution pattern. A beneficial effect of renal denervation in the sense of improved cortical perfusion and of the normalization of intrarenal circulation was suggested and indirectly proved on dogs with traumatic shock by GÖMÖRI et al. (1965) who found the PAH extraction to be almost normal.

Improved tissue perfusion and the normal pattern of intrarenal circulation in tourniquet shock after phenoxybenzamine treatment might suggest its therapeutic application in acute renal failure associated with every form of shock.

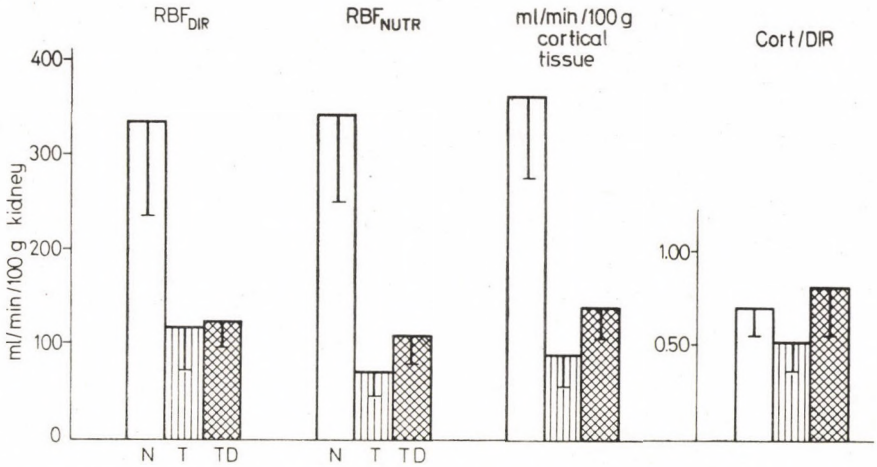


*Fig. 2.* Relation between $RBF_{dir}$ and $RBF_{nutr}$ in control condition and in tourniquet shock without, and with phenoxybenzamine

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# EFFECT OF SODIUM AND OSMOTIC CONCENTRATION ON RENIN RELEASE BY RENAL CORTICAL SLICES IN VITRO

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Renin release by canine renal cortical slices into the incubation medium of varying sodium content (10 to 200 mEq/l) and osmotic concentration (70 to 360 mosm/l) has been studied.

A linear correlation was found between renin release and sodium content (ranging between 10 and 90 mEq/l) in the incubation medium. Changes in the osmotic concentration at constant sodium level failed to affect renin release.

The mechanism of the regulation of renin secretion and release has not been clarified. On the basis of Goldblatt-type hypertension, renal ischaemia is supposed to be the stimulus of renin secretion. This is the basis of Tobian's (1964) baroceptor theory. However, hyponatraemic states are also associated with increased renin secretion (Brown et al. 1964). This has led to the assumption that renin release was triggered by the decreased intratubular sodium concentration at the macula densa (Gross et al. 1965; Vander 1967). Histochemical studies of the macula densa theory were carried out by Gomba et al. (1968). Administration of diuretics was followed by increased renin production (Fraser et al. 1965; Meyer et al. 1968; Vander and Carlson 1969).

Thurau et al. (1967, 1968), Cortney et al. (1966) and Schnermann et al. (1966) elicited afferent vasoconstriction in the sodium depleted animal by elevating the sodium level in the distal tubular fluid; this phenomenon might be due to renin release.

In previous experiments (Forgács et al. 1969; Châtel et al. 1970; Forgács and Gaál 1971) the infusion into the renal artery of a hypertonic solution of NaCl, Tris-Cl or urea, i.e. factors elevating sodium content in the distal tubule was followed by a constriction of the afferent arterioles and increased renin activity in renal venous blood in dogs kept on a sodium-low diet. Considering that the increase in the distal tubular sodium content in these acute experiments were accompanied by haemodynamic and perhaps also by neural changes, it seemed worthwhile to investigate the effect of the sodium and osmotic concentration on renin release by surviving renal cortical slices. Some details of these experiments have already been reported (Forgács and Gaál 1970).

## Methods

Mongrel dogs of either sex were anaesthetized with 30 mg/kg of pentobarbital. One hour later the left kidney was removed through a lumbar incision and placed into ice-cold physiological saline solution. Parallel to the surface, 0.4 mm thick cortical slices were cut and slices amounting of 100 to 200 mg wet weight were incubated in flasks.

The incubation medium had the following composition. In series "A" (increasing sodium content) dogs under pentobarbital anaesthesia were exsanguinated 24 hours after nephrectomy. The blood was collected in plastic tubes containing EDTA and centrifuged at 3000 *g* at 4°C. Five ml of this plasma served as renin substrate in each incubation medium. A final sodium content of 10 to 250 mEq/l (see the first line in Table I) was obtained by adding an 80 to 320 mEq/l solution of sodium chloride and/or a 5% solution of glucose. In series "B" (increasing osmolality at constant sodium content) a final 70 to 360 mosm/l was prepared by adding to the 5 ml plasma aliquots Tris-buffer pH 7.4 or distilled water. Sodium content in this series was 44.7 $\pm$ 2.4 mEq/l. After oxygenation for 5 minutes the tissue slices were incubated at 37°C for one hour.

After incubation, the cortical slices were removed, the incubating media were stored at —4°C overnight. After adjusting the pH to 5.5 and adding neomycin, incubation was carried out for 24 hours at 37°C. The enzymatic reaction was stopped by boiling for 10 minutes, followed by readjustment of the pH of the protein-free filtrate to 7.4 (KANEKO et al. 1967). The pressor activity of the renin-like substances (in the following: renin) in the medium was tested in vagotomized, mecamylamine-pretreated rats 16 hours after nephrectomy, using Hypertensin (CIBA) as standard and applying the four point assay. Renin activity in the medium was expressed in ng of angiotensin equivalent, related to 1 ml and gram of wet kidney weight. Recovery with this method was 30.38 $\pm$ 6.14% and reproducibility attained 9.98 $\pm$ 9.41% in 25 cases (FORGÁCS et al. 1971). As blanks, incubation medium without renal tissue and medium containing tissue without incubation were used. No pressor activity could be detected in either case.

Sodium content and osmotic concentration of the medium after the incubation were estimated by means of a flame-photometer and an Advanced type osmometer, respectively.

Throughout the experiments, slices from the same kidney were used in matched A and B type experiments but the different A and B type series originated from different kidneys. The results were evaluated statistically by means of Student's one-sample *t*-test.

## Results

Table I shows renin activity in the incubation media of varying sodium content. Renin activity in media containing 10 to 40 mEq/l of sodium was significantly weaker than in media of higher sodium content ($p < 0.01$). Renin activity in media containing 41 to 70 mEq/l sodium was significantly higher than in media of lower sodium content ($p < 0.02$), but significantly smaller than in media with higher sodium content ($p < 0.02$). There was no statistically significant difference in renin activity in media with a sodium content amounting to 71 to 90, 111 to 150, 151 to 170, and 171 to 200 mEq/l. Renin activity and a sodium content in the range of 10 to 90 mEq/l exhibited a linear correlation while no linearity was observed above a sodium content of 91 mEq/l (Fig 1).

Media with a sodium level exceeding 150 mEq/l had an osmotic concentration of 310 to 423 mosm/l. Changes in the osmotic concentration from 70 to 360 mosm/l at a constant sodium level had no effect on the renin activity of the incubation medium (Table II).

**Table I**

*Effect of sodium level in vitro on renin release*

| Sodium level of the medium (mEq/l) | 10—40 | 41—70 | 71—90 | 111—150 | 151—170 | 171—200 |
|---|---|---|---|---|---|---|
| Renin activity (ng/ml/g) | | | | | | |
| Mean | 139 | 384 | 765 | 941 | 920 | 840 |
| S.E. | 37 | 80 | 100 | 176 | 173 | 123 |
| n | 15 | 14 | 16 | 13 | 10 | 11 |

**Table II**

*Effect of osmotic concentration of the medium on renin release**

| Osmotic concentration (mosm/l) | 70—90 | 91—110 | 140—160 | 190—210 | 240—260 | 290—310 | 340—360 |
|---|---|---|---|---|---|---|---|
| Renin activity (ng/ml/g) | | | | | | | |
| Mean | 807 | 754 | 905 | 849 | 952 | 837 | 862 |
| S.E. | 136 | 72 | 154 | 93 | 117 | 84 | 87 |
| n | 9 | 11 | 10 | 11 | 10 | 10 | 12 |

* Sodium content of the media: 44.7 ± 2.4 mEq/l

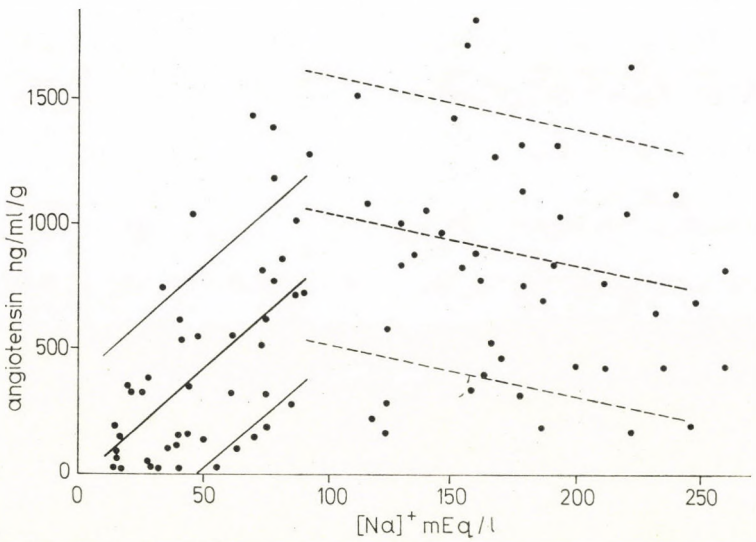


*Fig. 1.* Experimental values are indicated by points. The continuous line is the regression equation of renin activity and sodium content between 10 and 90 mEq/l values for the latter, $Y = -11 + 8.60\ (\pm 2.45)x$, $p < 0.01$. The broken line refers to regression of renin activity and sodium level between 91 and 250 mEq/l concentration values for sodium, $Y = 1246 - 2.05\ (\pm 1.78)x$, $p < 0.30$

## Discussion

Sodium concentration of the tubular fluid around the macula densa amounts to 40—70 mEq/l, as demonstrated in micropuncture experiments (SCHNERMANN et al. 1966). Thus, a sodium level of 40 to 70 mEq/l may well correspond to the physiological milieu of the renal cortical slices secreting and/or releasing renin. The present experiments revealed that a sodium content between 10 to 40 mEq/l reduced renin secretion, while sodium levels exceeding 70 mEq/l augmented it. No further increase was found between 91 and 200 mEq/l. Considering that an elevation of the sodium level above 150 mEq/l was associated by an increase in osmotic concentration, the exclusive role of sodium in renin release might be questioned. Changes in osmotic concentration between 70 and 360 mosm/l at a constant sodium level failed to affect renin activity. Since $F/P_{osm}$ values of 0.5—0.6, corresponding to 150—180 mosm/l, were found by micropuncture in the first part of the distal tubules, the range of 70 to 360 mosm/l, examined in the present study, seems to be wide enough for allowing the assumption that alterations in osmotic concentration have no effect on renin production and/or release.

Considering that the renin content of the kidneys may have differed prior to incubation, depending on previous salt intake and water balance, etc., the absolute values of renin activity may be of less meaning than the difference in renin activity of the matched incubates of the two series.

KARSUNKY et al. (1969) observed an intimate relation between sodium (10 to 150 mEq/l) and mannitol content (10 to 200 mmol/l) of the incubation medium and the renin activity of incubated rat renal cortical slices. Their data differ from ours in some respect but the species difference and the difference in the osmotically active substances applied should be taken into account.

The advantage of experiments in vitro is the elimination of some factors acting in vivo (e.g. vasomotor reactions, glomerular filtration, tubular transport, etc.). At the same time it is questionable to what an extent the responses of the surviving tissue reflect the regulation of renin release in vivo.

The possibility of studying renin release in vitro was first raised by BING (1944). ROBERTSON et al. (1966) and SZALAY and GYÉVAI (1967) demonstrated renin release in 9 to 15-day-old tissue cultures. YAMAMOTO et al. (1967) found maximum renin release by incubated canine cortical slices at pH 5 and at 25°C to 37°C; about 10% of the renin content of the tissue was released into the medium. According to BRAVERMAN et al. (1970), renin content in the medium depends on the renin stores in the renal cortex and its metabolism, as renin release by cortical slices of sodium depleted rats exceeded several times that of animals kept on a normal diet. DE JONG (1969) showed that renin content of the medium does not reflect the renin content of cortical slices but correlates with plasma renin activity. He found a reduced renin

release in vitro in rats after corticoid hypertension, and an increased one in animals with Goldblatt-type hypertension. MICHELAKIS et al. (1969) found an increased renin release by renal cortex cell suspensions after adding epinephrine, norepinephrine or cyclic AMP. The stimulation by these substances could be abolished by cyclohexenidine, an inhibitor of protein synthesis; this would indicate a synthesis of renin beside its secretion by the surviving tissue. BOŽOVIČ and EFENDIČ (1969) examined the effect of glucose on renin release.

These data suggest that the same mechanism is regulating renin production and release in vivo and in vitro. The present results give support to those experiments (FORGÁCS et al. 1969; CHÂTEL et al. 1970; FORGÁCS and GAÁL 1971) carried out in dogs, where the renal vasoconstriction elicited by a distal tubular sodium load was primarily attributed to intrarenal renin-angiotensin liberation.

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# THERMOREGULATORY RESPONSES TO HYPERCAPNIA IN THE NEW-BORN RABBIT

## COMPARISON WITH THE EFFECT OF HYPOXIA

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New-born rabbits, aged less than 2 days, 3 to 6 days, and 7 to 18 days were exposed to hypercapnia by breathing 6% $CO_2$ in air at an ambient temperature of 20°C. Oxygen consumption and colonic temperature were recorded continuously. The rabbits were transferred from an ambient temperature of 35°C, for 30 min to an ambient temperature of 20°C breathing air, then for another 30 min they were exposed to 6% $CO_2$ at the same ambient temperature, and ultimately the metabolic chamber was again ventilated with room air for a further 30 min.

No significant change in mean oxygen consumption was observed in response to breathing 6% $CO_2$ or to the subsequent restoration of room air. In contrast, body temperature responded to hypercapnia with a significant fall in the two younger age-groups, and the fall approached statistical significance in the oldest group. Between the change in oxygen consumption in hypercapnia and the concomitant change in body temperature the relationship was statistically significant in the youngest age-group only. In animals aged 3 to 6 days, statistically significant relationships were demonstrated *a)* between body temperature prior to hypercapnia and its subsequent change during hypercapnia; *b)* between body temperature before termination of hypercapnia and its subsequent change after restoration of air breathing; *c)* between the changes in body temperature during, and after the termination of, hypercapnia; *d)* between the changes in oxygen consumption during, and after termination of, hypercapnia; and *e)* between the change in body temperature in response to transfer from an ambient temperature of 35°C to one of 20°C, on the one hand, and its subsequent changes both during, and after the termination of, hypercapnia, on the other hand. In the oldest age-group, statistically significant relationships were found *a)* between the change in oxygen consumption in response to hypercapnia and the subsequent change after restoration of air breathing; *b)* between oxygen consumption prior to hypercapnia and its subsequent change in response to hypercapnia; and *c)* between the change in oxygen consumption in response to transfer from an ambient temperature of 35°C to one of 20°C and its subsequent change during hypercapnia. Implications of the results are discussed and a comparison with corresponding observations in response to hypoxia is undertaken to point out the differences between the responses to hypercapnia and hypoxia. Attention is drawn to the observation that relationships between the variables considered may be characteristic of a certain, relatively short, period of neonatal life and may be absent earlier and/or later.

Since the early observations of WESTENRYK (1901) and MAGYARY-KOSSA (1920) showing that hypercapnia reduced body temperature in the normal as well as in the febrile adult rabbit, little attention was paid to the question and the subject remained controversial for at least four decades. In man, for instance, hypercapnia was found both to elicit shivering (HENSEL 1949) and to inhibit shivering (BULLARD and CRISE 1961). More recent work showed, however, unequivocally that at ambient temperatures below the

thermoneutral zone, breathing $CO_2$ at concentrations of 4% or more, caused a reduction in heat production and a fall in body temperature in the adult rat (STUPFEL 1960a, b; SZEGVÁRI and VÁRNAI 1962; SZEGVÁRI et al. 1963). Until quite recently, no study had been available on the effect of hypercapnia on heat production and body temperature in new-born animals. In the new-born rat VÁRNAI et al. (1970b) have demonstrated that up to the age of 10 days the response differed markedly from that of the adult animal and approached this slowly during the next ten days, whereas the response to hypoxia resembled that of the adult animal from birth. Hypercapnia proved to be a useful tool in analysing thermoregulatory responses in the new-born rat (VÁRNAI 1970; VÁRNAI et al. 1970b) and hypoxia in both the new-born rat (VÁRNAI 1970; VÁRNAI et al. 1970a) and the new-born rabbit (VÁRNAI et al. 1971), one of the advantages being that thermoregulatory responses can be studied without changing the ambient temperature. Considering the differences in the response to hypoxia and hypercapnia in the new-born rat and, even more important, the interrelationships between changes in body temperature and heat production in response to exposure to cold and to hypoxia demonstrated in the new-born rabbit (VÁRNAI et al. 1970c, 1971), it was deemed necessary to study systematically the effect of hypercapnia and the changes following upon the termination of hypercapnia in the new-born rabbit.

## Methods

New-born rabbits of both sexes of the Institute's mixed outbred colony, aged from a few hours up to 18 days, were used. Oxygen consumption and $CO_2$ production were measured continuously by a Noyons-Kipp diaferometer as modified by DONHOFFER et al. (1958). The rabbits stayed in their mother's nest until they were transferred to the metabolic chamber, which was placed into a thermoneutral waterbath of 35°C. Thirty to sixty min later measurements were begun and continued for 30 min at the termoneutral ambient temperature, when the metabolic chamber was transferred to a waterbath of 20°C for another 30 min, then 6% $CO_2$ was supplied to the rabbits for 30 min at the same ambient temperature, after which room air was restored for 30 min without any change in environmental temperature. The change-over from air to 6% $CO_2$ was accomplished by attaching a double walled plastic bag containing 6% $CO_2$ in air to the inlet of the modified Noyons-Kipp apparatus (SZEGVÁRI and VÁRNAI 1962; VÁRNAI et al. 1970b). Colonic temperature was measured by a copper-constantan thermocouple, enveloped by a flexible nylon sheath, introduced to a distance of 4.5 to 6 cm from the anus, according to the size of the rabbit.

In the Tables, figures for oxygen consumption represent the mean of the relevant period; colonic temperatures refer, however, to the body temperature recorded at the end of the 30 min periods. In the Figures, dots and circles correspond to actual readings at that time; the galvanometers were read, however, every minute. The interrupted lines for oxygen consumption indicate interruption of measurements either until a new equilibrium had been reached after changing over from air to 6% $CO_2$ or vice versa, and/or for taking the base-line of the recording instrument. In a few instances the effect of breathing 12% $O_2$ in $N_2$ was observed prior to, or after exposure to, hypercapnia. Ultimately, the rabbits were returned to their mother's nest and in some cases were used again to study either the effect of transfer from an ambient temperature of 35°C to one of 20°C and/or the effect of hypoxia or hypercapnia at the latter environmental temperature.

For statistical evaluation, in addition to comparison of means by Student's two-tailed *t*-test, correlation and regression analysis was used (coefficient of correlation $r = \frac{\Sigma xy}{\sqrt{(\Sigma x^2)(\Sigma y^2)}}$;

$t = \frac{r}{\sqrt{1-r^2}}\sqrt{n-2}$ ; coefficient of regression $a = \frac{\Sigma xy}{\Sigma x^2}$ ; $s_a = \frac{1}{\Sigma x^2}\frac{\sqrt{(\Sigma x^2)(\Sigma y^2)-(\Sigma xy)^2}}{n-2}$ ; $t = \frac{a}{s_a}$). Body surface was calculated as the 2/3rd power of body weight.

## Results

Table I demonstrates the absence of a significant difference between the mean oxygen consumption prior to, during, and after termination of hypercapnia in all of the three age-groups. Even when the calculations were based on the mean of the differences observed in individual experiments in response to hypercapnia, and to the restoration of air breathing, no significant change in oxygen consumption could be established in any of the three age-groups (Table II). The change coming nearest to statistical significance was the increase in oxygen consumption during hypercapnia in the youngest age-group, but just in the opposite direction as had been expected.

Similarly, no statistically significant differences were found in mean colonic temperature before, during, and after the termination of, hypercapnia (Table III). Calculating, however, on the basis of the mean of the individual

**Table I**

*Oxygen consumption in ml/dm² · min at an ambient temperature of 20°C prior to exposure to hypercapnia, during breathing 6% $CO_2$, and after the restoration of air breathing (M±S.E.). In brackets, number of observations*

| Age | Prior to exposure to hypercapnia | p | During breathing 6% $CO_2$ | p | After restoration of air breathing |
|---|---|---|---|---|---|
| Less than 2 days (13) | 2.33 ± 0.13→ | >0.3 | ←2.58 ± 0.24→ | >0.8 | ←2.52 ± 0.25 |
| 3—6 days (14) | 2.19 ± 0.16→ | >0.7 | ←2.11 ± 0.16→ | >0.8 | ←2.17 ± 0.18 |
| 7—18 days (6) | 1.97 ± 0.23→ | >0.5 | ←1.80 ± 0.12→ | >0.6 | ←1.93 ± 0.23 |

**Table II**

*Change in oxygen consumption during hypercapnia and after restoration of air breathing in ml/dm² · min (M ± S.E.). In brackets, number of observations*

| Age | Change in $O_2$-consumption in response to hypercapnia | p | Change in $O_2$-consumption after restoration of air breathing | p |
|---|---|---|---|---|
| Less than 2 days (13) | +0.24 ± 0.12 | 0.1 > p > 0.05 | −0.06 ± 0.05 | >0.2 |
| 3—6 days (14) | −0.08 ± 0.12 | >0.5 | +0.06 ± 0.06 | >0.3 |
| 7—18 days (6) | −0.18 ± 0.13 | >0.2 | +0.13 ± 0.10 | >0.2 |

differences (Table IV), the decrease in body temperature in response to hypercapnia was unequivocally significant statistically in the two younger age-groups, and came near to statistical significance in the oldest age-group, the failure to reach significance probably having been due to the small number

**Table III**

*Colonic temperature at an ambient temperature of 20°C prior to exposure to hypercapnia, after breathing 6% $CO_2$ for 30 min, and 30 min after the restoration of air breathing. In brackets, number of observations*

| Age | Before exposure to hypercapnia M ± S.E. | p | After breathing 6% $CO_2$ for 30 min M ± S.E. | p | 30 min after restoration of air breathing M ± S.E. |
|---|---|---|---|---|---|
| Less than 2 days (13) | 36.52 ± 0.44→ | >0.1 | ←35.55 ± 0.57→ | >0.9 | ←35.63 ± 0.66 |
| 3—6 days (14) | 36.61 ± 0.26→ | >0.2 | ←35.83 ± 0.43→ | >0.9 | ←35.84 ± 0.49 |
| 7—18 days (6) | 37.86 ± 0.25→ | >0.4 | ←37.53 ± 0.34→ | >0.6 | ←37.74 ± 0.29 |

**Table IV**

*Change in body temperature in hypercapnia and after its termination (M ± S.E.). Ambient temperature, 20°C. In brackets, number of observations*

| Age | Change in hypercapnia | p | Change after restoration of air breathing | p |
|---|---|---|---|---|
| Less than 2 days (13) | −0.97 ± 0.44 | *<0.05* | +0.08 ± 0.13 | >0.5 |
| 3—6 days (14) | −0.78 ± 0.20 | *<0.01* | +0.02 ± 0.08 | >0.8 |
| 7—18 days (6) | −0.33 ± 0.16 | 0.1> p > 0.05 | +0.21 ± 0.09 | 0.1 > p > 0.05 |

of observations. After the restoration of air breathing, however, practically no change was observed in mean colonic temperature in the two younger age-groups, whereas in the oldest group the post-hypercapnic increase in body temperature approached statistical significance, as did the decrease during hypercapnia.

Since individual differences are naturally submerged in averages, a few individual observations are demonstrated, representing more or less frequent variants of the effects of breathing 6% $CO_2$. Fig. 1 demonstrates an observation made on the first day of life. Oxygen consumption, i.e. heat production, after a transient reduction regained during exposure to hypercapnia practically the same level as had been recorded prior to hypercapnia and practically did not differ from that observed after the restoration of air breathing. Body temperature was already declining prior to exposure to hypercapnia and no substantial change was observed in the rate of fall during hypercapnia, and

during the last ten minutes of breathing 6% $CO_2$ colonic temperature remained stable, the same level being maintained after the termination of hypercapnia.

Fig. 2 demonstrates an experiment in which a rabbit aged 2 days responded to hypercapnia with a considerable reduction in heat production

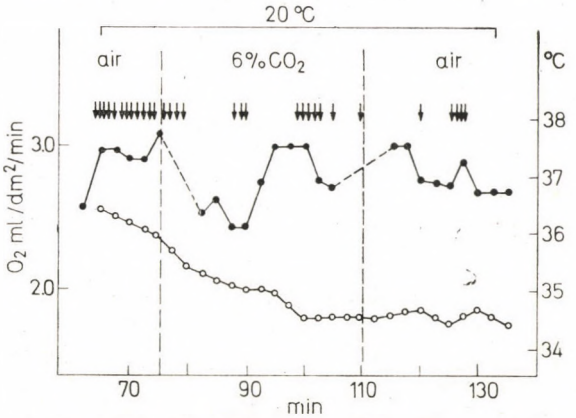


*Fig. 1.* May 11, 1964; rabbit aged 1 day, body weight 50 g. Dots: oxygen consumption; circles: body temperature. Arrows indicate motor activity. Abscissa: time elapsed since start of measurements

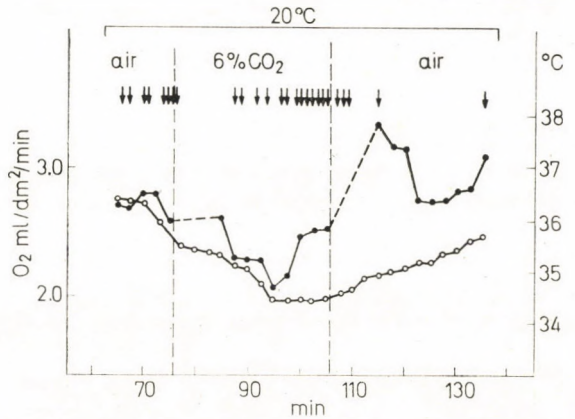


*Fig. 2.* April 7, 1964; rabbit aged two days, body weight 90 g. Dots: oxygen consumption; circles: body temperature. Arrows indicate motor activity. Abscissa: time elapsed since start of measurements

and a marked fall in body temperature, albeit during the last ten minutes of hypercapnia body temperature remained stable and heat production increased to a level only little lower than before hypercapnia. After the termination of $CO_2$ breathing, heat production increased well above the pre-exposure level and, simultaneously, body temperature increased steadily, both being rather infrequent events in the new-born rabbit. The steady increase in colonic temperature demonstrates, in addition, that the preceding

fall during hypercapnia was not due simply to a continuation of the decline observed during the last few minutes prior to hypercapnia.

Fig. 3 originates from an observation on a rabbit aged four days. Body temperature declined during, and increased after the termination of, hypercapnia without an appreciable change in heat production. Fig. 4 demonstrates an observation on a rabbit aged eleven days. Colonic temperature declined during breathing 6% $CO_2$, and increased when room air was supplied again to the animal. After an initial fall, oxygen consumption increased during hypercapnia slowly, and after restoring room air a further marked increase followed. Fig. 5 demonstrates an experiment on a rabbit aged 16 days. Heat production increased during hypercapnia accompanied by a small but definite decrease in body temperature.

Tables V and VI serve to show the distribution of the responses to breathing 6% $CO_2$ and to the subsequent restoration of air breathing. Evidently,

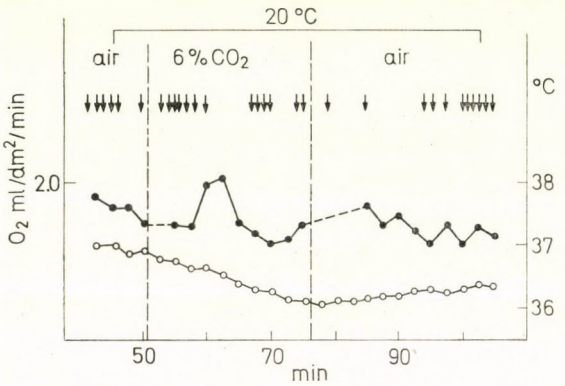


*Fig. 3.* February 24, 1966; rabbit aged 4 days, body weight 120 g. Dots: oxygen consumption; circles: body temperature. Arrows indicate motor activity. Abscissa: time elapsed since start of measurements

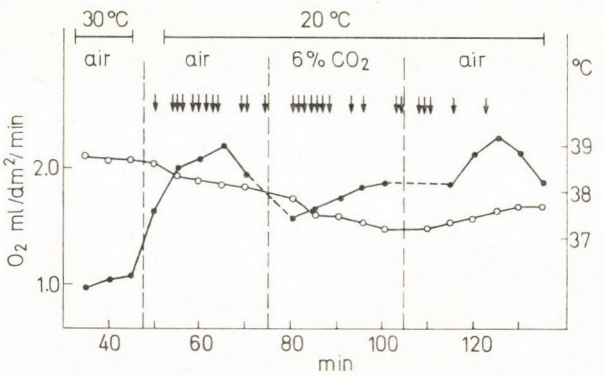


*Fig. 4.* January 4, 1967; rabbit aged 11 days, body weight 235 g. Dots: oxygen consumption; circles: body temperature. Arrows indicate motor activity. Abscissa: time elapsed since start of measurements

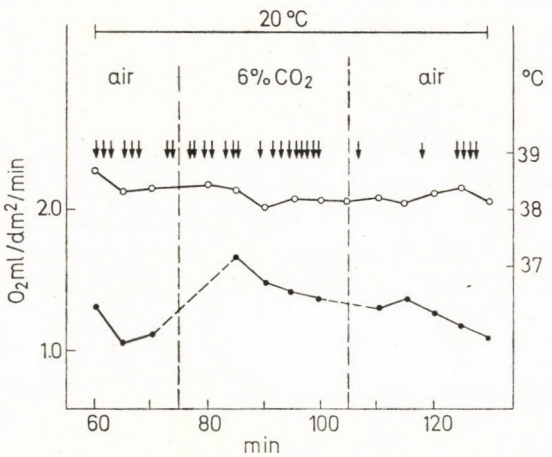


*Fig. 5.* April 15, 1966; rabbit aged 16 days, body weight 260 g. Dots: oxygen consumption, circles: body temperature. Arrows indicate motor activity. Abscissa: time elapsed since the start of measurements

the responses showed a greater variability, especially in body temperature, than the means would suggest. However, the relationship between the response to hypercapnia and to the subsequent restoration of air breathing could, naturally, not be revealed by Tables V and VI.

To uncover eventual relationships between changes in heat production and in body temperature in response to breathing 6% $CO_2$, and to the subsequent restoration of air breathing, as well as between these and the thermoregulatory changes in response to transfer from a thermoneutral ambient

**Table V**

*Change in oxygen consumption in response to breathing 6% $CO_2$ and to the termination of hypercapnia. In brackets, number of observations in the relevant age-groups*

| | | Change in oxygen consumption, per cent | | | | |
|---|---|---|---|---|---|---|
| | | Decrease | | Change ±5% | Increase | |
| | | greater than 10% | 5 to 10% | | 5 to 10% | greater than 10% |
| Less than 2 days (13) | in hypercapnia | 1 | 2 | 3 | 2 | 5 |
| | after hypercapnia | 1 | 3 | 5 | — | 3 |
| 3—6 days (14) | in hypercapnia | 3 | 3 | 5 | — | 3 |
| | after hypercapnia | — | 1 | 8 | 2 | 3 |
| 7—18 days (6) | in hypercapnia | 2 | — | 3 | — | 1 |
| | after hypercapnia | — | — | 3 | 1 | 2 |

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**Table VI**

*Change in body temperature in response to breathing 6% $CO_2$ and to the termination of hypercapnia. In brackets, number of observations in the relevant age-groups*

| Age | | Change in body temperature, °C | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Decrease | | | | Change ±0.1°C | Increase | |
| | | >2.0°C | 1.0 to 2.0°C | 0.25 to 1.0°C | 0.10 to 0.25°C | | 0.1 to 0.25°C | >0.25°C |
| Less than 2 days (13) | in hypercapnia | 2 | 2 | 3 | 1 | 1 | 4 | — |
| | after hypercapnia | — | — | 3 | 3 | 2 | 2 | 2 |
| 3—6 days (14) | in hypercapnia | 2 | 1 | 8 | 2 | — | 1 | — |
| | after hypercapnia | — | — | 2 | 1 | 5 | 3 | 3 |
| 7—18 days (6) | in hypercapnia | — | — | 3 | 1 | 1 | 1 | — |
| | after hypercapnia | — | — | — | — | 3 | — | 3 |

temperature to one of 20° C, correlation and regression analyses of the various parameters were carried out.

Table VII contains the results of correlation and regression analysis of the change in heat production and in body temperature in response to breathing 6% $CO_2$. A statistically significant relationship of these variables could be established in the youngest age-group only. Results for rabbits aged 3 to 6 days approached, but failed to reach, statistical significance, whereas no relationship whatsoever emerged in the oldest age-group.

In contrast, the relationship between the change in heat production and in colonic temperature after restoration of air breathing, showed definitely no relationship in the youngest age-group and almost reached statistical significance in the oldest age-group (Table VIII). The small number of observations in this age-group suggests that some additional experiments might have led to statistically significant results. Attention should be paid also to the reversal of the sign of the coefficients.

Table IX demonstrates in the two older age-groups a highly significant relationship between the changes in heat production during hypercapnia and the subsequent changes in oxygen consumption after restoration of air breathing, whereas no trace of such a relationship could be established in the youngest age-group. Although in the animals aged 3 to 6 days mean oxygen consumption was insignificantly reduced in hypercapnia and showed only a minimal increase after the termination of hypercapnia, it is evident that a greater reduction in heat production during hypercapnia had to be associated with a greater increase after the termination of $CO_2$ breathing, and an increase in heat production in response to hypercapnia had to be followed by a more or less commensurate decrease after restoring air breathing, at least in most of the animals.

Table X contains the corresponding data concerning the relationship of the changes in colonic temperature in response to breathing 6% $CO_2$ and to restoration of air breathing. Again, the relationship was highly significant statistically in the group aged 3 to 6 days, but not in the younger and older animals. However, a small reduction in body temperature during hypercapnia was associated with an increase after the termination of $CO_2$ breathing, whereas a marked fall in body temperature in response to hypercapnia was followed either by a further fall in colonic temperature after the termination of $CO_2$ breathing, or body temperature maintained the level reached during hypercapnia. The reversal of the sign of the coefficients in the oldest age-group probably deserves attention, since the coefficient of correlation is high, although not significant statistically.

As the next step, the relationships between oxygen consumption and body temperature prior to $CO_2$ breathing, and the subsequent changes during hypercapnia were examined. Table XI shows that, in the oldest group, a

**Table VII**

*Relationship between the change in heat production in response to hypercapnia (X) and the concomitant change in colonic temperature (Y). Ambient temperature, 20° C. In brackets, number of observations*

| Age | $\bar{X} \pm$ S.E. ml $O_2$ per $dm^2 \cdot min$ | $\bar{Y} \pm$ S.E. °C | Correlation | | | Regression | | |
|---|---|---|---|---|---|---|---|---|
| | | | r | t | p | a | t | p |
| Less than 2 days (13) | $+0.24 \pm 0.12$ | $-0.97 \pm 0.44$ | *+0.554* | *2.206* | *<0.05* | *+1.977* | *2.206* | *<0.05* |
| 3—6 days (14) | $-0.09 \pm 0.12$ | $-0.78 \pm 0.20$ | +0.465 | 1.819 | $0.1 > p > 0.05$ | +0.258 | 1.819 | $0.1 > p > 0.05$ |
| 7—18 days (6) | $-0.18 \pm 0.13$ | $-0.33 \pm 0.16$ | +0.172 | 0.350 | >0.7 | +0.143 | 0.350 | >0.7 |

**Table VIII**

*Relationship between the change in heat production after the termination of hypercapnia (X) and the concomitant change in colonic temperature (Y). Ambient temperature, 20°C. In brackets, number of observations*

| Age | $\bar{X} \pm$ S.E. ml $O_2$ per $dm^2 \cdot min$ | $\bar{Y} \pm$ S.E. °C | Correlation | | | Regression | | |
|---|---|---|---|---|---|---|---|---|
| | | | r | t | p | a | t | p |
| Less than 2 days (13) | $-0.06 \pm 0.05$ | $+0.29 \pm 0.25$ | −0.212 | 0.743 | >0.4 | −0.944 | 0.743 | >0.4 |
| 3—6 days (14) | $+0.06 \pm 0.06$ | $+0.02 \pm 0.08$ | −0.431 | 1.650 | >0.1 | −0.611 | 1.649 | >0.1 |
| 7—18 days (6) | $+0.13 \pm 0.10$ | $+0.21 \pm 0.09$ | +0.790 | 2.577 | $0.1 > p > 0.05$ | +0.780 | 2.576 | $0.1 > p > 0.05$ |

**Table IX**

*Relationship between the change in the oxygen consumption in response to hypercapnia (X) and the change in heat production after the termination of hypercapnia (Y). Ambient temperature, 20°C. In brackets, number of observations*

| Age | $\bar{X} \pm$ S.E. ml $O_2$ per $dm^2 \cdot min$ | $\bar{Y} \pm$ S.E. ml $O_2$ per $dm^2 \cdot min$ | Correlation | | | Regression | | |
|---|---|---|---|---|---|---|---|---|
| | | | r | t | p | a | t | p |
| Less than 2 days (13) | $+0.24 \pm 0.12$ | $-0.06 \pm 0.05$ | $-0.110$ | 0.383 | $>0.7$ | $-0.052$ | 0.386 | $>0.7$ |
| 3—6 days (14) | $-0.08 \pm 0.12$ | $+0.06 \pm 0.06$ | *−0.725* | *3.641* | *<0.01* | *−0.343* | *3.643* | *<0.01* |
| 7—18 days (6) | $-0.18 \pm 0.13$ | $+0.13 \pm 0.10$ | *−0.927* | *4.932* | *<0.01* | *−0.678* | *4.933* | *<0.01* |

**Table X**

*Relationship between the response of colonic temperature to hypercapnia (X) and the change in body temperature within 30 min after the termination of hypercapnia (Y). Ambient temperature, 20°C. In brackets, number of observations*

| Age | $\bar{X} \pm$ S.E. °C | $\bar{Y} \pm$ S.E. °C | Correlation | | | Regression | | |
|---|---|---|---|---|---|---|---|---|
| | | | r | t | p | a | t | p |
| Less than 2 days (13) | $-0.97 \pm 0.44$ | $+0.08 \pm 0.13$ | $+0.309$ | 1.076 | $>0.3$ | $+0.095$ | 1.076 | $>0.3$ |
| 3—6 days (14) | $-0.78 \pm 0.20$ | $+0.02 \pm 0.08$ | *+0.741* | *3.827* | *<0.01* | *+0.291* | *3.828* | *<0.01* |
| 7—18 days (6) | $-0.33 \pm 0.16$ | $+0.21 \pm 0.09$ | $-0.620$ | 1.580 | $>0.1$ | $-0.351$ | 1.580 | $>0.1$ |

statistically significant relationship could be established between oxygen consumption prior to hypercapnia and the reduction in heat production during $CO_2$ breathing: the greater the oxygen consumption prior to hypercapnia, the more marked the reduction in heat production during hypercapnia. No significant relationship between these two variables could be established in the other age-groups.

Between body temperature prevailing prior to hypercapnia and its subsequent change during hypercapnia (Table XII) a highly significant relationship was found in the group aged 3 to 6 days: the higher the body temperature prior to exposure to hypercapnia, the smaller the reduction in response to $CO_2$ breathing. No such relationship was found in the other age-groups.

Table XIII shows in the same age-group a relationship of similar significance as that demonstrated in Table XII, between body temperature reached by the end of breathing 6% $CO_2$ for 30 min and its subsequent change after the restoration of air breathing. Again, the reversal of the sign of the coefficients in the oldest age-group should be noted. In concordance with the relationships demonstrated in Tables X and XII, the higher the body temperature at the termination of hypercapnia, the greater the subsequent increase in response to the restoration of air breathing. Again, the results failed to establish statistically significant relationships in the other age-groups.

Ultimately, eventual relationships between changes in response to transfer from the thermoneutral temperature of 35°C to an ambient temperature of 20°C, on the one hand, and the changes in response to breathing 6% $CO_2$ and to the subsequent restoration of air breathing, on the other hand, were analysed. Table XIV demonstrates for the oldest age-group a highly significant relationship between the increase in oxygen consumption in response to transfer from an ambient temperature of 35°C to one of 20°C and the decrease in heat production in response to the subsequent exposure to hypercapnia without any further change in ambient temperature: the greater the increase in heat production in response to transfer to the cooler environment, the greater the reduction in oxygen consumption in response to breathing 6% $CO_2$. No indication of a similar relationship could be demonstrated in the two other age-groups.

In contrast to the increase in heat production in response to transfer from an ambient temperature of 35°C to one of 20°C, the concomitant change in body temperature showed no significant correlation with the subsequent change in oxygen consumption in response to breathing 6% $CO_2$ in any of the three age-groups (Table XV).

A remarkable and significant relationship was found in the animals aged 3 to 6 days between the change in body temperature in response to the transfer to the cooler environment and both the change in body temperature

**Table XI**

*Relationship between oxygen consumption at an ambient temperature of 20°C prior to hypercapnia (X) and the change in oxygen consumption in response to hypercapnia (Y). In brackets, number of observations*

| Age | $\bar{X} \pm$ S.E. $O_2$ ml per $dm^3 \cdot min$ | $\bar{Y} \pm$ S.E. $O_2$ ml per $dm^3 \cdot min$ | Correlation | | | Regression | | |
|---|---|---|---|---|---|---|---|---|
| | | | r | t | p | a | t | p |
| Less than 2 days (13) | $2.38 \pm 0.15$ | $+0.24 \pm 0.12$ | +0.423 | 1.619 | >0.1 | +0.350 | 1.620 | >0.1 |
| 3—6 days (14) | $2.19 \pm 0.15$ | $-0.08 \pm 0.12$ | −0.353 | 1.306 | >0.2 | −0.263 | 1.306 | >0.2 |
| 7—18 days (6) | $1.97 \pm 0.23$ | $-0.18 \pm 0.13$ | *−0.907* | *4.298* | *<0.02* | *−0.372* | *4.294* | *<0.02* |

**Table XII**

*Relationship between colonic temperature at an ambient temperature of 20°C prior to hypercapnia (X) and the change in colonic temperature in response to hypercapnia (Y) at the same ambient temperature. In brackets, number of observations*

| Age | $\bar{X} \pm$ S.E. °C | $\bar{Y} \pm$ S.E. °C | Correlation | | | Regression | | |
|---|---|---|---|---|---|---|---|---|
| | | | r | t | p | a | t | p |
| Less than 2 days (13) | $36.52 \pm 0.40$ | $-0.97 \pm 0.44$ | −0.093 | 0.309 | >0.7 | −0.102 | 0.310 | >0.7 |
| 3—6 days (14) | $36.61 \pm 0.26$ | $-0.78 \pm 0.20$ | *+0.720* | *3.595* | *<0.01* | *+0.566* | *3.597* | *<0.01* |
| 7—18 days (6) | $37.86 \pm 0.25$ | $-0.33 \pm 0.16$ | +0.290 | 0.606 | >0.5 | +0.195 | 0.606 | >0.5 |

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**Table XIII**

*Relationship between colonic temperature prior to termination of hypercapnia (X) and the change in body temperature 30 min after the termination of hypercapnia (Y). Ambient temperature, 20°C. In brackets, number of observations*

| Age | $\bar{X} \pm$ S.E. °C | $\bar{Y} \pm$ S.E. °C | Correlation | | | Regression | | |
|---|---|---|---|---|---|---|---|---|
| | | | r | t | p | a | t | p |
| Less than 2 days (13) | $35.55 \pm 0.51$ | $+0.08 \pm 0.13$ | +0.398 | 1.438 | >0.1 | +0.089 | 1.439 | >0.1 |
| 3—6 days (14) | $35.83 \pm 0.18$ | $+0.02 \pm 0.08$ | *+0.732* | *3.727* | *<0.01* | *+0.135* | *3.732* | *<0.01* |
| 7—18 days (6) | $37.54 \pm 0.34$ | $+0.21 \pm 0.09$ | −0.602 | 1.507 | >0.3 | −0.165 | 1.505 | >0.3 |

**Table XIV**

*Relationship between the change in heat production in response to transfer from an ambient temperature of 35°C to one of 20°C (X) and the change in oxygen consumption in response to hypercapnia (Y). In brackets, number of observations*

| Age | $\bar{X} \pm$ S.E. ml $O_2$ per $dm^2 \cdot min$ | $\bar{Y} \pm$ S.E. ml $O_2$ per $dm^2 \cdot min$ | Correlation | | | Regression | | |
|---|---|---|---|---|---|---|---|---|
| | | | r | t | p | a | t | p |
| Less than 2 days (13) | $+1.08 \pm 0.08$ | $+0.24 \pm 0.12$ | −0.052 | 0.172 | >0.8 | −0.082 | 0.172 | >0.8 |
| 3—6 days (11) | $+1.10 \pm 0.13$ | $-0.08 \pm 0.15$ | −0.078 | 0.236 | >0.8 | −0.089 | 0.236 | >0.8 |
| 7—18 days (6) | $+0.86 \pm 0.17$ | $-0.18 \pm 0.13$ | *−0.933* | *5.187* | *<0.01* | *−0.734* | *5.190* | *<0.01* |

during hypercapnia (Table XVI) and the change after the restoration of air breathing (Table XVII): the smaller the fall in colonic temperature in response to the transfer to the cooler environment, the smaller the reduction in hypercapnia and the greater the increase after the subsequent restoration of air breathing. Again, no significant relationship between the same variables could be established in the two other age-groups. In the case of the post-hypercapnic change in body temperature, there seemed to be no correlation whatever in the youngest and the oldest age-group (Table XVII).

*Comparison of the thermoregulatory effects of hypercapnia and hypoxia.* In adults of the species so far examined, the thermoregulatory responses to hypercapnia and hypoxia were found to be similar: at ambient temperatures below the thermoneutral zone both elicited a reduction in heat production and a fall in body temperature, followed after the termination of hypercapnia or hypoxia by an increase in heat production and a rise in body temperature. In the new-born rabbit, however, as well as in the new-born rat (VÁRNAI 1970; VÁRNAI et al. 1970a, b), marked differences between the responses to hypercapnia and hypoxia were apparent.

Table XVIII compares the responses of heat production to hypercapnia and to hypoxia, on the one hand, and the effects of restoring air breathing, on the other hand. The mean change in oxygen consumption in hypercapnia and in hypoxia differed highly significantly in the two younger age-groups, whereas no difference was observed between the respective means in the oldest age-group. Even more impressive was the difference between the changes in response to the restoration of air breathing. Compared with the change in heat production after the termination of hypercapnia, heat production increased highly significantly in all the three age-groups after the termination of hypoxia. This might have been expected since — at least in the two younger age-groups — mean heat production either increased or showed an insignificantly small decrease during hypercapnia. However, oxygen consumption was found to increase after the termination of hypoxia even in rabbits which responded to hypoxia with no reduction, or even with an increase in heat production (VÁRNAI et al. 1971).

Table XIX shows that although the mean fall in body temperature in response to hypercapnia was in all age-groups highly significantly smaller than that to breathing 12% $O_2$, it was, nevertheless, statistically significant in the two younger age-groups and approached statistical significance in the oldest age-group (Table IV). Despite the considerable fall in body temperature in response to breathing 6% $CO_2$, mean body temperature practically failed to increase after the termination of hypercapnia in the two younger age-groups and the increase approached statistical significance in the oldest group only. In contrast, after the termination of hypoxia, mean body temperature increased highly significantly in all the three age-groups.

**Table XV**

*Relationship between the change in body temperature in response to transfer from an ambient temperature of 35°C to one of 20°C (X), and the change in oxygen consumption during exposure to hypercapnia (Y). In brackets, number of observations*

| | $\bar{X} \pm$ S.E. °C | $\bar{Y} \pm$ S.E. ml $O_2$ per $dm^2 \cdot min$ | Correlation | | | Regression | | |
|---|---|---|---|---|---|---|---|---|
| | | | r | t | p | a | t | p |
| Less than 2 days (13) | −1.07 ± 0.38 | +0.24 ± 0.12 | +0.285 | 0.985 | >0.3 | +0.093 | 0.985 | >0.3 |
| 3—6 days (11) | −1.24 ± 0.32 | −0.08 ± 0.15 | +0.467 | 1.582 | >0.1 | +0.219 | 1.583 | >0.1 |
| 7—18 days (6) | −0.91 ± 0.16 | −0.18 ± 0.13 | +0.036 | 0.073 | >0.9 | +0.031 | 0.073 | >0.9 |

**Table XVI**

*Relationship between the change in body temperature in response to transfer from an ambient temperature of 35°C to one of 20°C (X), and the change in body temperature during exposure to hypercapnia (Y). In brackets, number of observations*

| Age | $\bar{X} \pm$ S.E. °C | $\bar{Y} \pm$ S.E. °C | Correlation | | | Regression | | |
|---|---|---|---|---|---|---|---|---|
| | | | r | t | p | a | t | p |
| Less than 2 days (13) | −1.07 ± 0.38 | −0.97 ± 0.44 | −0.207 | 0.702 | >0.4 | −0.242 | 0.703 | >0.4 |
| 3—6 days (11) | −1.24 ± 0.32 | −0.90 ± 0.24 | *+0.680* | *2.782* | *<0.05* | *+0.510* | *2.784* | *<0.05* |
| 7—18 days (6) | −0.91 ± 0.16 | −0.33 ± 0.16 | +0.392 | 0.851 | >0.4 | +0.425 | 0.852 | >0.4 |

**Table XVII**

*Relationship between the change in colonic temperature in response to transfer from an ambient temperature of 35°C to one of 20°C (X), and the change in body temperature after the termination of hypercapnia (Y). In brackets, number of observations*

| Age | $\bar{X} \pm$ S.E. °C | $\bar{Y} \pm$ S.E. °C | Correlation | | | Regression | | |
|---|---|---|---|---|---|---|---|---|
| | | | r | t | p | a | t | p |
| Less than 2 days (13) | $-1.07 \pm 0.38$ | $+0.08 \pm 0.13$ | +0.004 | 0.014 | >0.9 | +0.0015 | 0.015 | >0.9 |
| 3—6 days (11) | $-1.24 \pm 0.32$ | $-0.06 \pm 0.10$ | *+0.650* | *2.564* | *<0.05* | *+0.208* | *2.566* | *<0.05* |
| 7—18 days (6) | $-0.91 \pm 0.16$ | $+0.25 \pm 0.09$ | +0.070 | 0.140 | >0.8 | +0.041 | 0.140 | >0.8 |

**Table XVIII**

*Change in oxygen consumption during, and after the termination of, breathing 6% $CO_2$ and 12% $O_2$ ($M \pm$ S.E.). Ambient temperature, 20°C. In brackets, number of observations*

| Age | Change in oxygen consumption in ml $O_2$ per $dm^2$ per min | | | | | |
|---|---|---|---|---|---|---|
| | in hypercapnia | in hypoxia | p | after hypercapnia | after hypoxia | p |
| Less than 2 days | $+0.24 \pm 0.12$ (13) | $-0.44 \pm 0.09$ (20) | <0.001 | $-0.06 \pm 0.05$ (13) | $+0.82 \pm 0.12$ (20) | <0.001 |
| 3—6 days | $-0.08 \pm 0.12$ (14) | $-0.44 \pm 0.09$ (18) | <0.01 | $+0.06 \pm 0.06$ (14) | $+0.75 \pm 0.10$ (18) | <0.001 |
| 7—18 days | $-0.18 \pm 0.13$ (6) | $-0.19 \pm 0.09$ (20) | >0.9 | $+0.13 \pm 0.10$ (6) | $+0.54 \pm 0.08$ (20) | <0.01 |

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**Table XIX**

*Change in body temperature during, and after the termination of, breathing 6% $CO_2$ or 12% $O_2$ ($M \pm S.E.$). Ambient temperature, 20°C. In brackets, number of observation*

| Age | Change in colonic temperature (°C) | | | Change in colonic temperature (°C) | | |
|---|---|---|---|---|---|---|
| | in hypercapnia | in hypoxia | p | after hypercapnia | after hypoxia | p |
| Less than 2 days | $-0.97 \pm 0.44$ (13) | $-1.91 \pm 0.11$ (20) | $<0.05$ | $+0.08 \pm 0.13$ (13) | $+1.25 \pm 0.13$ (20) | $<0.001$ |
| 3—6 days | $-0.78 \pm 0.20$ (14) | $-1.85 \pm 0.14$ (18) | $<0.001$ | $+0.02 \pm 0.08$ (14) | $+0.83 \pm 0.21$ (18) | $<0.01$ |
| 7—18 days | $-0.33 \pm 0.16$ (6) | $-0.90 \pm 0.12$ (20) | $<0.01$ | $+0.21 \pm 0.09$ (6) | $+0.71 \pm 0.10$ (19) | $<0.001$ |

Fig. 6 demonstrates an individual experiment during which a rabbit aged 9 days was exposed to hypoxia and subsequently to hypercapnia. This animal showed in response to breathing 12% $O_2$ a marked reduction in heat production and a concomitant steep fall in colonic temperature. After the restoration of air breathing, oxygen consumption increased above the pre-hypoxia level and body temperature increased concurrently. Subsequently, 6% $CO_2$ was supplied to the rabbit. Oxygen consumption for some minutes persisted at the level reached after the termination of hypoxia and then

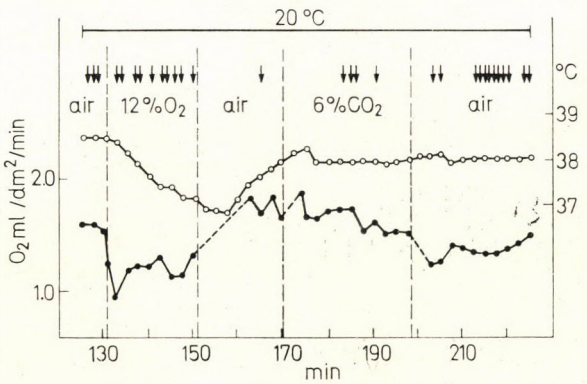


*Fig. 6.* June 5, 1962; rabbit aged 9 days, body weight 130 g. Dots: oxygen consumption; circles: body temperature. Arrows indicate motor activity. Abscissa: time elapsed since the start of measurements

declined to the pre-hypoxia level, and practically no change followed the restoration of air breathing. Body temperature during hypercapnia remained practically stable, the same level being maintained after room air had been restored to the rabbit. However, neither the mean, nor individual observations, revealed all the aspects of the thermoregulatory response. Therefore, it appeared to be of potential value to compare the results of correlation and regression analyses. Table XX serves, on the one hand, to facilitate synopsis for discussing the results obtained by exposing the rabbits to hypercapnia and, on the other hand, to compare these to the corresponding results obtained by exposure to hypoxia. Regression coefficients and their significances were omitted from Table XX for the sake of simplicity, since corresponding values of t and p were identical with those obtained by correlation analysis.

Table XX demonstrates convincingly that correlation analyses of the same parameters led to considerably different results in conjunction with hypercapnia and with hypoxia. A feature common to both was that a significant correlation was in many instances confined to one age-group only. Another striking result was that a significant correlation of the same parameters was often found in one age-group in conjunction with hypercapnia

## Table XX

*Synopsis of results presented (left) and comparison with those obtained by analysing the effects of breathing 12% $O_2$ (right). The numbers of Tables concerned with hypercapnia refer to the Tables in this paper, numbers of Tables concerned with hypoxia refer to published data for the two younger age-groups* (VÁRNAI *et al. 1971)*

| No. | | | | Age, days | | | |
|---|---|---|---|---|---|---|---|
| 1 | *$\Delta O_2$ in 6% $CO_2$——$\Delta T$ in 6% $CO_2$* (Table VII) | | | | *$\Delta O_2$ in 12% $O_2$——$\Delta T$ in 12% $O_2$* (Table IV) | | |
| | n | r | p | | r | p | n |
| | 13 | *+0.554* | *<0.05* | <2 | +0.271 | >0.2 | 20 |
| | 14 | +0.465 | 0.1>p>0.05 | 3—6 | +0.365 | >0.1 | 18 |
| | 6 | +0.172 | >0.7 | 7—18 | +0.281 | >0.2 | 20 |
| 2 | *$\Delta O_2$ after $CO_2$——$\Delta T$ after $CO_2$* (Table VIII) | | | | *$\Delta O_2$ after 12% $O_2$——$\Delta T$ after 12% $O_2$* (Table V) | | |
| | n | r | p | | r | p | n |
| | 13 | −0.212 | >0.4 | <2 | *+0.678* | *<0.001* | 20 |
| | 14 | −0.431 | >0.1 | 3—6 | +0.357 | >0.1 | 18 |
| | 6 | +0.790 | 0.1>p>0.05 | 7—18 | +0.395 | 0.1>P>0.05 | 20 |
| 3 | *$\Delta O_2$ in 6% $CO_2$——$\Delta O_2$ after 6% $CO_2$* (Table IX) | | | | *$\Delta O_2$ in 12% $O_2$——$\Delta O_2$ after 12% $O_2$* (Table VI) | | |
| | n | r | p | | r | p | n |
| | 13 | −0.110 | >0.7 | <2 | −0.032 | >0.8 | 20 |
| | 14 | *−0.725* | *<0.01* | 3—6 | −0.428 | 0.1>P>0.05 | 18 |
| | 6 | *−0.927* | *<0.01* | 7—18 | −0.283 | >0.2 | 20 |
| 4 | *$\Delta T$ in 6% $CO_2$——$\Delta T$ after 6% $CO_2$* (Table X) | | | | *$\Delta T$ in 12% $O_2$——$\Delta T$ after 12% $O_2$* (Table VII) | | |
| | n | r | p | | r | p | n |
| | 13 | +0.309 | >0.3 | <2 | −0.179 | >0.4 | 20 |
| | 14 | +0.741 | *<0.01* | 3—6 | −0.271 | >0.2 | 18 |
| | 6 | −0.620 | >0.1 | 7—18 | *−0.722* | *<0.001* | 20 |
| 5 | *$O_2$ at 20°C——$\Delta O_2$ in 6% $CO_2$* (Table XI) | | | | *$O_2$ at 20°C——$\Delta O_2$ in 12% $O_2$* (Table VIII) | | |
| | n | r | p | | r | p | n |
| | 13 | +0.423 | >0.1 | <2 | *−0.705* | *<0.001* | 20 |
| | 14 | −0.353 | >0.2 | 3—6 | *−0.767* | *<0.001* | 18 |
| | 6 | *−0.907* | *<0.02* | 7—18 | −0.359 | >0.1 | 20 |

6 *BT at 20°C——ΔT in 6% $CO_2$* (Table XII)

| n | r | p |
|---|---|---|
| 13 | −0.093 | >0.7 |
| 14 | *+0.720* | *<0.01* |
| 6 | +0.290 | >0.5 |

7 *BT in 6% $CO_2$——ΔT after 6% $CO_2$* (Table XIII)

| n | r | p |
|---|---|---|
| 13 | +0.398 | >0.1 |
| 14 | *+0.732* | *<0.01* |
| 6 | −0.602 | >0.3 |

8 *$\Delta O_2$ 35−20°C——$\Delta O_2$ in 6% $CO_2$* (Table XIV)

| n | r | p |
|---|---|---|
| 13 | −0.052 | >0.8 |
| 11 | −0.078 | >0.8 |
| 6 | *−0.993* | *<0.01* |

9 *ΔT 35−20°C——$\Delta O_2$ in 6% $CO_2$* (Table XV)

| n | r | p |
|---|---|---|
| 13 | +0.285 | >0.3 |
| 11 | +0.467 | >0.1 |
| 6 | +0.037 | >0.9 |

10 *ΔT 35−20°C——ΔT in 6% $CO_2$* (Table XVI)

| n | r | p |
|---|---|---|
| 13 | −0.207 | >0.4 |
| 11 | *+0.680* | *<0.05* |
| 6 | +0.392 | >0.4 |

11 *ΔT 35−20°C——ΔT after 6% $CO_2$* (Table XVII)

| n | r | p |
|---|---|---|
| 13 | +0.04 | >0.9 |
| 11 | *+0.650* | *<0.05* |
| 6 | +0.070 | >0.8 |

*BT at 20°C——ΔT in 12% $O_2$* (Table XI)

| | r | p | n |
|---|---|---|---|
| <2 | −0.077 | >0.7 | 20 |
| 3−6 | +0.150 | >0.5 | 18 |
| 7−18 | −0.022 | >0.9 | 21 |

*BT in 12% $O_2$——ΔT after 12% $O_2$* (Table XIV)

| | r | p | n |
|---|---|---|---|
| <2 | *+0.486* | *<0.05* | 20 |
| 3−6 | +0.229 | >0.3 | 18 |
| 7−18 | *−0.483* | *<0.05* | 20 |

*$\Delta O_2$ 35−20°C——$\Delta O_2$ in 12% $O_2$* (Table XVII)

| | r | p | n |
|---|---|---|---|
| <2 | *−0.655* | *<0.01* | 20 |
| 3−6 | *−0.772* | *<0.001* | 18 |
| 7−18 | *−0.729* | *<0.001* | 20 |

*ΔT 35−20°C——$\Delta O_2$ in 12% $O_2$* (Table XX)

| | r | p | n |
|---|---|---|---|
| <2 | −0.137 | >0.5 | 20 |
| 3−6 | −0.262 | >0.2 | 18 |
| 7−18 | −0.348 | >0.1 | 20 |

*ΔT 35−20°C——ΔT in 12% $O_2$* (Table XXI)

| | r | p | n |
|---|---|---|---|
| <2 | +0.214 | >0.3 | 20 |
| 3−6 | +0.067 | >0.7 | 18 |
| 7−18 | −0.391 | 0.1>p>0.05 | 21 |

*ΔT 35−20°C——ΔT after 12% $O_2$* (Table XXII)

| | r | p | n |
|---|---|---|---|
| <2 | *+0.517* | *<0.05* | 20 |
| 3−6 | *+0.553* | *<0.02* | 18 |
| 7−18 | +0.103 | >0.6 | 20 |

$O_2$ = oxygen consumption
$\Delta O_2$ = change in oxygen consumption
BT = body(colonic) temperature
ΔT = difference in body temperature
$O_2$ at 20°C = oxygen consumption at an ambient temperature of 20°C before exposure to hypercapnia or hypoxia
BT at 20°C = body temperature at an ambient temperature of 20°C prior to exposure to hypercapnia or hypoxia
$\Delta O_2$ 35−20°C = difference in oxygen consumption at ambient temperatures of 35°C and 20°C
ΔT 35−20°C = difference in body temperature at ambient temperatures of 35°C and 20°C

and in another one in conjunction with hypoxia or that a significant correlation could be established in conjunction with hypercapnia but not with hypoxia or vice versa.

## Discussion

*Oxygen consumption.* Comparison of mean oxygen consumption at an ambient temperature of 20°C prior to, during, and after the termination of, breathing 6% $CO_2$ failed to reveal changes of any significance (Table I). The same applied to the mean differences in heat production in response to hypercapnia and to restoration of air breathing, although the increase in oxygen consumption during hypercapnia observed in the youngest age-group approached statistical significance (Table II). This change was, however, in the opposite direction as would be expected. A somewhat different picture emerged when one considered the distribution of the changes shown in Table V and when looking at individual observations like those demonstrated in Figs 2 and 5, in which a marked decrease in heat production occurred in response to breathing 6% $CO_2$ followed after the termination of hypercapnia by a marked increase in heat production. Despite the minimal changes in mean oxygen consumption and the apparently inconsistent variability of individual observations, correlation and regression analysis revealed some statistically highly significant relationships in one or the other age-group.

Table IX (No. 3 in Table XX), for instance, showed a highly significant inverse relationship of the change in oxygen consumption during hypercapnia and the change following the termination of $CO_2$ breathing in the two older age-groups, without even an indication of a similar relationship in the youngest age-group. In contrast, the relationship between oxygen consumption at an ambient temperature of 20°C prior to exposure to hypercapnia and the change in heat production during $CO_2$ breathing (Table XI; No. 5 in Table XX) failed to reach statistical significance in the two younger age-groups, whereas a very close correlation with a correspondingly high statistical significance was found in the oldest age-group: the more heat was produced at an ambient temperature of 20°C prior to hypercapnia, the greater the reduction in heat production during hypercapnia. Another aspect of essentially the same relationship is demonstrated by Table XIV (No. 8 in Table XX). A statistically highly significant correlation was found in the oldest age-group: the greater the increase in oxygen consumption in response to transfer to the cooler environment, i.e. the greater the thermoregulatory heat production, the more pronounced the reduction in heat production during the subsequent exposure to hypercapnia. In contrast, not a trace of a similar relationship was found in the younger age-groups.

*Body temperature.* The fall in mean colonic temperature in response to hypercapnia failed to reach statistical significance in all of the three age-

groups. Practically no change occurred in mean colonic temperature after the restoration of air breathing in the two younger age-groups and a relatively small, statistically non-significant, increase in the oldest age-group (Table III). Basing the calculations on the mean of the changes observed in the individual experiments, a statistically significant fall was, however, observed during hypercapnia in the two younger age-groups, and statistical significance was approached by the oldest age-group. Considering the small number of observations, statistical significance would presumably have been reached if more observations had been available. Following the restoration of air breathing, however, the mean of the differences showed no change in the two younger age-groups, and an increase approaching statistical significance was observed in the oldest age-group only (Table IV). The absence or insignificance of an increase after the termination of hypercapnia has to be considered an important and perhaps the most distinctive feature of the effect of hypercapnia — at least in the two younger age-groups — since it indicates that hypercapnia exerted a sustained effect on the regulatory system controlling body temperature and heat production after air breathing had been restored.

Distribution of the changes in body temperature during, and after the termination of, hypercapnia indicated, however, a greater diversity of the responses as suggested prima facie by the means (Table VI). Again, correlation and regression analysis revealed relationships between changes in body temperature not apparent in the changes of mean colonic temperature or in the mean of the individual differences.

A highly significant relationship was found in rabbits aged 3 to 6 days between the change in body temperature during hypercapnia and the change in colonic temperature following the termination of $CO_2$ breathing: a smaller reduction or its absence in hypercapnia, was associated with a greater increase during the 30 min following the termination of hypercapnia, whereas a great fall in colonic temperature in hypercapnia was followed after the restoration of air breathing either by no change or by a further decline in colonic temperature. No significant relationship was found in the youngest age-group. The high coefficient of correlation (—0.620) found in the oldest group suggested that, if more observations would have been available, statistical significance might have been reached. It appears to be of some interest that, in contrast to the younger age-groups, in the oldest group the coefficients of correlation and regression were negative, i.e. the greater the fall in body temperature during hypercapnia, the greater the increase after the restoration of air breathing, a relationship which corresponds to that observed in adult animals of other species (Table X; No. 4 in Table XX).

A highly significant relationship, confined to animals aged 3 to 6 days was found also between the level of colonic temperature at an ambient temperature of 20°C and the subsequent change of colonic temperature in response

to hypercapnia (Table XII; No. 6 in Table XX), and between the level of body temperature at the termination of $CO_2$ breathing and the subsequent change in response to the restoration of air breathing (Table XIII; No. 7 in Table XX). The higher the colonic temperature prior to exposure to hypercapnia, the smaller its reduction in hypercapnia, and the higher the body temperature at the termination of $CO_2$ breathing, the greater its subsequent increase, while low body temperatures at the termination of hypercapnia were followed after the restoration of air breathing by a small increase, no change, or even by a further reduction in colonic temperature. The younger and the older age-groups showed no indication of a similar relationship between body temperature prior to hypercapnia and the subsequent change during hypercapnia (Table XII; No. 6 in Table XX), whereas between the level of body temperature at the termination of hypercapnia and the change subsequent to restoration of air breathing (Table XIII; No. 7 in Table XX) a high, but statistically not significant, negative coefficient (—0.602) was obtained for the oldest age-group. The same was the case when changes in body temperature during hypercapnia and those subsequent to restoration of air breathing were correlated (Table X; No. 4 in Table XX). A highly significant coefficient of correlation with a positive sign (+0.741) was obtained for the group aged 3 to 6 days, whereas a high, although statistically not significant, coefficient with a negative sign (—0.620) was obtained for the oldest age-group. This means, of course, that in the younger group the higher the body temperature at the termination of hypercapnia or the smaller the reduction in body temperature during hypercapnia, the greater the increase after the restoration of air breathing. In the oldest group, in contrast, the greater the fall in body temperature during hypercapnia and the lower the body temperature at the termination of hypercapnia, the greater the increase after the restoration of air breathing, i.e. the oldest group responded as could be expected from studies on adult animals of other species.

A significant correlation, again confined to the group aged 3 to 6 days, was found between the decrease in body temperature in response to transfer from an ambient temperature of 35° C to one of 20° C, on the one hand, and the subsequent change in colonic temperature during hypercapnia (Table XVI; No. 10 in Table XX) and after the restoration of air breathing (Table XVII; No. 11 in Table XX), on the other hand. A smaller fall in body temperature in response to exposure to the cool environment was associated with a smaller reduction or even its absence in response to $CO_2$ breathing, and with a greater increase following the termination of hypercapnia. No indication of a similar relationship was obtained in the other two age-groups.

*Interrelationships between heat production and body temperature.* A statistically significant correlation between the changes in heat production and in body temperature (Table VII; No. 1 in Table XX) was found in the youngest

age-group only; the coefficient of correlation approached statistical significance in rabbits aged 3 to 6 days, and there was no indication of a correlation in the oldest age-group. In contrast, the changes in heat production and in body temperature after the termination of hypercapnia (Table VIII; No. 2 in Table XX) displayed no significant correlation in the two younger age-groups, and only approached statistical significance in the oldest age-group. Considering, however, the high value of the coefficient of correlation (+0.790) in conjunction with the small number of observations the relationship between the two variables was most probably a real one.

No significant relationship was found between the decrease in body temperature in response to transfer from the thermoneutral environment to an ambient temperature of 20°C and the change in oxygen consumption in response to the subsequent exposure to hypercapnia in any of the age-groups (Table XV; No. 9 in Table XX).

From the responses of heat production and body temperature to hypercapnia, as well as from their interrelationship, it is fairly evident that the participation of the effector mechanisms, i.e. heat production and heat loss, varies considerably. They may change even in opposite directions: an increase in heat production may be associated with a fall in body temperature, i.e. an increase in heat production may be exceeded considerably by the concomitant increase in heat loss. It appears improbable that hypercapnia should exert its action primarily on the effector mechanisms, i.e. on the mechanisms directly governing heat loss and heat production. More probably, changes in these should be considered secondary to the changes elicited by hypercapnia in the function of higher integrative regulating mechanisms.

*The influence of age.* A striking result of the correlation and regression analyses was that a statistically significant relationship between any one pair of variables was often obtained in one age-group only (Table XX). Even when one considers the results only approaching statistical significance or, in the oldest age-group, the relationships with a correlation coefficient higher than 0.6, age appears to be a determining factor. In rabbits aged 3 to 6 days, the relationship of the change in heat production in response to hypercapnia and the concomitant change in body temperature could probably have reached statistical significance with further observations. The same applies to the oldest age-group regarding the relationships (a) between the change in heat production after the termination of $CO_2$ breathing and the concomitant change in colonic temperature ($r = +0.790$; Table VIII; No. 2 in Table XX); (b) between the change in body temperature during hypercapnia and the subsequent change after the restoration of air breathing ($r = -0.620$; Table X; No. 4 in Table XX); and (c) between body temperature at the termination of hypercapnia and the subsequent change after the restoration of air breathing ($r = -0.602$; Table XIII; No. 7 in Table XX). In all these instances, how-

ever, the signs of the coefficients of correlation and of regression were just the opposite of those obtained for both of the younger age-groups, and, therefore, point to a change with age in the relationship between the respective variables.

*Comparison between the thermoregulatory effects of hypercapnia and hypoxia and of the responses to restoration of air breathing.* The mean of the differences in oxygen consumption in response to hypercapnia and to hypoxia have been shown to differ highly significantly in the two younger age-groups, and to be practically identical in the oldest age-group. Even greater were the differences in the responses to the restoration of air breathing. Whereas after the restoration of air breathing an overshooting increase was observed in all age-groups after hypoxia, no overshoot was seen after hypercapnia even in the oldest age-group in which the mean change during exposure to hypercapnia was identical with that obtained in response to hypoxia (Table XVIII).

The differences in the response of heat production to hypercapnia and to hypoxia are even more striking when the concomitant changes in body temperature are considered. Albeit the mean decrease in body temperature in response to hypercapnia was significantly smaller than that observed in response to hypoxia, it was nevertheless statistically significant in the two younger age-groups and approached statistical significance in the rabbits aged 7 to 18 days (Table IV). It must, however, be remembered that, as demonstrated in Table VI, a number of rabbits failed to respond to hypercapnia with a fall in body temperature, whereas in response to hypoxia body temperature fell in every instance even when oxygen consumption increased during hypoxia. The most remarkable difference between the effects of hypercapnia and hypoxia probably was that despite the marked, statistically significant, fall in body temperature during hypercapnia, no significant increase in mean oxygen consumption followed the restoration of air breathing (Table II), nor did an appreciable change in mean body temperature occur in the two younger age-groups, and statistical significance was approached in the oldest age-group only (Table IV).

From our observations following the termination of hypoxia, the conclusion was reached that the fall in body temperature in the course of hypoxia constituted the stimulus which caused an overshooting increase in heat production and a marked rise in body temperature in every age-group. This conclusion was supported by the fact that heat production increased after the termination of hypoxia also in rabbits which responded to hypoxia with no decrease in oxygen consumption or even with an increase in heat production (VÁRNAI et al. 1971). In contrast, the fall in body temperature in response to hypercapnia did not seem to act as a decisive stimulus for an increase in heat production and body temperature — at least in the two younger age-groups (Tables XVIII and XIX). This was the more remarkable since, in the

new-born rat, in spite of the lack of a fall in body temperature under the effect of hypercapnia, colonic temperature increased significantly after the restoration of air breathing (VÁRNAI et al. 1970b).

Keeping in mind the limitations of comparing means and of demonstrating a few individual experiments, the correlation analyses were also compared. A glance at Table XX shows fairly convincingly that the relationships of the different variables were different in hypercapnia and hypoxia. For instance, a highly significant correlation could be established between the change in oxygen consumption during $CO_2$ breathing and the subsequent change in heat production after the restoration of air breathing in the two older age-groups, whereas there was no significant relationship between the same variables in conjunction with hypoxia (No. 3 in Table XX). Similarly, at the age of 3 to 6 days a highly significant coefficient of correlation with a positive sign was found between the change in body temperature in the course of hypercapnia and the change subsequent to the restoration of air breathing, whereas a non-significant coefficient of correlation with a negative sign was obtained between the corresponding variables in the case of hypoxia. In the oldest age-group, however, a highly significant correlation was established between the same variables in the case of hypoxia and a high coefficient of correlation was obtained in the case of hypercapnia, both with a negative sign (No. 4 in Table XX). Further, a highly significant correlation could be demonstrated in the oldest age-group between oxygen consumption at an ambient temperature of 20°C and the change in heat production in response to hypercapnia, and in the group aged 3 to 6 days between the change in body temperature in response to transfer from an ambient temperature of 35°C to one of 20°C and the subsequent change in response to hypercapnia, whereas no significant relationships could be demonstrated between the corresponding variables in conjunction with hypoxia (Nos 5 and 10 in Table XX). On the other hand, a significant relationship between certain variables appeared at an earlier age in conjunction with hypoxia than in the case of hypercapnia (Nos 5, 8 and 11 in Table XX).

On the whole, the results furnished convincing evidence for the earlier conclusion (VÁRNAI et al. 1970b) that the mechanism through which hypercapnia exerts its effect on the thermoregulatory mechanism is not identical with that responding to the stimulus of hypoxia and further evidence was presented that relationships characteristic of a certain period of neonatal life may be absent earlier and/or later. The variability of the response to hypercapnia in early neonatal life is probably of genetic origin, similarly to the variability of the response of body temperature to changes in environmental temperature, as has been suggested earlier (VÁRNAI et al. 1970c).

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# METABOLIC POOL OF HEART PROTEINS AND AMINO ACIDS IN COBALT-POISONED DUCKLINGS

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Accumulation of cobalt and concentration of free heart proteins and amino acids were studied in ducklings poisoned with cobalt.

1. Cobalt is accumulated intensively in heart tissue from a diet enriched with cobalt. A high concentration of cobalt in the diet does not involve an increase of the element in circulating blood.

2. No changes were found in the level of free heart proteins in ducklings poisoned with cobalt.

3. Specific and significant changes were observed in the concentration of free heart amino acids in experimental animals. The level of some amino acids decreased while of some amino acids increased.

It is generally accepted that a high concentration of cobalt changes many functions in the organism (VELIKÝ 1964), by blocking enzymatic systems (ALEXANDER 1969). The element is naturally accumulated in the organism during ontogenesis (MAREK et al. 1969) but after cobalt poisoning a greater accumulation occurs especially in the heart (SULLIVAN et al. 1968). The high cobalt concentration involves drastic changes in the heart of men (in heavy beer drinkers) and in experimental animals (ALEXANDER 1969; DERR et al. 1970; HALL and SMITH 1968; KESTELOOT et al. 1968; MORIN et al. 1967; SULLIVAN et al. 1968). It has also been shown that cobalt influences the metabolism of body proteins and amino acids (VELIKÝ 1964; KESTELOOT et al. 1968; PAULOV 1971).

On the basis of the above data we have studied in ducks poisoned with cobalt

1. the accumulation of cobalt in heart tissue;
2. the metabolic pool of free heart proteins; and
3. the pool of free heart amino acids.

## Methods

Two-day-old Peking ducklings were used in the experiments. They were allowed ad libitum a commercial Poussex diet enriched with cobalt in the form $CoCl_2 \cdot 6H_2O$ during three weeks (PAULOV 1971; Fig. 1).

The concentration of cobalt in the heart and blood plasma was analysed colorimetrically (STARE and ELVEHJEM 1953). The free proteins were obtained from heart tissue by extraction in 0.9% NaCl and evaluated by paper electrophoresis (PAULOV and DEMERS 1968). The free amino acids were obtained by extraction in 70% ethanol and analysed by two-dimensional paper chromatography (VESELOVSKÝ 1971).

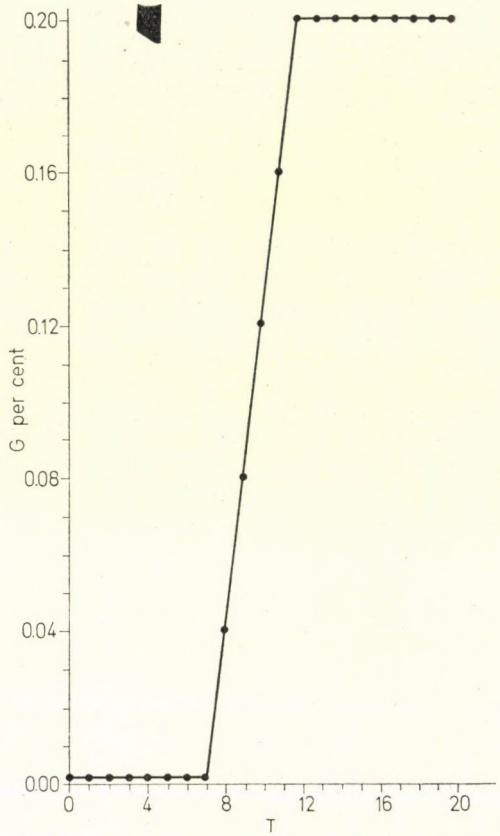


*Fig. 1.* System of intoxication of ducklings with cobalt. G % — concentration of cobalt in diet in g per 100 g; T — time in days

## Results

### 1. *Accumulation of cobalt in heart tissue*

A high concentration of cobalt was found in the heart of the ducklings poisoned with cobalt (Table I). The plasma cobalt level was not elevated.

**Table I**

*Accumulation of cobalt in the heart tissue of control (0) and experimental (1) ducklings*

| Group | Number of animals | μg Co/g fresh heart tissue |
|---|---|---|
| 0 | 7 | $5.28 \pm 0.63$* |
| 1 | 7 | $28.95 \pm 1.94$ |

* Significant difference between control and experimental animals, $p < 0.001$

## 2. *Distribution of free heart proteins*

Five free protein fractions were found in the heart of control and experimental ducklings; the two groups showed no difference in this respect (Table II). A schematic representation of the heart and serum protein spectrum is given in Fig. 2; it is seen that no changes occurred either in the relative or in the absolute values.

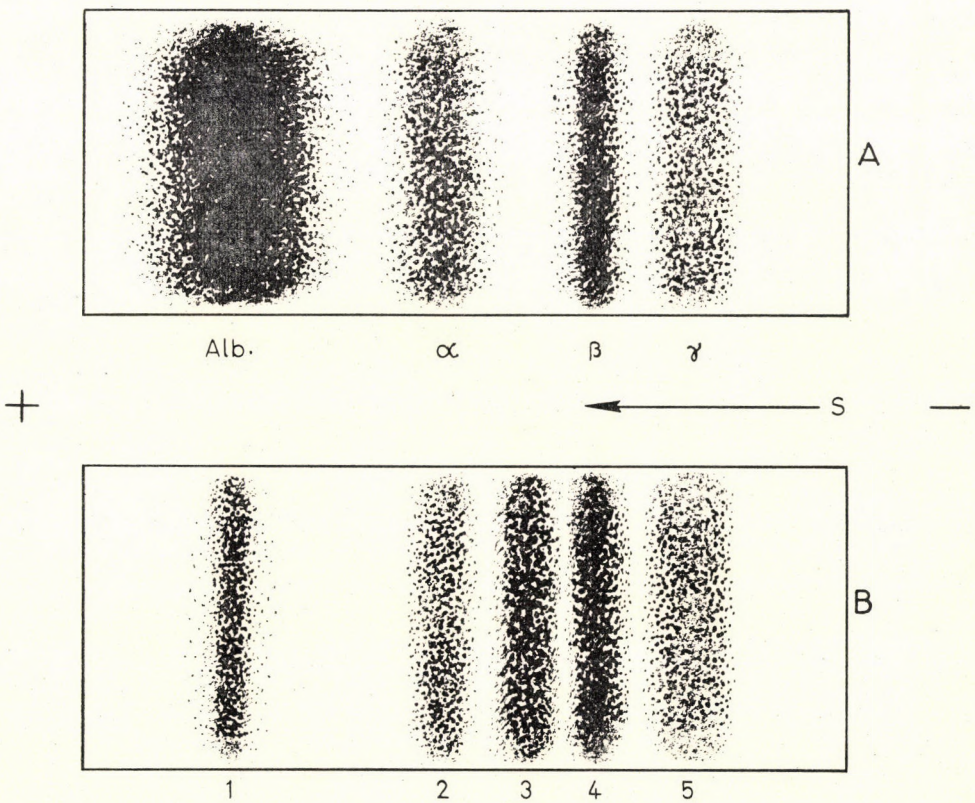


*Fig. 2.* Schematic representation of serum (A) and free heart (B) protein spectrum in ducklings. S — starting line and direction of mobility of protein components from cathode to anode

**Table II**

*Relative proportions of free heart protein fractions in control (0) and experimental (1) animals*

| Group | Fractions** | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| 0 | 11.9 ± 1.51 | 9.7 ± 1.35 | 23.9 ± 2.02 | 29.9 ± 2.54 | 24.6 ± 3.73* |
| 1 | 11.9 ± 2.11 | 9.3 ± 1.52 | 22.0 ± 2.62 | 31.3 ± 3.13 | 25.5 ± 3.06 |

* No difference between control and experimental animals, $p > 0.10$
** Fraction "1" is the component with serum albumin mobility

### 3. *Free amino acids in heart*

Specific and significant changes were observed in the level of free heart amino acids in the poisoned ducks. Data concerning these changes are given in Fig. 3. The concentration of Leu/Ileu, Meth/Val, Tyr, Ala, Gly/Ser, Asp, $GluNH_2$, Arg, His decreased while that of Thre, Glu and Try increased.

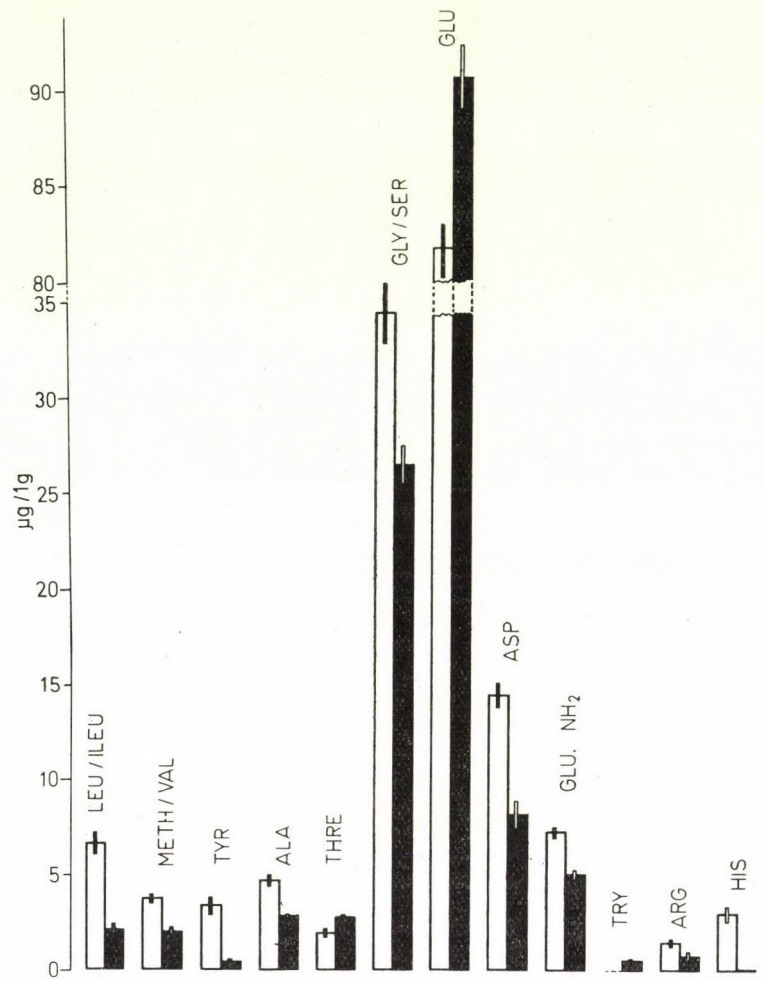


*Fig. 3.* Absolute free heart amino acid values (µg/g heart tissue) in control (□) and in cobalt intoxicated (■) ducks

## Discussion

It was found that the heart tissue of cobalt poisoned ducks accumulated from the diet ten times more cobalt than did the heart tissue of the control ducks. This result was in good agreement with the findings made on the

heart of chronic beer drinkers (SULLIVAN et al. 1968). It is assumed that cobalt exerts an unfavourable influence on the cardiac function. On the other hand, the plasma cobalt level did not increase, indicating the rapid elimination of the element from the circulation.

The metabolic free pool of heart proteins was unchanged in the animals poisoned with cobalt, while the serum protein level changed significantly in cobalt intoxication (KESTELOOT et al. 1968; PAULOV 1971).

The level of free amino acids in the heart of the experimental animals showed significant specific changes; of these, the decrease of some amino acids and the increase of others represents the specific adaptation of the organism to cobalt intoxication. These changes might be due to enzymatic factors (ALEXANDER 1969).

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# MUCOSAL PROTEOLYTIC ACTIVITY IN THE SMALL INTESTINE OF SUCKLING RATS

By

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High proteolytic activity was demonstrated in the lumen and mucosa of the small intestine of newborn and suckling rats. Mucosal proteolytic activity varied considerably over the length of the small intestine. Dividing this into 5 parts, activity was lowest in the 1st and 2nd segments in which the epithelial cells contained no vacuoles, while activity was highest in the 4th segment in which the supranuclear vacuoles of the epithelial cells were most pronounced. Mucosal proteolytic activity was found to be of at least 4 types corresponding to pancreatic protease activities and suggesting a pancreatic origin. It remains to be clarified how the immunoglobulins ingested with milk by the baby rats escape digestion.

In the distal part of the suckling rat's small intestine a special type of epithelial cell has been identified (CLARK 1959; BAINTNER and VERESS 1967), characterized by the presence of a large supranuclear vacuole and a tubular-vesicular system (WISSIG and GRANEY 1968; GRANEY 1968). These cells differ from the proximally located epithelial cells of the small intestine also functionally in that they are capable of absorbing proteins and certain indigestible colloids (CLARK 1959; BRAMBELL 1958; VON MÖLLENDORF 1924).

Previous studies (BAINTNER and VERESS 1970) have shown a distinct proteolytic activity of the mucous membrane in the distal part of the suckling rat's small intestine as well as a considerable decrease of this activity with the change of the intestinal epithelium from suckling to adult type. This finding appeared to correlate well with BRAMBELL's hypothesis (BRAMBELL 1966) that the supranuclear vacuoles represented "phagolysosomes", viz. digestive saccules in which the absorbed immunoglobulins were protected against digestion by specific receptors. It remained to be clarified whether the proteolytic activity measured by us was related to proteases absorbed together with the immunoglobulins from the intestinal lumen or to intracellular "phagolysosome" enzymes. An attempt was therefore made to identify the nature and origin of the proteolytic activity of the small intestinal mucosa.

## Methods

*Animals.* Albino rats newborn, 7-day- and 14-day-old were used.

*Preparation of extracts.* In one experiment (b) the small intestine was divided into 5 equal portions numbered in succession 1—5. In the other experiments, the small intestine was divided into 2 equal parts and exclusively the brown-coloured distal portion was examined.

The intestinal segments were slit open and cautiously rinsed with saline. The mucous membrane was scraped off in distilled water and the crude homogenate was centrifuged at 6000 *g* for 15 minutes. Aminopeptidase activity was determined in the sediment, while the other enzyme activities in the supernatant, which in the following will be termed intestinal mucosal extract. The pancreas was homogenized in distilled water and rough particles were removed by centrifugation.

*Enzyme activities.* Total proteolytic activity was estimated in Tris-HCl buffer pH 8 at 37°C, using Hammarsten-casein as substrate. Acid protease activity was determined in acetate buffer pH 3.75 at 37°C, with denatured haemoglobin as substrate. The proteins were precipitated by 10% trichloroacetic acid and the peptides retained in the supernatant were assessed by spectrophotometry at 280 mμ.

Tryptic and chymotryptic activity was determined according to SCHWERT and TAKENAKA (1955) at room temperature, using a Spektromom 201 spectrophotometer. Tryptic activity was estimated in Tris-HCl buffer pH 8, at 253 mμ, using N-α-benzoyl-L-arginine ethyl ester (BAEE) substrate (kindly supplied by Koch-Light Laboratories). Chymotryptic activity was determined at 237 mμ in Tris-HCl buffer pH 7, with acetyltyrosine ethyl ester (ATEE, Reanal) as substrate. Tryptic and chymotryptic activity was expressed in terms of μg enzyme related to the aqueous extract of the entire intestinal segment. Converting activity to μg enzyme, 12,000 BAEE units were regarded as equivalent to 1 mg trypsin and 14,000 ATEE units as equivalent to 1 mg chymotrypsin. For the sake of simplicity, the total BAEE-splitting activity measured was regarded as trypsin and the total ATEE activity as chymotrypsin. Aminopeptidase activity was determined in Tris-HCl buffer pH 8 at 37°C, using L-leucine-p-nitroanilide (Fluka) as substrate. Results were read spectrophotometrically at 420 mμ. Activity was expressed as μmol of substrate transformed in 1 minute, related to the entire intestinal segment.

*Other methods.* The extracts were examined for molecular weight distribution of proteolytic activity by chromatography on a Sephadex G—100 column, 400 mm long, 25 mm in diameter, using ANDREWS's method (ANDREWS 1964). The fractions were eluted in 0.1 *M* KCl containing 0.05 *M* Tris-HCl buffer pH 7.5. Fractions of 5 ml volume were collected by an automatic collector. In each fraction, proteolytic activity was determined with casein as substrate.

Tryptic activity was washed out from the ileal mucosal homogenate by means of 0.1 *M* Tris-HCl buffer pH 8.

The intestinal segments were weighed after slitting open and blotting with filter paper.

Soybean trypsin inhibitor (Reanal) was used at a final concentration of 0.2 mg/3 ml. Elevation of this concentration did not increase the inhibitory effect.

pH values were assessed by means of a Radelkisz titrimeter, using glass and calomel electrodes.

Pancreatectomy and sham-operation were performed under pentobarbital anaesthesia from a median abdominal incision.

## Results

*a)* Tryptic and chymotryptic activities of intestinal contents and mucosa were compared in the distal part of the small intestine of 8 rats, 14-day-old. Exclusively the not particle-bound activity extractable by a single washing was considered. The results are shown in Table I. Tryptic actvity was similar in both preparations, whereas chymotryptic activity was considerably higher in the intestinal contents than in the mucosa.

Proteolytic activity was of the same order in the small intestinal mucosa of newborn nonfed and older suckling rats.

*b)* The individual intestinal segments were examined for differences in mucosal proteolytic activity. The small intestine from 5 rats 14 days of age divided each into 5 equal segments and a mucosal extract was prepared from each segment. The fluid phase of the extract was assayed for tryptic, chymotryptic and acid protease activity, whereas the sediment for aminopeptidase activity. Acid protease activity was demonstrable only in traces. The weight

of the individual segments and the aminopeptidase activity measured in them tended to decrease in the distal direction without, however, showing parallelism (Fig. 1). In contrast, tryptic and chymotryptic activity of the segments tended to increase in distal direction, except for the first segment in which contamination by the adhering pancreas could not be excluded as well as for the 5th segment in which the activities tended to decline.

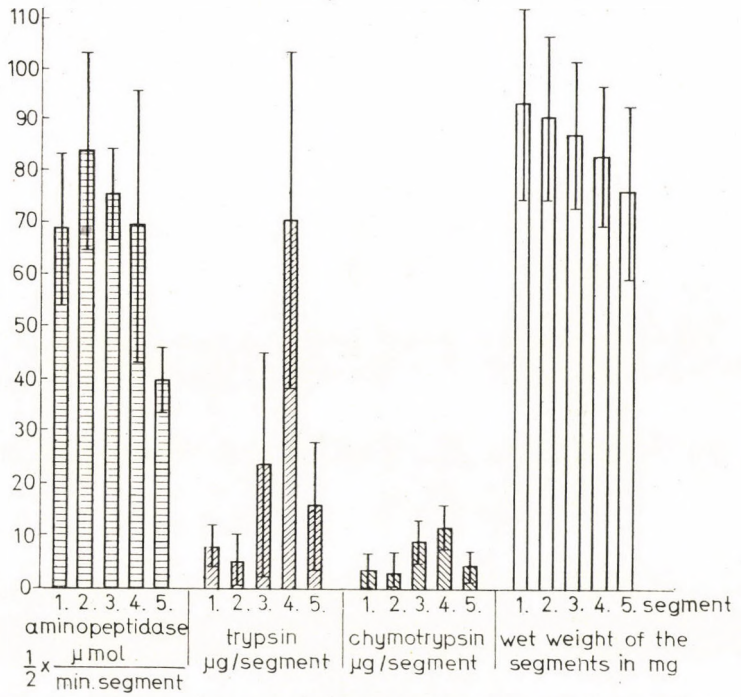


*Fig. 1.* Distribution of mucosal enzyme activities in successive small intestinal segments. Mean ± standard deviation

*c)* An attempt was made to remove by washing the tryptic activity of ileal mucosal scrapings from 14-day-old rats. The bulk of activity was readily removed in this manner, but a minor part, being particle-bound

**Table I**

*Proteolytic activity extracted from intestinal contents and mucosa by a single washing*

| | Intestinal contents | Intestinal mucosa |
|---|---|---|
| Trypsin | 39.7 ± 11.3 | 37.0 ± 17.0 |
| Chymotrypsin | 14.0 ± 3.5 | 3.5 ± 2.9 |

The figures indicate the amount in µg of (chymo)trypsin measured in the distal part of the small intestine
Mean ± standard deviation

persisted also after 6 washings. Conversely, the bulk of aminopeptidase activity remained particle-bound and only a minor part could be removed by washing.

*d)* An attempt was made to clarify whether the proteolytic activity of the small intestinal mucosa had originated from the pancreas or from local protease synthesis. No direct evidence could be obtained, as the pancreatic

**Table II**

*Effect of 16-hour starvation on proteolytic activity in small intestinal mucosa of newborn rats*

| | Control rats | Starved rats | Decrease of activity per cent |
|---|---|---|---|
| Chymotrypsin | 11.6 ± 2.4 | 0 | 100 |
| Trypsin | 51.4 ± 5.1 | 7.6 ± 1.5 | 85 |
| Aminopeptidase | 860 ± 140 | 740 ± 120 | 13 ($p < 0.01$) |

The figures indicate the amount in $\mu$g of (chymo)trypsin measured in the distal part of the small intestine and the amount in $\mu$mol of substrate transformed by aminopeptidase within 1 minute in the same segment
Mean ± standard deviation

**Table III**

*Effect of refeeding on mucosal tryptic activity in the distal part of the small intestine*

| | Number of animals | Trypsin |
|---|---|---|
| Control rats | 4 | 76 |
| After 16-hour starvation | 4 | 0 |
| 30 minutes after the beginning of refeeding | 4 | 0 |
| 1 hour after refeeding | 4 | 5 |
| 2 hours after refeeding | 4 | 29 |

duct could not be ligated in suckling rats. Pancreatectomy did not help either, as mucosal proteolytic activity disappeared also from sham-operated animals. Therefore, in further experiments, evidence was pursued by an indirect approach.

*e)* Of a 7-day-old rat litter, 4 animals were starved for 16 hours, while 3 animals were left with the mother. Next morning both groups of baby rats were killed and mucosal extracts were prepared from the distal portions of their small intestine. As a result of the fasting, mucosal tryptic and chymotryptic activity either disappeared or was reduced to a very low level, whereas aminopeptidase activity of the brush border showed hardly any change (Table II). This experiment was repeated on 20 rats of 2 litters with the modification that some of the starved littermates were allowed to suck for various times prior to sacrifice (Table III). On refeeding, tryptic and chymotryptic activity reappeared in the intestinal mucosa.

*f)* The pH-dependence of the tryptic activity of the ileal mucosal and pancreatic extracts was compared, and expressed in per cents related to the highest activity measured. The curves for the two types of extract run essentially parallel courses (Fig. 2). Characteristically, both preparations showed higher activity at pH 9 than at pH 8.

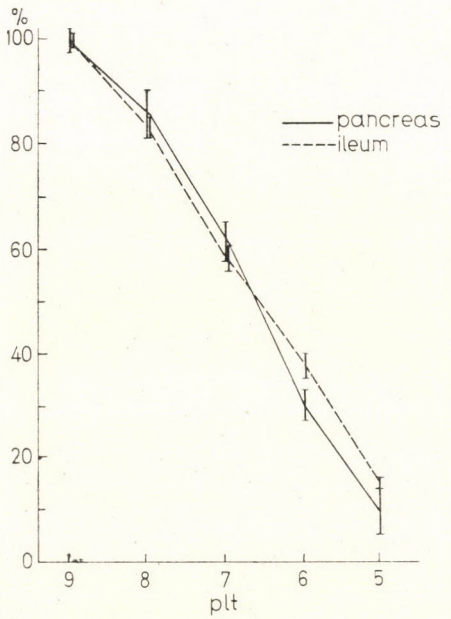


*Fig. 2.* pH-dependence of BAEE-splitting activity in pancreatic and ileal mucosal extracts

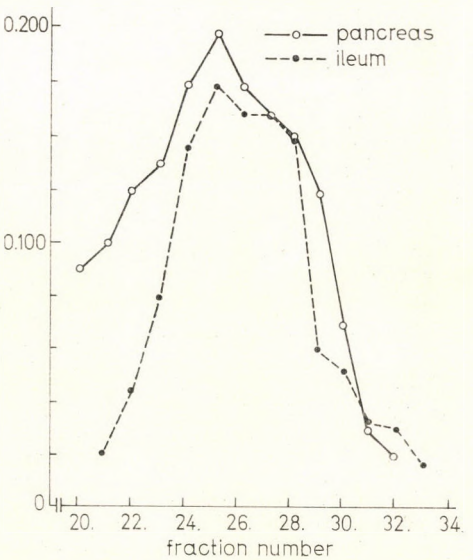


*Fig. 3.* Molecular weight distribution of total proteolytic activity in pancreatic and ileal mucosal extracts, determined on Sephadex G-100 column

*g)* Molecular weight distribution of proteolytic activity of pancreatic and ileal mucosal extracts was compared by chromatography on Sephadex G-100 column (Fig. 3). Consistently with GELOTTE's (1964) finding in pancreatic samples, proteolytic activity showed a single peak. The maximum of both examined substances and of the trypsin preparation used as reference standard appeared uniformly in the 25th fraction.

*h)* BAEE- and ATEE-splitting activities of the ileal mucosa were examined for suppressibility by soybean trypsin inhibitor. Inhibition was partial with both activities even with the inhibitor added in excess. The residual activity not suppressed by the inhibitor amounted to more than one half of the total activity, and rose further on incubation at 37°C for 2 hours (autolysis; Table IV). In the meantime, total activity decreased by 22.5

± 7.6%. The BAEE- and ATEE-splitting activities of the pancreatic extract could also be separated into two components each by means of trypsin inhibitor.

**Table IV**

*Differentiation of the types of BAEE-splitting activity in extracts of ileal mucosa*

| | Total activity per cent | Activity | |
|---|---|---|---|
| | | inhibited | not inhibited |
| | | by soybean trypsin inhibitor per cent | |
| Untreated extract | 100 | 22.4 ± 7.6 | 77.6 ± 14.4 |
| Extract incubated for 2 hours at 37°C | 100 | 16.0 ± 1.8 | 84.0 ± 6.5 |

## Discussion

As shown in Table I, both the lumen and the mucous membrane of the small intestine showed a high proteolytic activity in the suckling rats. Distribution of proteases over the length of the intestinal mucosa appeared to be related to the presence of supranuclear vacuoles in the intestinal epithelial cells; the more pronounced such vacuoles were in a given intestinal segment, the higher was its activity. Both vacuole size and proteolytic activity were highest in the 4th of the 5 intestinal segments (Fig. 1).

In vivo and in vitro studies were carried out to decide whether mucosal proteolytic activity was local or had originated in the pancreas.

In experiments in vivo, mucosal proteolytic activity behaved in the same manner as that of the expected pancreatic secretion in that it disappeared under the influence of abdominal incision or starvation for 16 hours, but reappeared after the baby rats had been allowed to suck again (Tables II and III). At the same time, the brush border-bound aminopeptidase activity (EICHHOLZ 1968) synthetized locally was hardly affected by the same treatments.

In experiments in vitro, certain characteristics of the proteolytic activity of the pancreatic extract and the ileal mucosa were compared. Both preparations behaved similarly in respect of solubility, pH-dependence, molecular weight distribution, substrate specificity, and sensitivity to trypsin inhibitor. In both extracts there were at least 4 different kinds of alkaline proteolytic activity, viz. trypsin inhibitor sensitive BAEE-splitting activity (true trypsin); trypsin inhibitor insensitive BAEE-splitting activity; trypsin inhibitor sensitive ATEE-splitting activity (true chymotrypsin); trypsin inhibitor

insensitive ATEE-splitting activity. This last activity is possibly identical with the pancreatic protease of URIEL and AVRAMEAS (1965).

The above indirect evidence unequivocally suggested the pancreatic origin of the small intestinal alkaline proteolytic activity. This offers an explanation also for the finding that the trypsin/chymotrypsin ratio was higher in the intestinal mucosa than in the lumen, owing to the inactivation of part of the chymotrypsin by trypsin during their presence in the intestinal epithelium or on its surface. The pancreatic origin of the proteolytic activity was not excluded by the failure to remove it completely by washing. Originally, the residual activity had probably been soluble, having been bound later on the mucous coating of the intestinal epithelium which is known to bind certain pancreatic enzymes such as trypsin, chymotrypsin (GOLDBERG et al. 1969a, b; WOODLEY and KENNY 1969), α-amylase (UGOLEV 1965) and lipase (IEZUITOVA et al. 1964).

A further problem was the higher uptake of pancreatic proteases by vacuolized cells. The explanation may be sought in the different dimensions of the cell surfaces. The surfaces of the microvilli showed no notable variation, as suggested by aminopeptidase activity assays (Fig. 1) and electronmicrographs (VERESS and BAINTNER, to be published). The difference may have been due rather to another surface increasing factor, the tubular-vesicular system (WISSIG and GRANEY 1968; GRANEY 1968) which occurs exclusively in the vacuolized cells. Further experiments will be needed to decide whether the enzymes would actually enter the tubular-vesicular system and pass therefrom into the supranuclear vacuole.

A high proteolytic activity of the intestinal epithelium was demonstrated in newborn rats prior to the first suckling and was found to persist during the first 3 weeks of life, until the decline of the capacity for immunoglobulin absorption (BAINTNER and VERESS 1970). Though the present findings failed to prove or to disprove the existence of the specific receptors postulated by BRAMBELL (1966), they have clearly shown that this hypothesis is in itself insufficient to explain the phenomenon of immunoglobulin absorption. As immunoglobulin absorption takes place exclusively in the medium and posterior segments of the small intestine, no indication of it being demonstrable in the anterior segment (CORNELL and PADYKULA 1969; CLARKE and HARDY 1970), a contact of the immunoglobulins with the proteolytic enzymes is unavoidable. The manner in which the immunoglobulins ingested with milk escape digestion remains to be clarified.

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# EFFECT OF RENAL PAPILLARY LIPIDS AND PROSTAGLANDIN $E_2$ ON CORTICOSTEROID PRODUCTION IN THE RAT*

By

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The effect of lipids of rat renal papillae on steroid production in vitro by the adrenals of dexamethasone-pretreated rats has been studied. A lipid extract from papillary homogenates significantly increased both aldosterone and corticosterone production rate.

Slices of papillae were incubated under different conditions and the steroidogenic effect of lipids released into the incubation medium was tested. Such an effect could be demonstrated if the papillae had been incubated in a medium with an ionic composition similar to that of papillary tissue of a moderately diuretic rat.

Synthetic prostaglandin $E_2$ brought about a slight but statistically significant increase in aldosterone and a highly significant increase in corticosterone production.

Changes in salt–water balance as well as changes in systemic blood pressure exert an influence on renal medullary circulation. Medullary blood flow is readily increased by moderate hypervolaemia and considerably reduced during dehydration. Variations in blood flow are followed by significant alterations of the ionic composition of the tissue. These ionic changes may be further enhanced by the countercurrent multiplier system. Thus, the renal papilla may be a receptor area for changes in salt–water balance.

The role of the kidney in the regulation of aldosterone secretion has been confirmed in several species. Nevertheless, experiments performed in rats (EILERS and PETERSON 1964; SPÄT et al. 1966, 1969; TAIT et al. 1970) have challenged the exclusive role of the renin-angiotensin system in mediating the renal effect. These results had led to the assumption of another renal substance regulating aldosterone secretion. The above-mentioned characteristics of medullary circulation have drawn our attention to this tissue as the production site of the postulated factor. On the analogy of the aldosteronotropic effect of angiotensin, our interest has been focussed on the vasoactive substances of the medulla, viz. the different prostaglandins (HICKLER et al. 1964; STRONG et al. 1966; LEE et al. 1967; DANIELS et al. 1967), the antihypertensive neutral lipid of MUIRHEAD et al. (1967) and the renin-preinhibitor (SMEBY et al. 1967), a special phospholipid.

The number of cytoplasmic lipid droplets in the interstitial cells of the renal papilla varies considerably with changes in haemodynamics and salt–

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water balance (NISSEN 1968a, b; TOBIAN et al. 1969; MUEHRCKE et al. 1969). It seemed therefore worthwhile to investigate the effect of renal papillary lipids and the effect of synthetic prostaglandin $E_2$ on adrenal steroid production.

## Methods

*Biological material*

Male rats, kept on semisynthetic pellets containing approximately 180 $\mu$Eq/g of sodium and 150 $\mu$Eq/g of potassium were injected with 1 mg of dexamethasone (Oradexon, Organon) in order to diminish the stress effects. 3 to 4 hours later they were decapitated and their adrenals were removed. Renal papillae were taken from female rats kept either on normal pellets and a 2% solution of NaCl in the last 24 hours ("Na-loading") or on peeled potatoes and distilled water for 7 days ("Na-depletion"). The rats' mean body weight and the diet applied in the different experimental arrangements are indicated in Table I.

*Extraction of papillary lipids*

After decapitating the rats, the kidneys were quickly excised and placed in physiological saline solution immersed in salted ice. The papillae, prepared from the cooled kidneys, were homogenized in 20 ml of chloroform–methanol 2 : 1 (v/v) per gram of tissue by means of a Potter-Elvehjem device, surrounded by salted ice. The homogenate was filtered through a Büchner's funnel. The filtrate was shaken with 0.2 vol of 0.05% $CaCl_2$ and centrifuged. The upper phase was discarded, the interlayer was washed 3 times with chloroform-methanol 0.04% $CaCl_2$ (3 : 48 : 47 v/v; FOLCH et al. 1957). The lower phase was evaporated in nitrogen stream at 35—40°C. The dry residue was dissolved in a few drops of 96% ethanol and stored overnight under nitrogen at —20°C. In control experiments a piece of muscle of identical weight from the abdominal wall or the tigh was extracted in the same way.

*Incubation of papillae*

Immediately after decapitation the kidneys were excised and placed in ice-cold physiological saline solution. After being cooled, both poles and the cortex on one side were removed and 0.3 mm thick slices were prepared with a Stadie-Riggs microtome. The sliced papilla was cleaned with fine scissors. The slices were stored in ice-cold saline till incubation. As a control, muscle slices of 0.3 mm thickness were prepared. Since, however, their thickness was not uniform, they were replaced later by 0.4 mm thick liver slices (FIELD 1948).

The papillae were incubated in media simulating conditions in vivo. Hydropenic state was simulated by the following medium: 254 m*M* NaCl + 26 m*M* $NaHCO_3$ + 19 m*M* KCl + + 1.2 m*M* $KH_2PO_4$ + 1 m*M* $MgSO_4$ + 5 m*M* $CaCl_2$ + 572 m*M* urea. The solution contained 200 mg glucose and 1 g human albumin (fraction V) per 100 ml, its calculated total osmolarity was approximately 1200 mosm/l. The following medium corresponded to the ionic composition of a papilla in a moderately diuretic state: 162 m*M* NaCl + 26 m*M* $NaHCO_3$ + 8.8 m*M* KCl + + 1.2 m*M* $KH_2PO_4$ + 1 m*M* $MgSO_4$ + 2.5 m*M* $CaCl_2$ + 92 m*M* urea. This solution, also containing 200 mg of glucose and 1 g of albumin per 100 ml, had a total osmolarity of 510 mosm/l. Both solutions were prepared on the basis of data obtained from micropuncture experiments and tissue composition assays in different species (WIRZ et al. 1951; MANITIUS et al. 1960; LASSITER et al. 1961; EIGLER et al. 1962; LEVITIN et al. 1962; GOTTSCHALK 1962—1963; GOTTSCHALK et al. 1963; LASSITER et al. 1963; JAMISON et al. 1967; GARDNER and VIERLING 1969; HAI and THOMAS 1969). Muscle and liver slices were incubated in Krebs-Ringer bicarbonate (KRB) solution containing 200 mg of glucose and 1 g of albumin per 100 ml. Incubation of papilla, muscle and liver slices in 95% $O_2$ + 5% $CO_2$ atmosphere at 38°C lasted for 2 hours. In one experimental series (Exp. No. IV) papillae were incubated in 5% $O_2$ + 5% $CO_2$ + + 90% $N_2$ atmosphere. The gas-mixtures were adjusted to a pH of 7.30 to 7.40 at 38°C in the incubation medium.

The incubation medium was extracted with 20 vol of chloroform-methanol (2 : 1, v/v) and purified as above. After drying over anhydrous $Na_2SO_4$, the chloroform phase was concentrated to a few ml in a rotating evaporator in vacuo, at 42°C. The residue was transferred into a glass-stoppered Wassermann-tube and evaporated to dryness in nitrogen stream at 35—40°C. The dry residue was stored overnight in a few drops of 96% ethanol in $N_2$ atmosphere at —20°C.

Incubation conditions in the different experimental series are indicated in Table I. *Prostaglandin* $E_2$ (Upjohn) was dissolved in 96% ethanol, to a concentration of 210 μg/ml. The solution was stored at −10°C under nitrogen atmosphere.

*Incubation of adrenal glands*

The rats were decapitated 3 to 4 hours after dexamethasone administration. Each incubation flask contained 3 pairs of adrenals in the first experimental group (Exp. I). In the other series, 8 rats were decapitated at the same time. The left adrenals of 4 rats and the right adrenals of the other 4 rats were incubated together in the same flask, and vice versa. In this way, two flasks each containing 8 adrenals, were regarded as matched incubates. Incubation was undertaken in 10 ml of KRB-glucose solution containing 1 g of human albumin (fraction V) per 100 ml, at 38° C, in a 95% $O_2$ + 5% $CO_2$ atmosphere at pH 7.30 to 7.40 in the incubation medium. At the end of the 2nd hour the medium was withdrawn for analysis and incubation was continued in a fresh medium for another two hours.

The substrates under study (tissue extracts or $PGE_2$) were transferred into the KRB-solution in ethanol. The final concentration of ethanol in the flasks with or without added substances was invariably 0.5% (v/v). The ethanol-insoluble lipids were dissolved by the albumin-containing medium itself. Tissue extracts or prostaglandin were added to the adrenal glands always in the 2nd incubation period.

*Determination of steroids*

The incubation medium was extracted with 3 × 20 ml of chloroform. The chloroform extract was washed with 2 ml 0.1 *n* NaOH and 3 × 5 ml of distilled water. After drying over $Na_2SO_4$ it was flash-evaporated in vacuo at 40—42°C. The dry residue was dissolved in methylene chloride and transferred quantitatively to Whatman's No. 1 paper. The material was run overnight at room temperature in Bush A system. Then, following vapour saturation for 90 minutes, the material was chromatographed in o Bush B5 system at 38°C for 2—2 1/2 hours. After developing with alkaline tetrazolium blue (Merck), the formazane spots were eluted with a 7 : 3 mixture of ethyl acetate and methanol and determined photometrically in an Opton spectrometer, at 540 nm. Aldosterone and corticosterone were determined using varying amounts of reference standards (Organon), run parallel with the material. Recovery of 2.5 μg of aldosterone from 10 ml saline containing 1% albumin and 0.5% ethanol, attained 73.3% (S. D. 14.4%, n = 10).

To identify aldosterone and corticosterone extracted from the incubation medium, separate experiments were performed with authentic cortisol, aldosterone, corticosterone and 11-deoxycorticosterone standards. The spots were localized by UV absorption, soda fluorescent reaction and tetrazolium blue reaction.

*Expression of results*

According to our observations, aldosterone production rates of contralateral adrenal glands matched better if not referred to adrenal weight. For this reason results were expressed in μg/adrenal/hr (in the tables: ng/adrenal/hr).

Since slices of papilla lost solids during incubation, tissue weights refer to the initial wet state.

Statistical probabilities were determined by the paired sample *t*-test.

## Results

### *Steroid production as a function of time* (Exp. I)

Adrenal glands of dexamethasone pretreated rats, incubated in KRB-glucose solution containing 1% (w/v) albumin and 0.5% (v/v) ethanol exhibited a 30% decrease in aldosterone production rate ($p < 0.01$) and an 18% decrease in corticosterone production rate ($p < 0.01$) in the 2nd two-hour incubation period, as compared to the first one (Fig. 1).

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**Table I**

*Experimental conditions*

| Experiment | Adrenal donors | | | Substrate added to adrenals | Papilla donors | | | Incubation conditions of papilla |
|---|---|---|---|---|---|---|---|---|
| | No. of rats | mean body weight, g | Na-balance | | No. of rats | mean initial body weight | Na-balance | |
| I. steroid production in course of time | 24 | 137 | repletion | — | — | — | — | — |
| II. adrenal effects of papillary lipid extracts | 72 | 193 | repletion | papilla or muscle | 36 | 144 | loading | Extraction without previous incubation |
| III. adrenal effects of papillary lipids released into "antidiuretic" medium | 40 | 127 | repletion | papilla or muscle | 24 | 178 | depletion | 1200 mosm/l 95% $O_2$ + 5% $CO_2$ |
| IV. adrenal effects of papillary lipids released during hypoxic conditions | 40 | 186 | repletion | papilla or liver | 30 | 207 | depletion | 1200 mosm/l 5% $O_2$ + 5% $CO_2$ + + 90% $N_2$ |
| V. adrenal effects of papillary lipids released into "diuretic" medium | 40 | 172 | repletion | papilla or liver | 30 | 157 | depletion | 510 mosm/l 95% $O_2$ + 5% $CO_2$ |
| VI. adrenal effects of $PGE_2$ | 40 | 179 | repletion | $PGE_2$ 3 $\mu M$ | — | — | — | — |

*Effect on steroid production of lipid extract of papillary homogenates* (Exp. II)

Papillary lipids prevented the decline in both aldosterone and corticosterone production rate (Fig. 2). Production rate of both hormones were significantly higher after the addition of papillary lipids than in the presence of muscle lipids ($p < 0.01$; Table II).

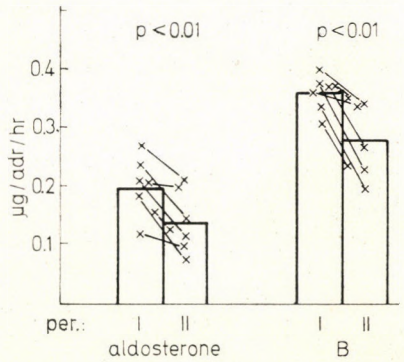


*Fig. 1.* Aldosterone and corticosterone (B) production rates of adrenals from dexamethasone-pretreated rats in two consecutive 2-hour periods

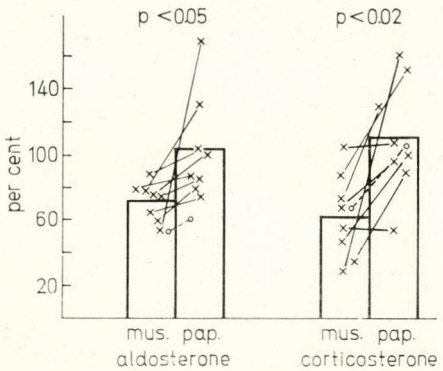


*Fig. 2.* Effect of lipid extracts from muscle and papillary homogenates on steroid production rate, expressed in per cent of the control (first) period. (Broken line represents the experiments in the 7th line of Table II.)

*Release of steroid stimulating lipids in vitro*

Exp. III: Papillary lipids extracted from the incubation medium of 1200 mosm/l had no effect on aldosterone production. Corticosterone production rate exhibited a decrease in course of time, despite the addition of either muscle or papillary lipids (Table III). The decline in corticosterone output was, however, significantly smaller ($p < 0.05$) in the presence of papillary lipids.

**Table II**

*Effect of lipids of muscle and papillary homogenates on aldosterone and corticosterone production rate in vitro**

| Muscle lipids | | | | | Papillary lipids | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Average adrenal weight, mg/adr. | Aldosterone | | Corticosterone | | Average adrenal weight, mg/adr. | Aldosterone | | Corticosterone | |
| | first period (A) | second period (B) | first period (C) | second period (D) | | first period (E) | second period (F) | first period (G) | second period (H) |
| 19.8 | 238 | 181 | 363 | 200 | 19.8 | 205 | 204 | 387 | 204 |
| 21.0 | 225 | 198 | 231 | 156 | 21.1 | 215 | 219 | 406 | 388 |
| 20.8 | 369 | 288 | 206 | 94 | 21.3 | 313 | 406 | 113 | 113 |
| 22.0 | 106 | 69 | 606 | 169 | 20.3 | 169 | 125 | 156 | 250 |
| 19.6 | 81 | 44 | 219 | 75 | 18.6 | 63 | 106 | 200 | 175 |
| 21.1 | 331 | 244 | 350 | 369 | 20.8 | 312 | 266 | 463 | 494 |
| 19.3** | 350** | 181** | 506** | 338** | 18.8** | 200** | 119** | 325** | 344** |
| 19.0 | 300 | 181 | 506 | 368 | 19.5 | 272 | 216 | 437 | 562 |
| 19.3 | 259 | 205 | 356 | 312 | 19.9 | 284 | 243 | 390 | 593 |
| mean 20.3 | 239 | 176 | 355 | 218 | 20.2 | 229 | 223 | 319 | 341 |
| S.E. 0.37 | 36 | 29 | 50 | 42 | 0.33 | 30 | 32 | 50 | 67 |

Significance: A *vs.* B: $p < 0.01$; B *vs.* F: $p < 0.01$; C *vs.* D: $p < 0.001$; D *vs.* H: $p < 0.01$

* Steroid values expressed in ng/adr/hr. Substrate concentration: 18.2 (range: 17.3—19.0) mg of extracted muscle or papilla, per ml of adrenal incubation medium

** Due to unusual differences between control aldosterone values this line has been omitted from statistical calculations

**Table III**

*Effect of lipids released from incubated muscle and papilla on aldosterone and corticosterone production rate in vitro. (Papilla incubated in a medium of 1200 mosm/l.)**

| Muscle lipids | | | | | Papillary lipids | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Average adrenal weight, mg/adr. | Aldosterone | | Corticosterone | | Average adrenal weight, mg/adr. | Aldosterone | | Corticosterone | |
| | first period (A) | second period (B) | first period (C) | second period (D) | | first period (E) | second period (F) | first period (G) | second period (H) |
| 15.6 | 172 | 144 | 419 | 263 | 14.9 | 166 | 125 | 419 | 319 |
| 15.1 | 200 | 190 | 456 | 281 | 14.8 | 150 | 113 | 356 | 275 |
| 16.5 | 241 | 200 | 331 | 275 | 16.0** | 204 | 169** | 282 | 259** |
| 15.0 | 150 | 91 | 369 | 313 | 14.6 | 91 | 47 | 313 | 294 |
| 15.1 | 97 | 38 | 231 | 128 | 15.9 | 119 | 47 | 275 | 263 |
| mean 15.5 | 172 | 132 | 361 | 252 | 15.2 | 146 | 100 | 329 | 282 |
| S.E. 0.24 | 24 | 31 | 39 | 33 | 0.29 | 19 | 24 | 27 | 12 |

Significance: A *vs.* B: $p < 0.02$; E *vs.* F: $p < 0.01$; C *vs.* D: $p < 0.02$; G *vs* H: $p < 0.1$

* Steroid values expressed in ng/adr/hr. Substrate concentration: 13.0 (range: 9.9—13.9) mg of incubated muscle and 15.1 (range: 10.7—17.0) mg of incubated papilla slices per ml of adrenal incubation medium

** Adrenals were incubated in 5 ml medium in the 2nd period

Exp. IV: When papillae were incubated in the previous medium under hypoxic conditions, the lipids released into the medium had no effect on steroid production (Table IV).

Exp. V: Papillary lipids released into the incubation medium of 510 mosm/l failed to abolish the fall in aldosterone production. However, in four of five cases the decrease was smaller than on the addition of lipids released from liver slices into KRB-solution. Corticosterone production rate increased to 120% ($p < 0.2$) after the addition of papillary lipids in the 2nd period, while it decreased by 22% in the presence of liver lipids. The difference between the corresponding incubates was significant ($p < 0.01$; Table V and Fig. 3).

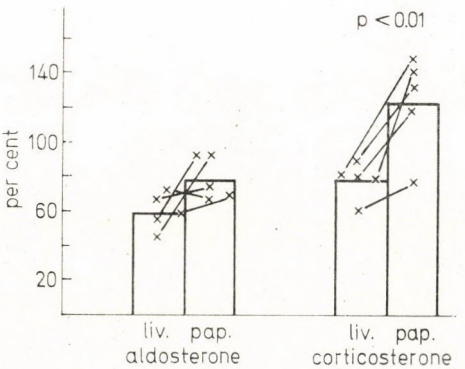


*Fig. 3.* Effect of lipids released from liver and papillary incubates on steroid production rate, expressed in per cent of the control period

**Table IV**

*Effect of lipids released from incubated liver and papilla on aldosterone and corticosterone production rate in vitro. (Papilla incubated in a medium of 1200 mosm/l, in 5% $O_2$ + 5% $CO_2$ + 90% $N_2$ atmosphere.)**

| | Liver lipids | | | | | Papillary lipids | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Average adrenal weight, mg/adr. | Aldosterone | | Corticosterone | | Average adrenal weight, mg/adr. | Aldosterone | | Corticosterone | |
| | | first period (A) | second period (B) | first period (C) | second period (D) | | first period (E) | second period (F) | first period (G) | second period (H) |
| | 20.6 | 381 | 281 | 463 | 306 | 20.6 | 312 | 281 | 444 | 388 |
| | 20.1 | — | 200 | 419 | 338 | 19.5 | 256 | 206 | 444 | 319 |
| | 20.1 | 300 | 231 | 338 | 325 | 20.1 | 300 | 222 | 338 | 313 |
| | 19.6 | 294 | 238 | 394 | 306 | 20.0 | 244 | 200 | 318 | 356 |
| | 20.5 | 294 | 238 | 319 | 369 | 20.4 | 313 | 250 | 500 | — |
| mean | 20.2 | 317 | 238 | 387 | 329 | 20.1 | 285 | 232 | 409 | 344 |
| n | 5 | 4 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 4 |
| S.E. | 0.17 | 21 | 13 | 26 | 19 | 0.19 | 15 | 15 | 35 | 14 |

Significances: A *vs.* B: $p < 0.01$; E *vs.* F: $p < 0.01$; C *vs.* D: $p < 0.2$; G *vs.* H: $p < 0.3$

* Steroid values expressed in ng/adr/hr. Substrate concentration: 25.3 mg of incubated tissue per ml of adrenal incubation medium

**Table V**

*Effect of lipids released from incubated liver and papilla on aldosterone and corticosterone production rate in vitro. (Papilla incubated in a medium of 510 mosm/l.)**

| Liver lipids | | | | | Papillary lipids | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Average adrenal weight, mg/adr. | Aldosterone | | Corticosterone | | Average adrenal weight, mg/adr. | Aldosterone | | Corticosterone | |
| | first period (A) | second period (B) | first period (C) | second period (D) | | first period (E) | second period (F) | first period (G) | second period (H) |
| 19.5 | 263 | 175 | 344 | 275 | 19.5 | 232 | 169 | 344 | 406 |
| 20.5 | 275 | 125 | 281 | 250 | 21.6 | 213 | 194 | 319 | 419 |
| 21.8 | 263 | 188 | 366 | 219 | 20.5 | 241 | 157 | 369 | 281 |
| 20.0 | 194 | 113 | 244 | 200 | 19.6 | 169 | 116 | 231 | 344 |
| 18.8 | 169 | 94 | 238 | 188 | 18.8 | 234 | 213 | 256 | 363 |
| mean 20.1 | 233 | 139 | 295 | 226 | 20.0 | 217 | 170 | 304 | 363 |
| S.E. 0.51 | 21 | 18 | 26 | 16 | 0.48 | 13 | 17 | 26 | 25 |

Significances: A *vs.* B: $p < 0.01$; E *vs.* F: $p < 0.02$; C *vs.* D: $p < 0.05$; G *vs.* H: $p < 0.2$; D *vs.* H: $p < 0.01$

* Steroid values expressed in ng/adr/hr. Substrate concentration: 26.6 mg of incubated tissue per ml of adrenal incubation medium

*Effect of prostaglandin $E_2$ on steroid production* (Exp. VI)

Second-period aldosterone production rate was slightly higher in the presence of $PGE_2$ (3 $\mu M$) than without it ($p < 0.02$). $PGE_2$ increased corticosterone production rate by 56% as compared to the first period ($p < 0.01$), the difference between outputs in the 2nd period being highly significant ($p \ll 0.001$; Table VI).

## Discussion

The interstitial cells in the renal papilla display an ultrastructure of secretory character (cf. LATTA et al. 1967; MUEHRCKE et al. 1969). The number of the special cytoplasmic lipid droplets is higher after salt or water loading than after sodium restriction or dehydration (NISSEN 1968a, b; OSVALDO-DECIMA and LATTA 1969). Preliminary experiments (SPÄT and BOJESEN, unpublished data) suggested that these droplets contained an aldosterone stimulating substance and the increase in the number of lipid droplets after sodium repletion might reflect the inhibition of release of such an aldosteronotropic substance. For this reason, previous loading with NaCl had been applied with the purpose of increasing the tissue concentration of the hypothetical substance when papillary homogenates were used for extraction (Exp. II). On the other hand, sodium depletion had been carried out when papillae were

**Table VI**

*Effect of $PGE_2$ on aldosterone and corticosterone production rate in vitro**

| | – | | | | $PGE_2$ | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Aldosterone | | Corticosterone | | Aldosterone | | Corticosterone | |
| | Average adrenal weight, mg/adr. | first period (A) | second period (B) | first period (C) | second period (D) | Average adrenal weight, mg/adr. | first period (E) | second period (F) | first period (G) | second period (H) |
| | 18.6 | 281 | 150 | 413 | 275 | 21.4 | 250 | 169 | 369 | 488 |
| | 19.9 | 269 | 163 | 356 | 325 | 19.9 | 216 | 181 | 278 | 526 |
| | 20.0 | 275 | 206 | 331 | 294 | 18.1 | 244 | 225 | 338 | 513 |
| | 18.1 | 225 | 206 | 300 | 250 | 19.3 | 200 | 219 | 325 | 450 |
| | 17.0 | 147 | 119 | 313 | 206 | 17.0 | 119 | 163 | 275 | 469 |
| mean | 18.7 | 239 | 169 | 343 | 270 | 19.1 | 206 | 191 | 317 | 489 |
| S.E. | 0.56 | 25 | 17 | 20 | 20 | 0.75 | 22 | 11 | 18 | 15 |

Significance: A *vs.* B: $p < 0.05$; B *vs.* F: $p < 0.02$; C *vs.* D: $p < 0.05$; G *vs.* H: $p < 0.01$; D *vs.* H: $p \ll 0.001$

* Steroid values expressed in ng/adr/hr. Concentration of $PGE_2$ : 3 $\mu M$ (= 1.05 $\mu$g/ml)

incubated (Exps III—V), since an augmented lipid synthesis prior to decapitation was preferred to the accumulation of lipids.

Adrenal glands from dexamethasone-pretreated rats synthetized significantly less steroid in the third and fourth hour of incubation than in the first 2 hours (Fig. 1). The decline in the hormone production rate in vitro as a function of time has been observed by several authors, examining adrenals from either intact or hypophysectomized rats (cf. TAIT et al. 1970). Therefore, any substance preventing this spontaneous decay was regarded as stimulatory.

Production of aldosterone and corticosterone in adrenal incubates was significantly higher in the presence of papillary extract than of muscle lipids (Exp. II). Adrenal glands from rats without steroid pretreatment also produced more aldosterone and corticosterone when papillary extract had been added to the solvent, as demonstrated by our preliminary experiments applying the double isotopic dilution method for aldosterone and corticosterone determination (BOJESEN and SPÄT, unpublished observations). These data suggest that the observed difference in the present experiments was due to the effect of papillary lipids.

For a further clarification of the steroidogenic effect, the release of the respective substance from the papilla had to be demonstrated. This problem was approached by incubating papillae of sodium depleted rats and by testing the adrenal effects of lipids extracted from the incubation medium. Since the renal papilla greatly differs from other tissues in ionic composition and this is highly dependent on the diuretic state, papillary slices were incubated in two kinds of medium. The first one, of 1200 mosm/l, corresponded in ionic compo-

sition to the papillae of hydropenic rats (Exp. III). Release of any aldosteronotropic lipid into this medium could not be detected. Nevertheless, lipids extracted from this medium reduced the decline in corticosterone production rate, as compared to controls (Table III).

Experiments using various methods have pointed to the low oxygen tension in the papilla in situ, as compared to other tissues (RENNIE et al. 1958; KRAMER et al. 1960, 1961; ULLRICH et al. 1961; LEONHARDT and LANDES 1963; AUKLAND 1964). Anaerobic metabolism is of great importance, especially in the interstitial cells (GYÖRGY et al. 1928; DICKENS and WEIL-MALHERBE 1936; STERNBERG et al. 1956; KEAN et al. 1961, 1962; LEE et al. 1962; BERNANKE and EPSTEIN 1965; GLOOR and NEIDITSCH-HALFF 1965; NISSEN, personal communication). With this in view, papillae were incubated under hypoxic conditions (Exp. IV), but the released lipids failed to affect steroid synthesis (Table IV).

Variations in osmotic concentration between 300 and 1200 mosm/l are also influencing the metabolic processes in the papilla (LEE et al. 1962; KEAN et al. 1962; BERNANKE and EPSTEIN 1965). For this reason, the incubaton medium of 1200 mosm/l was replaced by one of 510 mosm/l, which approximated the composition of papillae in rats in a moderately diuretic state. In four of five cases the reduction of aldosterone production rate was smaller in the presence of lipids released from papillae than in the presence of control liver lipids, albeit the difference was not significant statistically. However, corticosterone output was significantly stimulated by the papillary lipids.

The class of prostaglandins consists of hormonal compounds of ubiquiter occurrence in the mammalian organs. According to literary data, the lipid extracts of the renal medulla probably contain prostaglandin $E_2$, $A_2$ (medullin), and $F_{2\alpha}$, although $PGA_2$ — at least in part — may be an artefact of isolation (STRONG et al. 1966; LEE et al. 1967; DANIELS et al. 1967). Partially purified lipid extracts of medullary homogenates displayed a $PGE_2$ and $PGA_2$-like sustained vasodepressor effect, when injected to vagotomized pentolinium-treated rats (SPÄT, unpublished data). Therefore, in subsequent experiments we have investigated the adrenal effects of synthetic $PGE_2$.

Prostaglandin $E_2$ at a concentration of 3 $\mu M$, slightly but significantly stimulated aldosterone and highly significantly corticosterone production rate (Table VI). In previous experiments a superfusion of $PGE_2$ (3—6 $\mu M$, 0.4 ml/min) failed to increase aldosterone output of the adrenals of sodium-depleted rats, irrespective whether or not ACTH was added (1.25 $\mu$g/ml; BOJESEN and SPÄT, unpublished data). In the present experiments, adrenals were obtained from sodium-repleted, dexamethasone-pretreated rats, and this may greatly account for the differences in the results.

We are not aware of any paper dealing with the effect of prostaglandins on aldosterone synthesis. Our results concerning their effect on corticosterone

production are in agreement with the data of FLACK et al. (1969), according to which a superfusion of $PGE_2$ and, less effectively, of $PGE_1$ and $PGF_{2\alpha}$ (1.4 to 2.8 $\mu M$) increased corticosterone output from the decapsulated adrenals of hypophysectomized rats. In other experiments, $PGE_1$ was effective in vivo only in the presence of an intact pituitary (PENG et al. 1970), and failed to augment corticosterone production in vitro (BUTCHER 1968; RAMWELL 1968). $PGA_1$ and $PGF_{2\alpha}$ also appeared ineffective in vivo (PENG et al. 1970). On the basis of these data, the steroidogenic effect of our papillary extract may be attributed to $PGE_2$ in the first place, if to a prostaglandin at all.

The question arises whether $PGE_2$ formed in the kidney may play any role in the regulation of corticosteroid hormones. The adrenals indeed extract and accumulate $PGE_1$ (SAMUELSSON 1964). Considering, however, that the pulmonary tissue efficiently extracts $PGE_2$ from the circulation (FERREIRA and VANE 1967) and metabolizes 15-hydroxyprostaglandins in vitro (ÄNGGÅRD and SAMUELSSON 1966), the role of prostaglandins of renal origin in the regulation of adrenocortical function is questionable.

SHAW and RAMWELL (1967) demonstrated that ACTH promoted the formation of $PGE_2$ and $PGF_{2\alpha}$ by rat adrenal homogenates. This finding together with the present ones allow to assume that prostaglandin $E_2$ — either as a regulator or as a transmitter substance — plays a role in the regulation of corticosteroid biosynthesis.

If the stimulation of steroid output by papillary lipids was really due to prostaglandins, the prostaglandin dehydrogenase activity of the kidney (NISSEN and ANDERSEN 1968) may well account for the smaller steroidogenic effect of extracts from papillary incubates as compared to that of papillary homogenates. Nevertheless, at this moment it cannot be ascertained whether the aldosterone stimulating factor in papillary extracts was $PGE_2$ or some other substance.

## Acknowledgement

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# COMPENSATORY HYPERTROPHY OF ADRENAL, OVARY AND THYROID GLANDS IN OBESE RATS CAUSED BY VENTROMEDIAL HYPOTHALAMIC LESIONS

By

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Compensatory hypertrophy of adrenal, ovary and thyroid glands was studied in female rats with midline hypothalamic lesions at the level of the ventromedial nucleus. Unilateral removal of peripheral target organs and placements of electrolytic lesions were made in one stage of operation. Food and water intake as well as gain of body weight showed a marked increase after hypothalamic lesions. In comparison to the degree of compensatory hypertrophy in sham-operated rats, there was no difference in compensatory hypertrophy in the rats with hypothalamic lesions showing characteristic obesity. Pituitary weight was slightly reduced in obese rats. It was concluded that pituitary functions controlled by feedback mechanisms of the peripheral hormones are unimpaired in hypothalamic obese rats.

Data concerning the changes in the function of the pituitary–target organs following ventromedial hypothalamic lesions associated with an increase of food intake and with obesity, are controversial. Since the pioneer observation of HETHERINGTON and RANSON (1942) that hypophysectomy did not prevent the obesity induced by ventromedial hypothalamic lesions, some data have suggested the role of pituitary function in the development of the syndrome. Thus, REDDING et al. (1966) reported that gold-thioglucose treated mice did not gain weight more rapidly than only hypophysectomized mice and concluded that the presence of the pituitary was necessary for eliciting body weight changes associated with ventromedial hypothalamic lesions. On the other hand, there are data to indicate that thyroid hypofunction subsequent to ventromedial hypothalamic lesions would be a causal factor in the development of obesity (MESS 1951). Other authors suggested a sex difference in hyperphagia and increase in body weight following ventromedial hypothalamic lesions and found a lack of obesity in male rats with such hypothalamic damage (see VALENSTEIN et al. 1967). Again others reported an increase of the plasma growth hormone and prolactin concentration in rats with ventromedial hypothalamic lesions or gold-thioglucose treatment (FROHMAN and BERNARDIS 1968; BROWNING et al. 1965).

In the present investigation we have studied the effect of ventromedial hypothalamic lesions associated with obesity on the compensatory hypertrophy of three endocrine glands in female rats.

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## Methods

A total of 22 female albino rats was used in the study. All surgical procedures were carried out under hexobarbital anaesthesia. Bilateral hypothalamic lesions were induced by anodal polarization with 1.0 mA current intensity for 10 seconds. Unilateral removal of the thyroid and adrenal glands as well as of the ovary was performed during the same operation. A group of rats with an electrode inserted in the ventromedial hypothalamus without electrolytic lesion served as sham-operated controls to study the compensatory hypertrophy of endocrine glands. The animals were killed by decapitation on the 14th postoperative day, then the endocrine glands were weighed with 0.2 mg accuracy on a torsion balance. Food and water intake as well as body weight were recorded daily during the 14-day postoperative period.

At the end of the experiments the brain was fixed in 10% formol solution and the localization of lesions was checked in 100 micron thick frozen sections.

## Results

Symmetrical bilateral lesions were located in the vicinity of the third ventricle at the level of the ventromedial nucleus which damaged in a different degree the complexity of the nucleus (see Fig. 1). No lesions were found which had destroyed the median eminence region or extended in the anterior hypothalamic area.

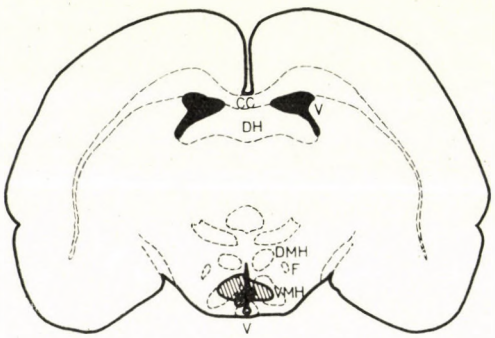


*Fig. 1.* Site of electrolytic lesions destroying the ventromedial nucleus and the periventricular area and inducing obesity. Smallest and maximal extents of the lesions are indicated by the shadowed area. Abbreviations: CC — corpus callosum; DH — dorsal hippocampus; DMH — n. hypoth. dorsalis medialis; F — fornix; V — ventricle; VMH — n. hypoth. ventromedialis

In contrast to the sham-operated rats which showed a slight decrease in food intake and body weight on the first postoperative days, rats with ventromedial hypothalamic lesions exhibited a typical overeating phase which was associated with a marked increase in body weight. A significant difference in food and water intake as well as in gain of body weight between the two groups was observed during the 14 postoperative days (Figs 2 and 3).

Compensatory hypertrophy of the three endocrine glands was statistically significant in both the sham-operated and the lesioned groups. There was no difference in the degree of compensatory hypertrophy between the two

groups (Fig. 4). Also, a combined index of the compensatory hypertrophy of the three endocrine glands which was plotted on the gain of body weight during the postoperative period failed to indicate a functional relation between the

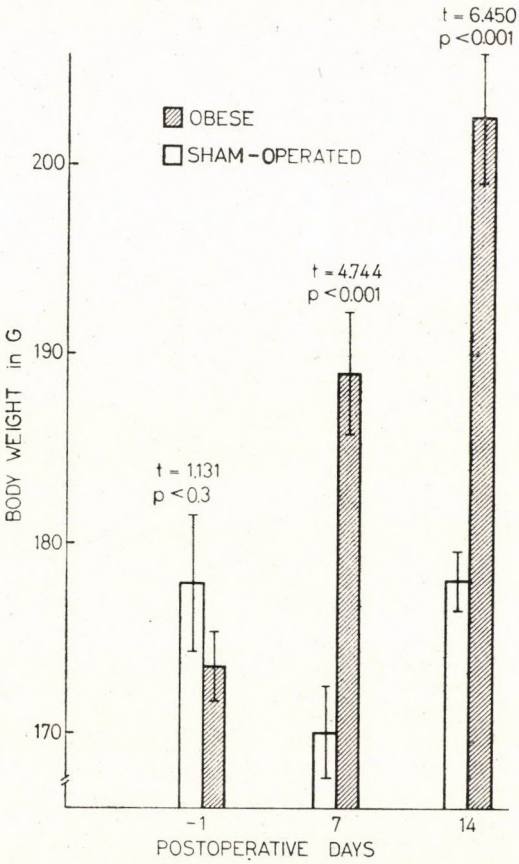


*Fig. 2.* Body weight of obese and sham-operated rats during 14 postoperative days

degree of compensatory hypertrophy and of obesity (see Fig. 5). However, despite the lack of an impaired compensatory hypertrophy of the endocrine glands in hypothalamic obese rats, pituitary weight was significantly less in the obese rats than in the sham-operated ones (Fig. 4).

## Discussion

The present findings have confirmed the view that the hyperphagia induced by ventromedial hypothalamic lesions may be regarded as result from the impairment of the nervous organization involved in the control of feeding

behaviour. Participation of pituitary function in such events seems to be unlikely. Considering the fact that after unilateral removal of three endocrine glands a normal degree of compensatory hypertrophy ensued, this capacity of pituitary function in obese rats excludes the possibility that an altered secre-

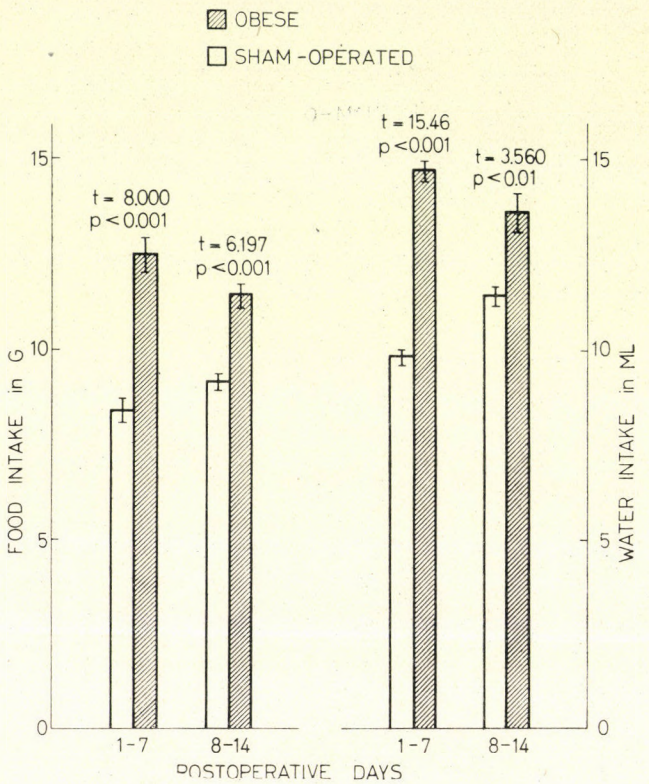


*Fig. 3.* Food and water intake of obese and sham-operated rats during 14 postoperative days

tion of pituitary tropic hormones might be involved in the preliminary phase of hyperphagia characterized as overeating by TEPPERMAN et al. (1943). The involvement in hypothalamic obesity of humoral factors appears also unlikely, at least in the light of the feedback regulation of pituitary function demonstrated by the compensatory hypertrophy of three endocrine glands. The present findings confirmed the observation of HERVEY (1959) who united young rats parabiotically and after lesioning the ventromedial hypothalamus of one partner found no hyperphagia or an excess gain in body weight in the other partner.

The present data indicate that the pituitary functions involved in the control of thyroid, adrenal and gonadal functions remained unimpaired after

ventromedial hypothalamic lesions which were followed by obesity. Small lesions in the vicinity of the third ventricle and damaging the ventromedial nucleus only partially seemed to suffice to induce hyperphagia and obesity without interfering with the control of the hypophysiotropic area. There are

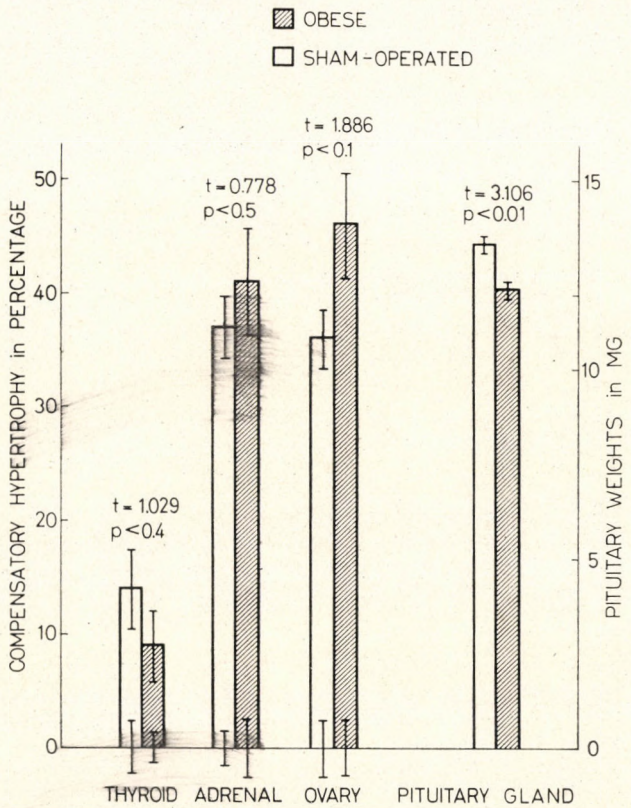


*Fig. 4.* Percentual changes in the weight of the thyroid, adrenal glands and ovary during compensatory hypertrophy in obese and sham-operated rats. Standard error of the initial mean endocrine weights is indicated on baseline. Columns on the right side are showing the changes in pituitary weight

observations suggesting a hypofunction of the thyroid or gonadal hormone secreting elements at the pituitary–target organ levels. Such changes may be attributed to differences in the degree of damages extending to a greater territory of the hypophyseotropic function of the ventromedial hypothalamic region. Thus, fairly large lesions in the ventromedial hypothalamus are necessary to interfere with the secretion of tropic hormones (e.g. for TSH, see SOULARIAC et al. 1954; GANONG and HUME 1955; REICHLIN 1960; etc.). Similarly, lesions extending over the larger part of the anterior hypothalamus may be followed by various degrees of impairment of gonadotropin and adrenocortico-

tropin secretion and associated with hyperphagia and obesity (HILLARP 1949; GREER 1953; KOBAYASHI et al. 1959; KORDON and BACHRACH 1959; etc.). On the other hand, the decrease in pituitary weight in the obese rats indicated that in the absence of any obvious change in the feedback adjusted control of pituitary function, lesions of the ventromedial hypothalamus had impaired the hypothalamo-hypophyseal connections.

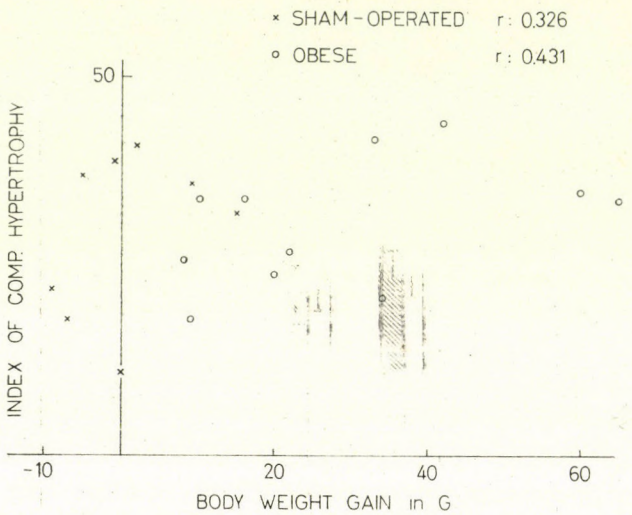


*Fig. 5.* Combined index expressing the mean percentual changes in the weight of the ovary, thyroid and adrenal glands during compensatory hypertrophy, plotted on gain of body weight of obese and sham-operated rats. Correlation coefficients indicate no relation between the two parameters

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# EFFECT OF GONADOTROPHINS ON EXTINCTION OF AN AVOIDANCE CONDITIONED REFLEX AND EXPLORATORY BEHAVIOUR IN THE RAT

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The effect of human chorionic gonadotrophin (HCG) and pregnant mare serum gonadotrophin on the extinction of conditioned avoidance responses, intertrial responses during extinction and exploratory behaviour has been studied in rats. HCG facilitated the extinction of avoidance conditioned reflex, decreased the intertrial responses during extinction and exploratory behaviour.

Pregnant mare serum gonadotrophin had no significant effect on any of the above mentioned behavioral parameters.

Certain changes in endocrine function are accompanied by different psychological disturbances and functional disorders of the autonomic nervous system. Many of these symptoms develop in early pregnancy or in the menopause. In both cases an increased gonadotrophic hormone secretion is a characteristic change. In the first case chorionic gonadotrophin, in the latter one pituitary gonadotrophin secretion is higher.

In the last few years a number of data has accumulated concerning the effect of peptides on neural function. It has been shown that ACTH and ACTH fragments, lysine vasopressin, and melanocyte stimulating hormone can alter the avoidance behaviour of the rat (MURPHY and MILLER 1955; LEVINE and JONES 1965; DE WIED 1965, 1966; DE WIED and BOHUS 1966; KORÁNYI and ENDRŐCZI 1967), which effect is not mediated by the adrenals (MILLER and OGAWA 1962).

Since human data showed a relation between a high gonadotrophin level and the neural symptoms, it seemed worthwhile to examine the effect of exogenous gonadotrophic hormones on neural function in animal experiments. In the present study the effect of human chorionic gonadotrophin and pregnant mare serum gonadotrophin has been investigated on two behavioral patterns, on avoidance behaviour and exploratory behaviour in the rat.

## Methods

Seventy-one white R-Amsterdam inbred adult female rats weighing 130—150 g were used in the experiments. In order to avoid the gonad stimulating effect of gonadotrophins, three weeks prior to beginning of conditioning the animals were ovariectomized.

Avoidance conditioning was performed according to our published earlier methods (TELEGDY et al. 1968) in a box measuring 45 cm by 25 cm with a grid floor through which electric shock (70 V, 1.0 mA, A.C. of 50 c/s) could be applied. To avoid the shock the animals had to jump onto a bench placed 6 cm above the grid on the wall of the box. The conditioning stimulus (CS) was the sound of a buzzer. The stimulus was presented for 5 seconds before the shock (unconditioned stimulus, US) had been delivered through the grid, switching on the current every other second. When the animals had avoided the shock by jumping upon the bench, the US and CS were terminated. After ten seconds the animals were gently placed upon the grid. Ten conditioning trials were given every day with 60 seconds intertrial intervals until the animals had reached the criterion of a minimum 70% avoidance response during 4 consecutive days. Subsequently, an extinction trial was run for 21 days or until the animals had reached the 30% avoidance response for 3 consecutive days. During extinction, CS was terminated after 5 seconds and when the animals had not performed a response it was not followed by the US. During extinction, the intertrial interval of CS was 60, 15, 30, 45, 30, 45, 60 seconds.

During conditioning and extinction, the intertrial responses were also recorded.

Exploratory behaviour was tested in a rectangular open field for three consecutive days at the beginning of the conditioning trial and at the end of the extinction trial for 3 minutes in each instance (LÁT 1965). Results were expressed in mean stimulation rate obtained from the three days' average expressed per minute.

Gonadotrophin treatment was carried out with human chorionic gonadotrophin (HCG "Choriogonin", Richter, Budapest) 500 I.U./day/animal, and with pregnant mare serum gonadotrophin (PMS "Gestyl", Organon Oss) 100 I.U./day/animal from the first day of the extinction trial and given always after conditioning. For gonadotrophin treatment, the animals were distributed randomly into a gonadotrophin and a control group. The control group received the same volume of saline every day.

Statistical analysis was carried out by the Mann-Whitney test and Student's *t*-test.

## Results

Human chorionic gonadotrophin treatment significantly enhanced extinction ($p < 0.01$; Fig. 1). Intertrial activity during extinction was also significantly lower ($p < 0.01$) in the HCG-treated group than in the control group (Fig. 2).

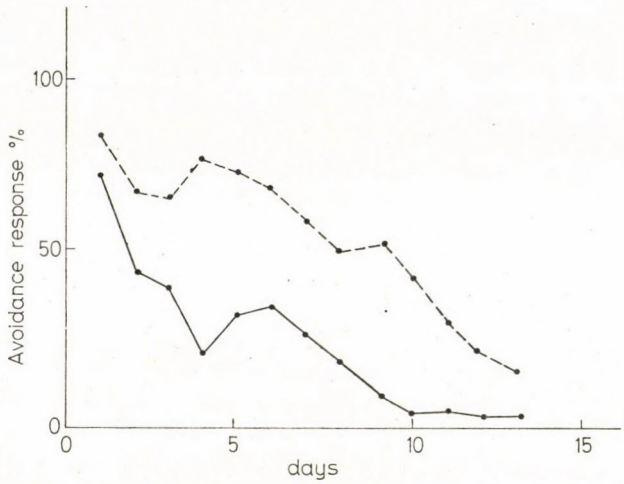


*Fig. 1.* Effect of HCG on extinction of conditioned avoidance reflex. Solid line: HCG-treated group (16); broken line: control group (15). In brackets, number of animals

The exploratory reaction in the human chorionic gonadotrophin-treated group was about 50% lower ($p < 0.01$) than in the control group (Fig. 3).

Pregnant mare serum gonadotrophin treatment caused a slight enhancement of extinction but the difference was not significant ($p > 0.05$). No difference could be observed in intertrial activity and exploratory reaction.

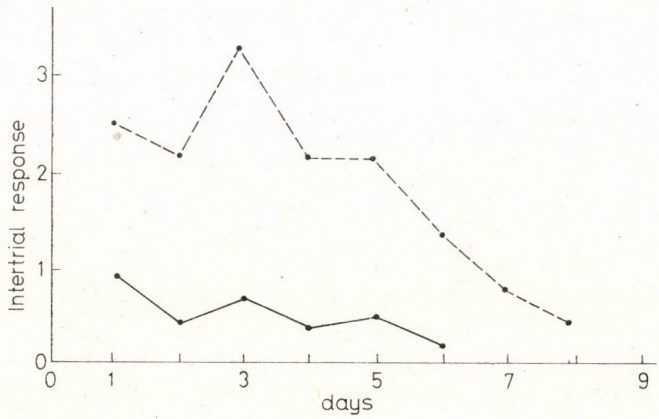


*Fig. 2.* Effect of HCG on average intertrial responses during extinction. Solid line: HCG-treated group; broken line: control group

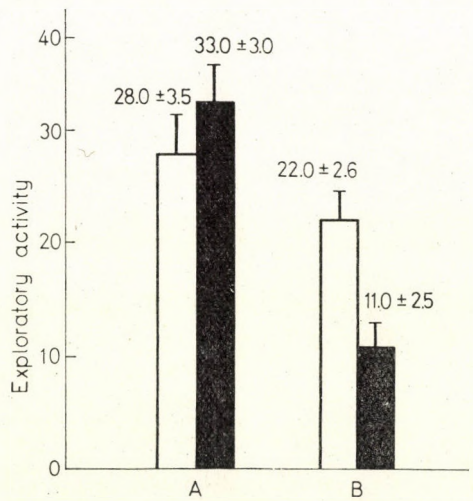


*Fig. 3.* Effect of HCG on exploratory behaviour in an open field. A: control group; B: HCG-treated group; white bar: before treatment; black bar: after treatment. Mean ± standard error

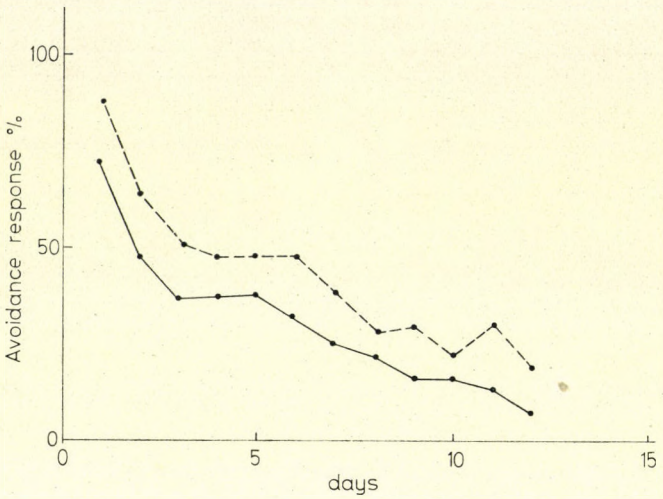


*Fig. 4.* Effect of PMS on extinction of conditioned avoidance reflex. Solid line: PMS-treated group (20); broken line: control group (20). In brackets, number of animals

## Discussion

Earlier we have shown (TELEGDY et al. 1968) that in adult rats castration does not influence avoidance behaviour, which means that the gonadotrophin hypersecretion caused by castration is not enough to bring about any change in the extinction of a conditioned avoidance response.

Human chorionic gonadotrophin in high dose (500 I.U. daily) significantly facilitated the extinction of the conditioned avoidance response, decreased the intertrial responses and exploratory behaviour. HCG in the rat has a LH-like activity, causing hyperovulation. PMS in this species is very similar biologically (c.f. WILSON and ZARROW 1962) although not identical immunologically (FLUX and LI 1965). In a dose of 100 I.U. daily it had no effect on the behavioral reaction studied. This may indicate that the phenomena induced by HCG are specific to HCG or, which is more likely, they are due to the higher doses applied. A small dose does not influence but a high one changes the activity of the neural system which leads to alterations of certain behavioral reactions.

The mechanism of action is not clearly understood. It has been shown that corticosteroids have a similar action; they facilitate the extinction of conditioned avoidance response and decrease intertrial activity (DE WIED 1966; BOHUS and ENDRŐCZI 1965). These changes can be considered an effect of decreased drive reduction. This concept is further supported by our findings in which the exploratory reaction was also decreased. Since these animals were

ovariectomized before conditioning, it is clear that the action of gonadotrophin is not mediated by sexual hormones of ovarian origin. In view of the similarity of the results obtained by adrenocortical hormones (BOHUS and ENDRŐCZI 1965; DE WIED 1966) the role of the adrenals has to be clarified. Clinical data do not indicate a stimulatory influence of HCG on adrenocortical secretion in humans (BATRINOS et al. 1962) and in early pregnancy corticoid secretion is not higher than in normal subjects (SCHÜLLER 1957).

It is obvious that HCG in the rat is a non-species specific gonadotrophin. Its administration in high doses elicited phenomena of which it could not be decided whether they were due to a physiological or rather to a pharmacological effect. However, in humans during the first part of pregnancy no other essential hormonal changes can be observed than a high HCG level which is often associated with emotional disturbances. In the menopause, too, a high gonadotrophin level associated with neural disturbances is characteristic. Our finding seems to indicate that gonadotrophin which in a certain dose is able to alter animal behaviour might be, at least partly, responsible for the ne ral symptoms observed in humans in such cases in which gonadotrophin secretion is high.

## Acknowledgement

We are indebted to Mrs. M. ÁDÁM for skilful technical assistance.

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# FURTHER DATA ON THE EFFECT OF HUMAN CHORIONIC GONADOTROPHIN ON AVOIDANCE AND EXPLORATORY BEHAVIOUR IN THE RAT

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The effect of human chorionic gonadotrophin (HCG) on acquisition, extinction of conditioned avoidance reflex, intertrial activity and exploratory behaviour has been studied in the rat.

HCG had no effect on acquisition of conditioned avoidance reflex and intertrial activity during acquisition. HCG treatment, applied from the first day of conditioning, significantly facilitated extinction, decreased intertrial activity and exploratory behaviour. The effect of HCG on behavioral patterns was not mediated by the gonads and adrenals.

Inactivation of the gonadotrophic potency of HCG induced a diminution of the effect on behavioral reactions.

It has been reported earlier (TELEGDY and RÓZSAHEGYI 1971) that human chorionic gonadotrophin (HCG) facilitates the extinction of avoidance conditioned reflex, decreases the intertrial responses during extinction and exploratory reaction of rats. There arose some further questions concerning the effect of HCG on behavioral reactions. One of these was how HCG influenced the acquisition of avoidance and whether there was any difference in HCG on extinction and exploratory behaviour if HCG treatment had started on the first day of avoidance conditioning. Since the effect of HCG observed by us was very similar to the corticosteroid action reported by others (DE WIED 1966; BOHUS and LISSÁK 1968) it became necessary to elucidate the effect of HCG on adrenal function when the treatment was given during avoidance conditioning. Finally, it was also unclear whether there was some correlation between the so-called "gonado-trophic" activity and the activity exerted on the above-mentioned behavioral patterns.

In the present study we have attempted to collect further information about these questions.

## Methods

In the experiments 56 white adult female R-Amsterdam rats were used. They were ovariectomized three weeks prior to conditioning in order to avoid the action of gonadotrophins mediated by the gonads.

Avoidance conditioning was performed according to our method (TELEGDY et al. 1968). For conditioning, a box measuring 45 cm by 25 cm with a grid floor through which electric current (70 V, 1.0 mA, A.C. of 50 c/s) could be applied. To avoid the shock, the animal had to jump on a bench on the wall of the box. The conditioning stimulus (CS), the light of a 40 W bulb, was given 5 sec prior to the unconditioned stimulus (US). If the animal did not jump onto

the bench in five seconds an electric shock was delivered through the grid for 10 sec or until the animal had performed the avoidance reflex. Ten conditioning trials were applied daily till the animal had reached the criterion of learning, a 70% avoidance response during four consecutive days.

Learning was evaluated as follows.

1. Appearance of conditioned response (ACR): number of trials making the animal to avoid the electric shock with or without reinforcement.
2. Conditioning trial (CT): number of trials necessary to reach the 70% avoidance response during four consecutive days.
3. Reinforcement trials (RT): number of reinforcements given in the conditioning period.
4. Time of reinforcement (TR): total duration of reinforcements in the conditioning period, expressed in seconds.

After reaching the criterion of learning, an extinction trial was run till the animals had reached the 30% avoidance response in three consecutive days. In the extinction trial, no unconditioned stimulus was used.

During avoidance conditioning, intertrial activity was also recorded.

The exploratory reaction was tested in an open field measuring the ambulance (crossing) rate at the beginning of conditioning and at the end of extinction during three consecutive days in each case for three minutes. From the total ambulance rate scored in each three days session an average was calculated and expressed per minute.

Human chorionic gonadotrophin (Choriogonin, Richter, Budapest) treatment was carried out using 500 I.U./day/animal according to the experimental schedule, given always after conditioning.

In order to study the effect of inactivated HCG on avoidance behaviour, HCG was inactivated by boiling for 60 minutes according to WIDE (1962). For treatment, an equivalent amount of 500 I.U. of inactivated HCG was given daily to each animal.

The effect of HCG on adrenocortical function was tested in vivo, estimating corticosterone secretion from the adrenal venous blood according to our earlier method (TELEGDY and ENDRŐCZI 1965).

The experiments were carried out in the following groups.

1. Effect of HCG treatment on acquisition, intertrial activity, extinction and open field behaviour. 500 I.U./day/animal of HCG was given from the first day of conditioning till the end of extinction (10 animals). The 10 control animals received the same volume of saline. At the end of the experiment, adrenal corticosterone secretion was measured in the gonadotrophin-treated and in the control group.
2. Effect of inactivated HCG on extinction, intertrial and exploratory behaviour. The equivalent amount of 500 I.U. inactivated HCG was given from the first day of the extinction trial to 19 animals. The control animals (17) received the same volume of saline.

Statistical evaluation was done by the Mann-Whitney test and Student's *t*-test.

## Results

HCG treatment had no effect on acquisition (Fig. 1). The detailed evaluation of learning showed no difference (Table I).

HCG treatment, carried out from the first day of conditioning, significantly facilitated extinction ($p < 0.01$; Fig. 2) as in our earlier study (TELEGDY and RÓZSAHEGYI 1971).

**Table I**

*Evaluation of the effect of HCG on learning of conditioned avoidance reflex*

| | ACR | CT | RT | TR/sec |
|---|---|---|---|---|
| Control | $12 \pm 2$* | $100 \pm 5$ | $47 \pm 9$ | $130 \pm 11$ |
| HCG-treated | $12 \pm 1$ | $97 \pm 9$ | $52 \pm 6$ | $147 \pm 15$ |

* Mean $\pm$ standard error

Intertrial activity did not differ significantly from the control group and was weaker during the extinction trial (Fig. 3). The exploratory reaction at the end of extinction was lower in the gonadotrophin-treated than in the control group ($p < 0.001$; Fig. 4).

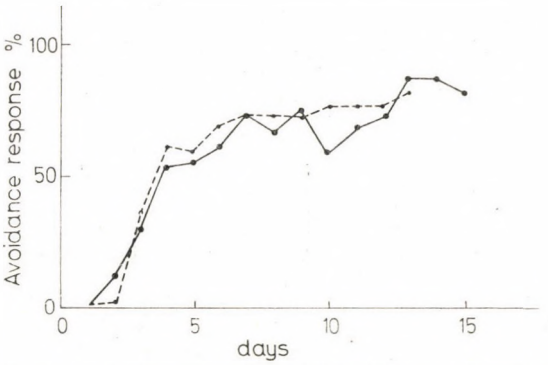


*Fig. 1.* Effect of HCG on acquisition of conditioned avoidance reflex. Solid line: HCG-treated group; dotted line: control group

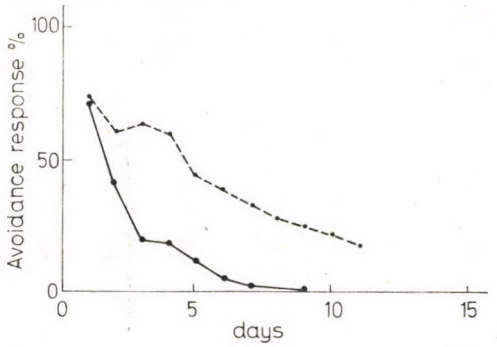


*Fig. 2.* Effect of HCG treatment on extinction of conditioned avoidance reflex. Solid line: HCG-treated group; dotted line: control group

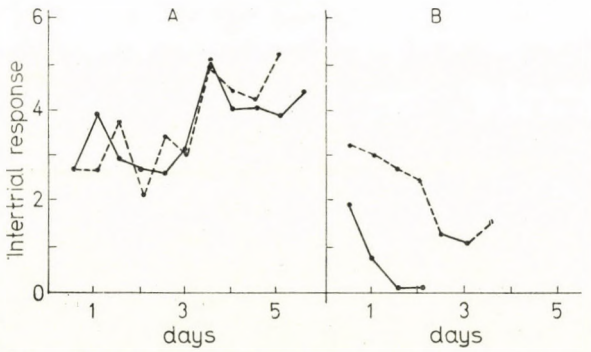


*Fig. 3.* Effect of HCG on intertrial responses during acquisition (A) and extinction (B) of conditioned avoidance reflex

Adrenocortical corticosterone secretion did not differ significantly from the control value (Fig. 5).

HCG, after inactivation, had no influence on extinction (Fig. 6), inter-trial responses (Fig. 7) and exploratory behaviour (Fig. 8).

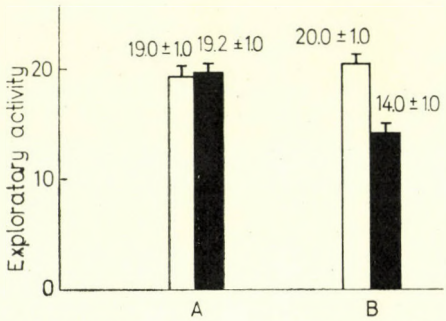


*Fig. 4.* Effect of HCG on exploratory behaviour in an open field. A: Control group; B: HCG-treated group. White bar: prior to treatment; black bar: after treatment. Mean ± standard error

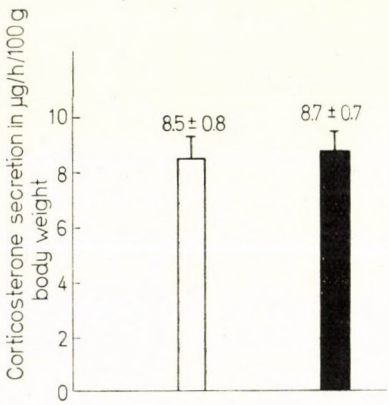


*Fig. 5.* Effect of HCG on corticosterone secretion. White bar: control group; black bar; HCG-treated group

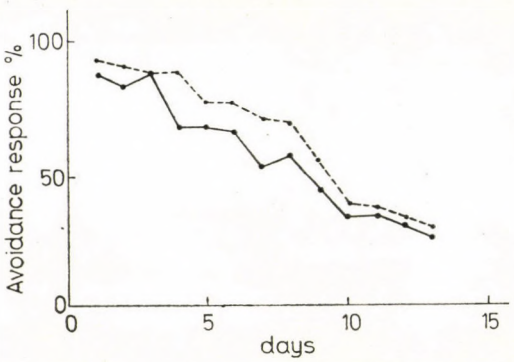


*Fig. 6.* Effect of inactivated HCG on extinction of conditioned avoidance reflex. Solid line: HCG-treated group; dotted line: control group

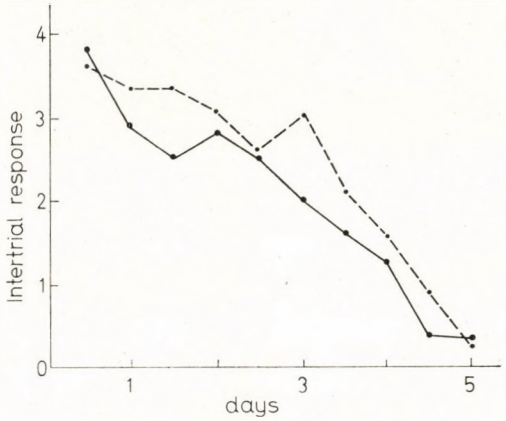


*Fig. 7.* Effect of inactivated HCG on intertrial responses during extinction. Solid line: HCG-treated group; dotted line: control group

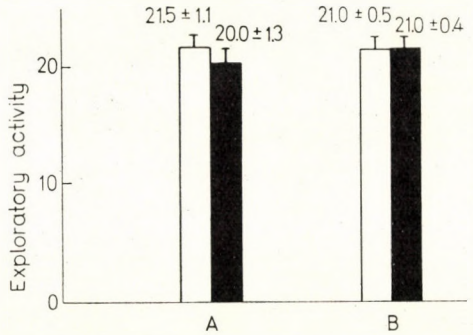


*Fig. 8.* Effect of inactivated HCG on exploratory behaviour in an open field. A: Control group; B: HCG-treated group; white bar: prior to treatment; black bar: after treatment. Mean ± standard error

## Discussion

The present results have confirmed our earlier findings (TELEGDY and RÓZSAHEGYI 1971). It seems that HCG has no effect on acquisition and intertrial activity during acquisition in a simple avoidance conditioning, but facilitates extinction. There was no difference in the result of HCG treatment when it was applied from the beginning of conditioning (present results) or from the beginning of extinction (TELEGDY and RÓZSAHEGYI 1971). The case was essentially similar for the exploratory reaction. The results obtained with HCG were roughly similar to those reported for corticosteroids (DE WIED 1966; BOHUS and LISSÁK 1968). Since HCG has no effect on corticosteroid secretion, the mediating role of the adrenals could also be ruled out.

After inactivation of the "gonadotrophic" potency of HCG, the effect on behavioral processes can also be diminished. This indicates that the structure of the HCG molecule responsible for the gonadotrophic activity might be responsible for the "neurotrophic" activity of HCG. Neither the nature of this so-called "neurotrophic" activity nor its mechanism of action is known. Further experiments have to be performed to elucidate this problem.

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# CURRENT–VOLTAGE RELATIONS OF THE GIANT NEURONE SOMA MEMBRANE OF LYMNAEA STAGNALIS

By

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Excitation processes of the membrane of giant neurones present in the central nervous system of *Lymnaea stagnalis* have been investigated by means of voltage clamp. The general picture of the ionic currents did not differ from that observed in other structures. On voltage clamping lasting for more than 1 second, rhythmically recurring signals could be observed superimposed on the outward current. These signals might have originated from distant parts of the membrane where the potential was not fixed.

When the preparation was placed into Na-free solution, the initial inward current ceased in most of the neurones. Currents originating from distant parts of the membrane ceased within a shorter time.

The effect of previous depolarization or hyperpolarization, further that of TEA on the ionic currents has also been studied. Polarization influenced both the inward and the outward current, TEA mainly the outward current.

Equilibrium potential of Na-current and K-current was determined from the voltage-ampere characteristics. The former did not differ from the peak action potential, while the latter was 23—25 mV more negative than the resting potential.

The soma of giant neurones of molluscs is a structure suitable for studying the mechanisms involved in the generation of action potential (OOMURA et al. 1961; GERASIMOV et al. 1964, 1965; KOSTYUK 1964, 1968; MEVES 1968; GEDULDIG and JUNGE 1968). The geometry of these neurones is such that with certain limitations an area of fixed potential can be produced on the soma (MAGURA 1967) and thus the voltage clamp method can be applied in studies of excitation processes of the membrane (HAGIWARA and SAITO 1959b; HAGIWARA et al. 1961; CHAMBERLAIN and KERKUT 1967, 1969; MAGURA and KRYSHTAL 1970).

Several authors have shown that the ionic mechanisms involved in action potential generation show certain species specificity in molluscs.

The present paper reports on some characteristics of the mechanisms responsible for action potential generation in the soma of the giant neurones of *Lymnaea stagnalis*. The voltage clamp method was applied and the nature of the ionic currents flowing through the membrane analyzed with the membrane potentials fixed at different levels.

## Methods

The experiments were performed on giant neurones of *Lymnaea stagnalis*, using mainly cells of the left parietal ganglion identified by YARMIZINA et al. (1968). The figures presented show the data obtained in these studies. Some giant neurones of the abdominal ganglion were

also investigated. The isolated peripharyngeal ganglion ring was placed into normal saline containing 60 m*M* NaCl, 2 m*M* KCl and 10 m*M* CaCl. When the effect of TEA was studied, part of the NaCl was changed for an osmotically equivalent quantity of TEA-Cl. In the Na-free solution saccharose was used instead of NaCl.

Two glass microelectrodes were inserted into the soma of the neurone. The first of these served for inducing changes in the membrane potential by electric current, the second for leading off the membrane potential. The electrodes were filled with 3 *M* KCl solution. The resistance of the polarizing electrode was less than 10 M$\Omega$. The apparatus used for voltage clamping was similar to that described by HAGIWARA and SAITO (1959a). Technical characteristics of the apparatus allowed to perform short clampings for 1 second and also long ones for several minutes.

Voltage clamp applied on the soma of giant neurones involves some difficulties due to the capacity of the membrane and to the high resistance of the polarizing microelectrode. Consequently, the capacitative current appearing at the initial phase when the membrane potential is changed would often obscure the first phase of the component of the ionic current. The time constant of this capacitative current is approximately $\frac{C \cdot R}{a}$ where C = cell membrane capacity, R = resistance of the polarizing electrode, a = amplification of the feed-back mechanism (HAGIWARA and SAITO 1959a).

If the soma is about 150 $\mu$ in diameter, the membrane capacity is approximately $10^{-2}$ nF. If the resistance of the polarizing electrode is 10 M$\Omega$ and amplification of the feed-back system 5000-fold, the time constant of the capacitative current is about 0.5 msec.

Conditions of producing areas of clamped voltage on the soma of giant neurones vary considerably from neurone to neurone depending on the geometry of the cell. Consequently, sometimes small periodic inward currents become superimposed on the late outward current. Most probably the activity of the axon is responsible for these small currents (see Fig. 3).

Considering the above, the method applied in these experiments seemed adequate for determining certain current–voltage relations, but unsuited for the exact quantitative presentation of the parameters of the excitable membrane as did HODGKIN et al. (1952) in their studies on the giant axon.

## Results

Components of the ionic current flowing through the membrane during voltage clamp, further the current–voltage function did not differ significantly from data obtained on other excitable structures (Figs 1 and 2). Most probably the Na-ion had the main role in carrying the initial inward current. However, it was often difficult to determine the carrier ions of this current. If Na was completely removed from the perfusing solution, the initial inward current often disappeared (Fig. 1B) but in some cases Na removal did not influence significantly the inward current. In these cases Na ions could have still remained in the area surrounding the cell or, else, the Ca ions may also have a role in carrying the inward current (GERASIMOV et al. 1964, 1965; GEDULDIG and JUNGE 1968).

Nerve excitation studies have shown that the axon responds more sensitively to the removal of extracellular Na, and in Na-free solution the axon stops to generate action potential within a few minutes. In the present voltage clamp studies, when the preparation was placed in normal solution, small periodic inward currents could be observed superimposed on the late outward current (see Methods). On the removal of Na from the solution these currents ceased (Fig. 3). Most probably this phenomenon was due to the fact that action potential generation stopped in the areas of the axon beyond the control of the experiment.

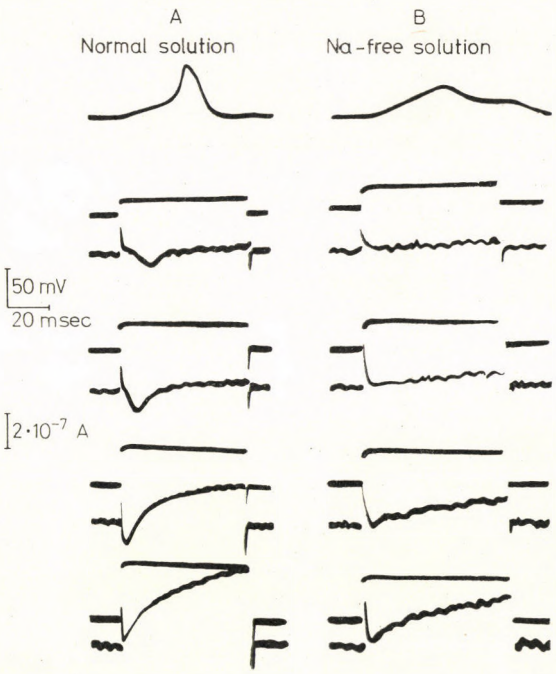


*Fig. 1.* Membrane currents during depolarizing impulses in normal and in Na-free solutions. Above: action potentials. Right: cessation of early inward current in Na-free solution

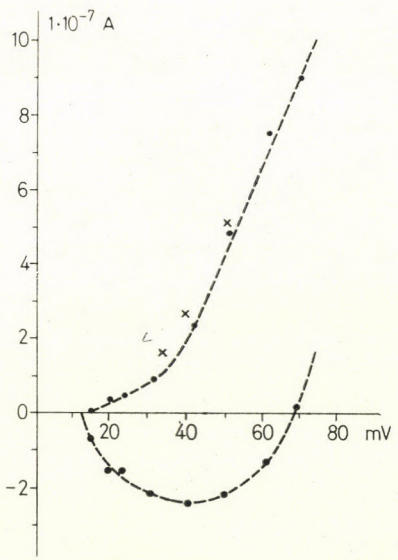


*Fig. 2.* Relation between diverted membrane potential, early inward current and outward current (black circles). Crosses indicate outward current in Na-free solution

The equilibrium potential of the initial inward current did not differ considerably from the maximum of action potential (see Fig. 1) Similar observations were made on the giant neurones of *Onchidium verruculatum* (HAGIWARA and SAITO 1959b) and on *Helix pomatia* (MAGURA and KRYSHTAL 1970).

The late outward current decreased in some cases when clamping lasted longer than 1 sec. HAGIWARA et al. (1961) obtained similar data on the nerve cell of *Onchidium verruculatum*, NAKAJIMA and KUSANO (1966) on the supramedullary neurones of *Spheroides maculatus*. The phenomenon could be inter-

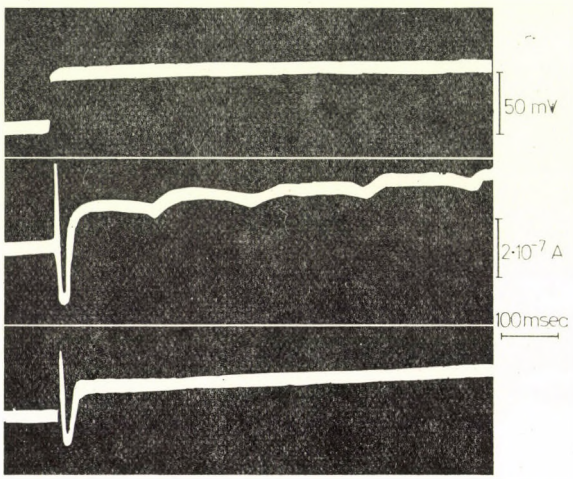


*Fig. 3.* Area-clamp insufficiency. Above: diverting of membrane potential. Below: membrane current in normal and Na-free solutions. (Records in Na-free solution were taken 5 min after perfusion.) Recurring small inward currents are seen superimposed on the much more pronounced outward current. The inward currents disappear in Na-free solution

preted as an inactivation of the K-mechanism. In subsequent experiments the membrane was depolarized or hyperpolarized for a certain time prior to the measurements (to be termed conditioning depolarization or hyperolarization in the following). Conditioning depolarization for more than 10—15 sec induced a marked decrease in the initial inward current, but decreased also the late outward current. This effect is demonstrated in Fig. 4 which shows voltage clamping at the same membrane potential level. Only the level of the starting membrane potential differs in the three records; in case *a)* it corresponds to the resting potential, in case *b)* to conditioning depolarization, in case *c)* to hyperpolarization.

Conditioning hyperpolarization induced a marked increase in the initial phase of the outward current. The same was observed by HAGIWARA et al. (1961) on the neurones of *Onchidium verruculatum*.

If voltage clamping was performed at a definite depolarization level and then the membrane potential was suddenly reversed to the resting level and

reclamped there, often an exponentially decreasing outward current appeared (Fig. 5). This phenomenon could be interpreted as a residue of the membrane's high K-conductivity. Thus, a tendency exists which is operative in approximating the K-equilibrium potential. The exponential drop reflects the decrease of the K-conductivity of the membrane towards the K-equilibrium potential. At the moment when the membrane potential is reversed to the resting level, the intensity of the given outward current is the function of the amplitude of the square impulse applied for fixation. The fact has to be reckoned with that

*Fig. 4.* Effect of conditioning polarization of membrane potential (15 sec) on membrane current under voltage clamp. The three curves were recorded from the same cell. Broken line indicates the level of resting potential

the maximum of current intensity is somewhat reduced owing to the capacitative artefact.

In Fig. 5 the intensity of the outward current is indicated by small triangles. The deviation of the fixed membrane potential from the resting potential is plotted on the ordinate. The points on the ordinate show the intensity of the outward current at the moment when the membrane potential has returned to the resting level. The straights connecting the triangles with the points represent the so-called instantaneous voltage–ampere function. The angle enclosed by the straights and the abscissa indicates the K-conductivity of the membrane. The points of intersection of the extended straights on the abscissa give approximately the value of the K-equilibrium potential. It can be seen in the figure that K-equilibrium in this case was 23—25 mV more negative than the resting potential. TEA added to the solution exerted a marked effect on the system of the somatic membrane of the neurones which is responsible for the so-called delayed rectification (HAGIWARA and SAITO 1959b). When TEA concentration of the solution reaches approximately 15 m*M*, the soma of the giant neurones generates action potentials that are delayed in time. The most expressed change can be observed on the descending phase where the impulse width of the spike is extended. Sometimes at the onset of the TEA effect the descending phase forms a plateau on which recurring potentials appear (Fig. 6B). In other cases spikes resembling the action potentials of the myocardium can be seen (Fig. 6C). The ascending phase of the action potential also changes under the effect of TEA. There is a decrease in the time constant of the rise. Thus, it may be assumed that

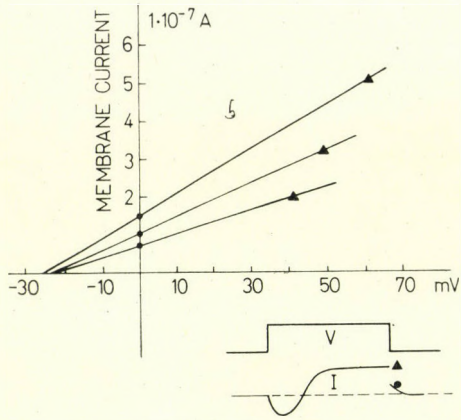


*Fig. 5.* Momentary current–voltage function. After the late outward current had reached a certain value, membrane potential was stabilized at a new level. This new level corresponds to the resting potential. The steepness of each of the straights gives the conductivity of the membrane at the moment indicated by the small triangles

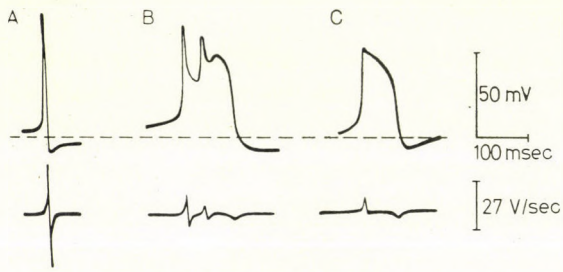


*Fig. 6.* Effect of tetraethyl ammonium on the action potential. Action potential A: in normal solution; B and C: in solution containing 15 m*M* TEA. Records below show the differentials of the potentials

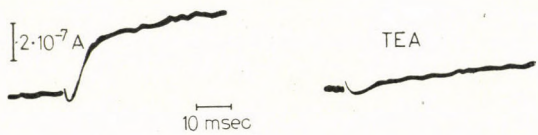


*Fig. 7.* Effect of TEA on membrane current under voltage clamp. Diverting of membrane potential on both records, 60 mV. Changes in inward and outward currents are seen. The curves were recorded from the same cell

TEA affects both the K-current and the Na-current transmitting systems, reducing the activity of both systems.

Under voltage clamp when the membrane potential is fixed at the depolarization level, TEA considerably decreases the late outward current. At the same time the early inward current decreases, though to a lesser degree (Fig. 7). Evaluating this latter effect it has to be considered that the quantity of Na-ions in the solution containing TEA is below the physiological level.

## Discussion

Ionic current flow through a given membrane could not be assessed quantitatively from the experimental data, as the extent of the area on which the membrane potential of the giant neurones have been fixed could not be defined exactly. Still, some conclusions could be drawn from the results as to the mechanisms involved in generating the action potential in the soma of the neurone. First of all, it is the differing response of the soma from that of the axon to the removal of extracellular Na, that is connected with these mechanisms. The soma is able to generate action potential for a shorter or longer period in Na-free solution, whereas the generation of spike potential in the axon ceases rapidly. A possible explanation for this marked difference would be a more efficient barrier on the surface of the soma inhibiting the loss of Na-ions by diffusion more effectively than is the case in the periaxonal area. The possibility has also to be considered that the ionic mechanism of action potential generation is different on the soma from that of the axon. The data of GERASIMOV et al. (1965) and of KOSTYUK (1968) indicate that the Na-ions are the carriers of the inward ionic current in the giant soma of *Lymnaea stagnalis*. Although the results of the present experiments are in agreement with these observations, in certain cases the possibility cannot be disregarded that Ca ions participate in carrying the inward current (MAGURA 1969). Conditioning polarization has a marked effect on the inward and also on the outward current. Depolarization inactivates not only the Na-carrier system, but also the K-carrier system. Some of our unpublished observations indicate the same. Signs of K-inactivation could also be observed when the rhythmic generation of action potentials was studied. K-inactivation is apparently able to stabilize the level of action potential overshoot also when the Na-carrier mechanism is definitely inactivated. Conditioning hyperpolarization promotes the generation of the early outward current. This current can most probably be regarded as a K-current (HAGIWARA et al. 1961). In conditioning hyperpolarization an early activation of the K-carrier system can be the cause underlying the marked decrease in the amplitude of the action potential (MAGURA and KRYSHTAL 1970). This effect of conditioning hyperpolarization was observed only in the giant neurones of molluscs.

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# *Pharmacologia*

## EFFECTS OF THEOPHYLLINE ON CORTICOSTERONE SECRETORY AND ADRENAL GROWTH RESPONSE TO ACTH IN VIVO

By

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In the hypophysectomized rat, acute theophylline increases the basal and the ACTH-stimulated corticosterone secretion. Chronic theophylline intensifies thymic involution and exerts no effect on adrenal growth in response to ACTH. The apparent synergism of theophylline and ACTH in vivo favours current suggestions that adenosine 3',5'-cyclic monophosphate is an intracellular mediator of the steroidogenic action of ACTH.

Adenosine 3',5'-cyclic monophosphate (cAMP) is now known to be the intracellular mediator of many actions of different hormones (ROBISON et al. 1968), including ACTH (HAYNES 1958; HAYNES et al. 1959; GRAHAME-SMITH et al. 1967). Substances inhibiting the enzymatic breakdown of cAMP probably potentiate the hormonal effects mediated by this nucleotide. Methylxanthine derivatives such as theophylline have been found to inhibit cyclic 3',5'-nucleotide phosphodiesterase activity in different mammalian tissue (BUTCHER and SUTHERLAND 1962) and rat adrenal homogenates (REDDY and STREETO 1968).

The present work describes an attempt to establish whether theophylline influences steroidogenesis, adrenal growth, and thymus involution in response to ACTH in the hypophysectomized rat.

### Methods

CFE male rats weighing approximately 120 g were hypophysectomized by the transauricular approach (GAY 1967), and others of 60 g b.w. were adrenalectomized bilaterally. All were housed in air-conditioned animal quarters (24 ± 1°C and 60—70% relative humidity) and maintained on commercial laboratory chow. The hypophysectomized rats were offered 5% glucose and the adrenalectomized group 1% saline solution as a substitute for drinking water. Completeness of hypophysectomy was checked at autopsy. In the chronic experiments, serial sections prepared from the sella of randomly selected animals were examined histologically.

Three hours after hypophysectomy the rats were given a single 10.0 mg/100 g b.w. dose of theophylline or an equivalent volume of physiological saline intraperitoneally. At the same time urethane anaesthesia was introduced (0.1 g/100 g b.w., i.m.). Twenty minutes later both

groups received an intravenous injection of ACTH (1.0 ng Synacthen/100 g). Blood was withdrawn from the aorta at times stated in Fig. 1. Plasma corticosterone concentration was estimated by the method of GUILLEMIN et al. (1959).

Rats which had been hypophysectomized 4 days earlier were given combined treatment with theophylline and ACTH once daily for further 4 days in doses and by routes as listed in Tables I and II. Twenty-four hours after the last injection the animals were decapitated and the thymus and the adrenals were removed and weighed on a torsion balance.

Adrenalectomized rats were treated with theophylline and hydrocortisone for 5 days as shown in Table III. The treatment was started on the first postoperative day. The animals were decapitated on the 6th day, the thymus was removed and weighed on a torsion balance.

All experiments followed a randomized block design. Analysis of variance and SHEFFÉ's (1953) multiple comparison method were used for statistical evaluation.

The following substances were used: theophylline ethylene diamine ("Diaphyllin venosum", Richter, Budapest), ACTH for intravenous use(Synacthen, CIBA), for prolonged treatment (Cortrophine-Z, Organon, Oss., Holland), hydrocortisone (Hydrocortison, Richter, Budapest).

## Results

In the rats which had been pretreated with theophylline 3 hours after hypophysectomy and were given an injection of ACTH 20 minutes later, the plasma corticosterone level at 0 time and throughout the secretory response to ACTH was significantly higher ($p < 0.01$) than in those pretreated with physiological saline (Fig. 1). The same was the case with animals similarly pretreated 24 hours after hypophysectomy (theophylline, $30.4 \pm 3.1$ $\mu$g/100 ml, $n = 11$; against saline, $9.0 \pm 0.5$ $\mu$g/100 ml, $n = 11$, without ACTH). Theophylline did not interfere with corticosterone determination; it produced no fluorescence, nor did it modify that of corticosterone.

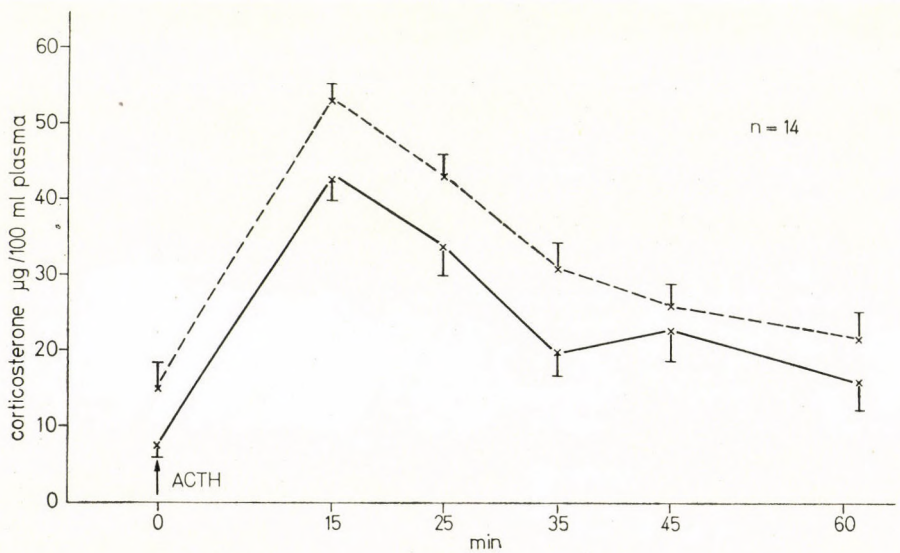


*Fig. 1.* Corticosterone secretory response to intravenous ACTH (1.0 ng Synacthen/100 g), in theophylline (x- - - -x) and saline (x ——— x) pretreated rats 3 hrs after hypophysectomy

**Table I**

*Effect of chronic theophylline treatment on adrenal weight in response to ACTH in hypophysectomized rats*

| Group | Treatment* | Dose** | Adrenal weight, mg | | Significance, p |
|---|---|---|---|---|---|
| | | | 0.9% NaCl 0.2 ml subcutaneously A | ACTH 0.6 U subcutaneously B | |
| 1. | 0.9% NaCl | 0.2 ml | 15.5 ± 0.7 (11)*** | 22.3 ± 1.1 (10) | 1A-1B < 0.005 |
| 2. | theophylline | 10.0 mg | | 24.0 ± 1.0 (11) | 1B-2B > 0.10 |
| 3. | 0.9% NaCl | 0.2 ml | 14.8 ± 0.6 (10) | 20.3 ± 0.8 (10) | 3A-3B < 0.005 |
| | | | | | 3A-4A > 0.10 |
| 4. | theophylline | 7.5 mg | 14.9 ± 0.7 (8) | 22.5 ± 1.5 (9) | 4A-4B < 0.005 |
| | | | | | 3B-4B > 0.10 |

* intramuscularly
** 100 g daily for 4 days
*** $\bar{x} \pm$ S.E. (n)

The data on the effects of chronic, combined treatment with theophylline and ACTH on adrenal growth and thymic involution are presented in Tables I and II. This experiment was carried out in two series at two dose levels of theophylline, 10 mg and 7.5 mg/100 g/day. The higher dose in itself killed the animals in the course of the treatment. The lower dose in itself exerted no effect on adrenal weight, while ACTH increased it significantly ($p < 0.005$). The combined effect of theophylline and ACTH was not superior to that

**Table II**

*Effect of chronic theophylline treatment on thymus weight in response to ACTH in hypophysectomized rats*

| Group | Treatment* | Dose** | Thymus weight, mg | | Significance, p |
|---|---|---|---|---|---|
| | | | 0.9% NaCl 0.2 ml subcutaneously A | ACTH 0.6 U subcutaneously B | |
| 1. | 0.9% NaCl | 0.2 ml | 341 ± 22 (11)*** | 210 ± 17 (10) | 1A-1B < 0.01 |
| 2. | theophylline | 10.0 mg | | 117 ± 11 (11) | 1B-2B < 0.05 |
| 3. | 0.9% NaCl | 0.2 ml | 349 ± 24 (10) | 230 ± 16 (10) | 3A-3B < 0.01 |
| | | | | | 3A-4A > 0.10 |
| 4. | theophylline | 7.5 mg | 314 ± 28 (8) | 117 ± 13 (9) | 4A-4B < 0.01 |
| | | | | | 3B-4B > 0.10 |

* intramuscularly
** 100 g daily for 4 days
*** $\bar{x} \pm$ S.E. (n)

of ACTH alone. Theophylline in itself left thymic weight unaffected, but ACTH reduced it significantly ($p < 0.01$). Whereas the higher dose of theophylline potentiated the thymolytic action of ACTH significantly ($p < 0.05$), the smaller one merely revealed a tendency toward potentiation.

With the object of reaching a conclusion as to whether theophylline increased the production of corticosterone in response to ACTH or potentiated

**Table III**

*Effect of chronic theophylline treatment on thymus weight in response to hydrocortisone in adrenalectomized rats*

| Group | Treatment* | Dose** | Thymus weight, mg | | Significance, p |
|---|---|---|---|---|---|
| | | | 0.9% NaCl 0.2 ml subcutaneously A | Hydrocortisone 0.2 mg subcutaneously B | |
| 1. | 0.9% NaCl | 0.2 ml | 402 ± 25 (8)*** | 249 ± 19 (7) | 1A-1B < 0.01 |
| | | | | | 1A-2A > 0.10 |
| 2. | theophylline | 7.5 mg | 344 ± 11 (12) | 248 ± 25 (11) | 2A-2B < 0.01 |
| | | | | | 1B-2B > 0.10 |

* intramuscularly
** 100 g daily for 5 days
*** $\bar{x} \pm$ S.E. (n)

the effect of the corticosteroid on thymic involution, we studied theophylline for its effect on the thymolytic action of chronic hydrocortisone treatment. We found that hydrocortisone caused a significant thymic involution ($p < 0.01$) which concomitant theophylline failed to aggravate (Table III). Hence the conclusion is offered that theophylline intensified the thymolytic action of ACTH by increasing corticosterone production.

## Discussion

In the present work it has been shown that in hypophysectomized rats acute theophylline increases the corticosterone secretion in response to, and in the absence of, ACTH. In the experimental framework used by us, chronic theophylline exerts no influence on adrenal growth. On the other hand, it aggravates ACTH-induced thymic involution. SZEBERÉNYI and FEKETE (1966) have demonstrated that theophylline increases corticosterone production in response to ACTH and cAMP in vitro. HALKERSTONE et al. (1966) suggest that theophylline has two antagonistic actions: on the one hand, it enhances the steroidogenic action of ACTH by inhibiting the breakdown of cAMP and, on the other hand, it reduces this hormonal action by the inhibition of protein synthesis, the latter being essential for the steroidogenic action (FERGUSON

1963; FARESE 1964; GARREN et al. 1965). Our present finding that theophylline simulates and intensifies the steroidogenic action of ACTH in the hypophysectomized rat, makes it seem probable that the inhibitory effect of the drug on protein synthesis in vivo asserts itself less emphatically.

The fact, established on the basis of two parameters, that theophylline acts synergistically with ACTH constitutes additional evidence that cAMP is an intracellular mediator of the steroidogenic action of ACTH.

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# THE EFFECT OF CHRONIC DROPERIDOL TREATMENT ON PITUITARY-ADRENOCORTICAL FUNCTION IN THE RAT

By

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The effect of droperidol on pituitary-adrenocortical function has been studied in the rat. Total corticosteroid, corticosterone and aldosterone production was estimated in vitro. Thirty-day droperidol treatment significantly increased total corticosteroid and corticosterone production, while aldosterone secretion did not change significantly.

Droperidol is widely used as a neuroleptic with an anti-shock effect. Considering the role of the pituitary-adrenocortical system in adaptation, it seemed interesting to study the effect of droperidol on that system.

It has been shown earlier that in the rat a single dose of droperidol induced maximal corticosteroid output and increased the stress-induced ACTH-release in vitro (DEBRECENI et al. 1969). In humans, droperidol exerted a stimulating effect on the pituitary-adrenocortical system at the supra-pituitary level (DEBRECENI et al., in press).

In the present work, the effect of chronic droperidol treatment on pituitary-adrenocortical function has been studied in the rat.

## Methods

A total of 120 adult female rats weighing 200—250 g was used in the investigation. The animals were kept on a standard diet and at constant temperature and divided into a control and a droperidol-treated group.

The test animals received 2.5 mg/kg b.w. droperidol (Janssen Pharmaceutica, Beerse, Belgium) intraperitoneally daily for 30 days, while the control animals received 0.5 ml physiological saline daily for the same period.

Pituitary-adrenocortical function was assessed after 30-day treatment. Thirty minutes following the last injection the rats were decapitated, the adrenals were removed, cleaned and weighed on torsion balance. Adrenal slices were incubated in Krebs-Ringer-bicarbonate solution containing 200 mg/100 ml glucose. Incubation was carried out for 2 hours at 38°C, in a mixture of 95% $O_2$ and 5% $CO_2$ (SAFFRAN et al. 1952; SÓLYOM et al. 1969).

Adrenal corticosteroid production was determined in vitro by the method of VAN DER VIES et al. (1960). Total corticosteroid production was expressed in $\mu$g/100 mg adrenal/hour used as an index of pituitary ACTH-release (VAN GOCH et al. 1963).

After evaporation the methylene chloride extract, aldosterone and corticosterone were separated by paper chromatography, developed by tetrazolium blue, and the spots corresponding to aldosterone and corticosterone were eluated and estimated spectrophotometrically, using a Spectromom 201 apparatus (SPÄT et al. 1968).

## Results

After 30-day treatment there was no significant difference in adrenal weight between the two groups: it was 61.3 mg in the control and 63.6 mg in the droperidol-treated rats.

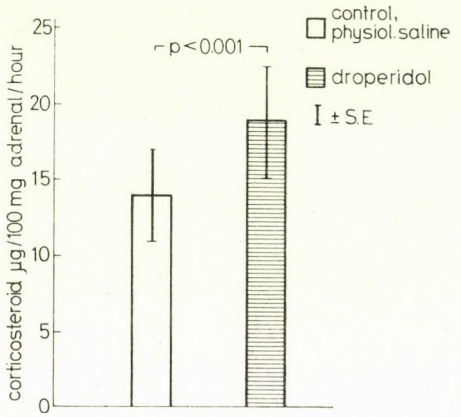


*Fig. 1.* Effect on corticosteroid production of 30-day droperidol treatment

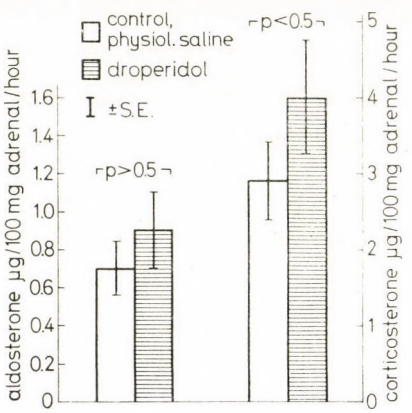


*Fig. 2.* Effect on aldosterone and corticosterone production of 30-day droperidol treatment

Fig. 1 shows the total corticosteroid production of the rats treated with droperidol. A moderate, 33% increase in corticosteroid production occurred in the droperidol treated rats, as compared with the control ones ($p < 0.001$).

Fig. 2 shows the values for aldosterone and corticosterone of the control and the droperidol-treated groups. The increase in corticosterone production amounted to 34% ($p < 0.05$), while that of aldosterone, 22% ($p > 0.05$), as compared with the controls.

## Discussion

In previous experiments, when we investigated the effect of a single dose of droperidol, we found the drug to increase pituitary-adrenocortical activity in the rat.

The question then was whether prolonged droperidol treatment induced a morphological and/or functional change in the adrenals.

There was no difference in adrenal weight between the saline-treated controls and the droperidol-treated animals, consequently the treatment did not induce an atrophy or hypertrophy of the adrenals.

Treatment with droperidol caused adrenal hyperfunction after 30 days; one month after treatment there was a difference in the in vitro corticosteroid production between the saline and droperidol-treated groups. Droperidol induced a significant increase in corticosteroid production after 30-day treatment.

In a previous study we have shown that droperidol exerted its stimulating effect on the adrenal cortex not directly but at the suprapituitary level, by inducing ACTH hypersecretion (DEBRECENI et al., in press). It was therefore assumed that droperidol increases not only the mobilization of ACTH but also its synthesis; this has now been proved by the present results.

After 30-day treatment with droperidol without increased synthesis of ACTH we would have found an ACTH depletion in the pituitary. In contrast, adrenals displayed hyperfunction, which proved in an indirect way the increased ACTH synthesis.

The effect of droperidol on pituitary-adrenocortical function as compared with that of the other major tranquillizers showed a striking difference: prolonged reserpine or chlorpromazine treatment resulted in a marked ACTH depletion of the pituitary, with a diminution of the stress-induced response (MAICKEL et al. 1961; SMITH et al. 1963). SULMAN and WINNIK (1956) found a diminution in adrenal weight in rats after treatment with chlorpromazine in a dose of 10 mg/kg daily for 3 months.

In our opinion the influence of droperidol exerted on the pituitary-adrenocortical system inducing ACTH hypersecretion is in accordance with the shock-preventive action of the drug. This is a decisive part of the complex central nervous effect of droperidol which plays an important role in the adaptation of the organism.

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# *Recensiones*

G. HORN and R. A. HINDE (Eds)

## Short-Term Changes in Neural Activity and Behaviour

Cambridge University Press, 1970. VII + 628 pages, with 232 figures. Price: £ 10 8s; $ 28.50

The volume contains the proceedings of a Conference sponsored by King's College Research Centre, Cambridge. The four main sections of the book include twenty papers presented by outstanding authors. The main sections are, I. Behavioural Considerations; II. Neural Basis of Habituation; III. Neural Basis of Plastic Changes Other than Habituation; IV. Epilogue. Changes in neuronal activity and their relationship to behaviour, reflect the interesting subject of the Conference.

The rapid technical improvement has made it possible to analyse the neural activity connected with behavioural changes at successively finer levels, like the behavioural, neural, neuronal, subcellular and molecular aspects. From this follows that habituation, a relatively simple type of behavioural change in which the units are similar at lower levels of analysis, has a central role in the discussions. The book offers a comprehensive review of the interpretation of habituation and this is a promising prospect to a better understanding of the other aspects of learning in the not too distant future.

The book will be of interest first of all to neurophysiologists, but is highly recommended to neurobiologists interested in the most fundamental problems of cerebral function.

K. LISSÁK

M. J. MELDMAN

## Disease of Attention and Perception

International Series of Monographs in Experimental Psychology. Gen. ed.: H. J. EYSENCK. Pergamon Press, 1970.

The role and importance of attention in psychological thinking varied from time to time. At the first sight the title and size of MELDMAN's book would not suggest more than a study of attention from the traditional psychiatric point of view. Perusal of the book, however, soon convinces that the enterprise goes far beyond this aim. It is not simply one of the alternative approaches to psychopathological events but an attempt to elaborate a new concept in psychiatry, the axis and basic coordinating principle of which is attention.

Recently, the meaning of attention as well as of the related notions such as arousal, activation, orientation, exploration have undergone a basic change mainly under the impact of neurophysiological discoveries (activating system). At the same time, the concepts concerning the mechanisms of higher integrative processes have also changed considerably. The consequences offered by neurophysiological evidence have been adopted surprisingly rapidly by experimental psychologists. This unusual readiness of acceptance seems to be explained by assuming that the recognition of the importance of the arousal-attention dimension proceeded latently, also in the fellow branches of neurosciences; we might say it was in the air of contemporary neurological thinking. After all, the fact pointed out by MELDMAN in the introduction, that the traditional conceptions of psychiatry remained essentially untouched by this conceptual revolution is somewhat astonishing and worth consideration. What the author is attempting in his book is not less than to make steps in filling up this gap. It is a pioneering undertaking to confront the bulk of facts and principles of the arousal-attention function gathered so far with

some basic phenomena of psychiatry, in order to be able to reclassify mental and perceptual disorders as diseases of attention.

The conception is developed in ten chapters roughly covering the traditional classification of psychological phenomena. This arrangement seems to have some disadvantages since it often requires to disperse coherent experimental evidence into different chapters. Nevertheless, it does not leave many gaps for the sceptical reader to evade the point by means of conventional argumentation.

The first chapter opens with a wittily composed collection of definitions as well as a systematic registration of the phenomena related to attention, making the reader aware of the complexities of the problem. The second chapter gives an excellent survey of the attention models, the third recapitulates the basic neuromorphological aspects of attention. The next three chapters disclose an impressively rich and very clearly presented material concerning motivational, emotional and cognitive aspects of attention. The seventh chapter is a survey of facts connected with receptive and efferent functions of attention. The main point of the last three chapters concerns pathological manifestations of attention. An attempt is made here to develop a general theory of disease as well as a demonstration of the usefulness of the concept by the method of "attentional profile".

Among the impressions raised by the book there are two which must particularly be emphasised. The amount and diversity of the presented facts seems to prove in itself that the arousal-attention function represents that particular psychological aspect from where the highest integrative functions of the brain can most advantageously be attacked. This assertion should not lessen our appreciation of the exceptional integrative abilities of the author attested by selection and interpretation of the apparently inhomogeneous bulk of data. This requires the comprehension and speaking of the languages of independent branches of science, an ability and ambition not often shown by contemporary authors. One of the greatest merits of MELDMAN's book is that in the course of mediating between different scientific approaches including such diverse fields as neurophysiology and psychoanalysis, the facts remain correct and interesting for both expert and newcomer.

A second impression is that we learned more about psychophysiology and less about psychiatry than the title of the book has promised. If that is what present-day psychiatry has needed then our second impression is not less positive than the first one. The suspicion might, however, arise that there may be some basic deficiencies in our up-to-date psychophysiological attention theories which impeded further psychiatrical implications. With the statement made in the course of the description of the attention models, viz. that "FREUD found it hard to give any mechanical (automatic) explanation of the origin of attention" we feel the author has touched on the weakest point of the present-day psychophysiological conceptions of attention. The physiologist on the one hand must be aware that this momentary answer to the question does not go much beyond FREUD's notions, and on the other hand that the first approach of this problem rests on physiology. What we know now is a faint idea that the solution of the question lies somewhere in the borderland of the basic mechanisms of learning and motivation. Such considerations among others urge the reviewer to recommend warmly to read this book not only to psychiatrists but also to neurophysiologists.

E. GRASTYÁN

R. PORTER and JULIE KNIGHT (Eds)

## Energy Metabolism in Trauma

A Ciba Foundation Symposium. J. and A. Churchill, London 1970. 202 pages. Price: £ 2.50 (50s)

This volume contains the records of a multidisciplinary symposium covering studies on patients with severe burns and trauma, and experiments with scalded and traumatized animals, ranging from changes in thermoregulation and overall metabolism to details of intermediary protein, carbohydrate and lipid metabolism. Despite their wide range, the contributions are welded into a coherent whole as the result of careful preparation and highly skilled editing in the best tradition of the Ciba Symposia. The discussions not only contribute valuable additional material, but by raising various points help to focus attention to some essential problems. The fact that in a few instances a trifle more teleological thinking pervaded the discussion as might appeal to some readers, does not detract from the value of the volume, which is highly recommended both to experimental workers and to clinicians concerned with the treatment of burns and with the care of traumatized patients.

SZ. DONHOFFER

S. SEVITT and H. B. STONER (Eds)

## The Pathology of Trauma

A Symposium organized by the Royal College of Pathologists. Supplement No. 4 of vol. 23 of the Journal of Clinical Pathology, London 1970. 214 pages. Price: £ 2.50

This Symposium followed the Ciba Symposium on "Energy Metabolism in Trauma" and the close collaboration between the organizers ensured that the two meetings have complemented each other, covering together most of the main problems of the field. Some overlapping was unavoidable and hence the chapter on metabolic changes deals on 40 pages with some of the problems discussed in greater detail at the Ciba Symposium. The other chapters deal with circulatory effects including electronic and computer monitoring in shock, thrombosis, embolism, coagulation and fibrinolysis, fat embolism, and the pathology of head injuries. On the whole, this volume is more clinically oriented than that of the Ciba Symposium. The contributions are of high quality and so are the reproductions. The discussions have not been included.

SZ. DONHOFFER

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# РЕЗЮМЕ

## ДАННЫЕ К ВОПРОСУ АРТЕРИО-ВЕНОЗНЫХ СОУСТЬЕВ ПОЧКИ. IX. ДЕЙСТВИЕ ТУРНИКЕТНОГО ШОКА НА ВНУТРИПОЧЕЧНОЕ КРОВООБРАЩЕНИЕ

З. НАДЬ, П. БЕНЧАТ и К. ТОРНЯИ

Авторы исследовали влияние турникетного шока на внутрипочечное кровообращение у собак. Ими было установлено, что соответственно шоковому состоянию кровяное давление уменьшалось, минутный объем значительно снижался и повышалось периферическое циркуляторное сопротивление. Значительное снижение ренальной фракции минутного объема указало на редистрибуцию системной циркуляции.

Тотальный почечный кровоток, измеренный с помощью электромагнитного прибора, снижался приблизительно на одну треть нормальной величины. Снижение питательного (nutritiv) кровотока, определенного с помощью поглощения изотопа Rb, выявило еще более выраженную ишемию. Уменьшение частного $RBF_{nutr}/RBF_{dir}$ (нормаль: 0,97; здесь: 0,649) подтверждает мнение авторов, что в турникетном шоке значительная часть тотального почечного кровообращения обходя паренхиму почки, через артериовенозные соустья попадает обратно в системную циркуляцию, и таким образом усиливает почечную ишемию.

## ДАННЫЕ К ВОПРОСУ АРТЕРИО-ВЕНОЗНЫХ СОУСТЬЕВ ПОЧКИ. X. ДЕЙСТВИЕ ДИБЕНЗИЛИНА НА ВНУТРИПОЧЕЧНОЕ КРОВООБРАЩЕНИЕ ПРИ ТУРНИКЕТНОМ ШОКЕ У СОБАК

З. НАДЬ, А. МЕСАРОШ и Л. ВАШЛАКИ

У собак в турникетном шоке изучалось действие дибензилина, введенного в почечную артерию (1,5 мг/кг). Наблюдалось нормальное внутрипочечное кровообращение, несмотря на то, что тотальный почечный кровоток, кровяное давление, минутный объём и фракция почечная минутного объёма уменьшались соответственно шоковому состоянию. Дибензилин препятствовал возникновению внутрипочечной редистрибуции и ишемии почечной паренхимы, которая является следствием почечной вазоконстрикции и открывающих в результате этого артерио-венозных соустьев.

## ВЛИЯНИЕ НАТРИЕВОЙ И ОСМОТИЧЕСКОЙ КОНЦЕНТРАЦИИ ИНКУБАЦИОННОЙ ЖИДКОСТИ НА ВЫСВОБОЖДЕНИЕ РЕНИНА ИЗ СРЕЗОВ КОРЫ ПОЧКИ

И. ФОРГАЧ и К. ГАЛ

Высвобождение ренина из срезов почечной коры собак было исследовано в различных инкубационных жидкостях, в которых изменялись содержание натрия (10—200 миллиэкв/л) и осмотическая концентрация (70—360 миллиосмол/л).

Установлено, что

1. с изменением концентрации натрия (10—90 миллиэкв/л) линейно изменяется высвобождение ренина;

2. изменение осмотической концентрации при постоянной концентрации натрия не оказывает влияния на высвобождение ренина.

## ДЕЙСТВИЕ ГИПЕРКАПНИИ НА ТЕРМОРЕГУЛЯЦИЮ НОВОРОЖДЕННЫХ КРОЛИКОВ И СРАВНИВАНИЕ ПОСЛЕДНЕГО С ДЕЙСТВИЕМ ГИПОКСИИ

И. ВАРНАИ М. ФАРКАШ и С. ДОНХОФФЕР

Потребление кислорода и температура ободочной кишки измерялись непрерывно у кроликов, возраст которых 1—2 дня, 3—6 дней, или 7—18 дней, при температуре окружающей среды 35°С, и которых потом помещали в среду с температурой 20°С, где они дышали 30—30 минут воздухом, потом 6%-ым углекислым газом и опять воздухом. Средние величины потребления кислорода не изменялись значительно ни при гиперкапнии, ни после гиперкапнии. И напротив температура тела значительно снижалась у двух младших возрастных групп, и у старшей возрастной группы снижение также приближалось к статистическому значению. Между изменением потребления кислорода при гиперкапнии и сопровождающим его изменением температуры тела можно было наблюдать корреляцию только у самой младшей возрастной группы. В группе возрастом 3—6 дней между следующими величйнами была показана статистически значительная корреляция: 1) между температурой тела перед гиперкапнией и ее изменением при гиперкапнии; 2) между температурой тела, измеренной при окончании гиперкапнии и ее изменением в течение следующих 30 минут; 3) между изменениями температуры тела при гиперкапнии и после гиперкапнии; 4) между изменениями потребления кислорода при гиперкапнии и после его окончания; 5) между изменением температуры тела при перенесении животных из среды с 35°С в среду с 20°С и изменением температуры тела после этого при гиперкапнии или после гиперкапнии. У старшей возрастной группы статистически значительная корреляция наблюдалась 1) между потреблением кислорода перед гиперкапнией и его изменением под гиперкапнией; 2) между разницей потребления кислорода при 35°С и 20°С, и изменением потребления кислорода при гиперкапнии. Сравнивая действия гипоксии и гиперкапнии можно видеть, что исследованные корреляции не параллельны при гипоксии и гиперкапнии. Значительная корреляция между некоторыми параметрами часто ограничивалась только коротким периодом новорожденных и у младших или у старших животных не наблюдалась.

## МЕТАБОЛИЧЕСКИЕ ЗАПАСЫ СЕРДЕЧНЫХ ПРОТЕИНОВ И АМИНОКИСЛОТ НА УТЯТАХ ПРИ Co-ИНТОКСИКАЦИИ

Ш. ПАУЛОВ, Й. ВЕСЕЛОВСКИ и Й. М. ДЕМЕРС

Аккумуляция кобальта и концентрация свободных сердечных протеинов и аминокислот были исследованы на утятах при интоксикации кобальтом. Получены следующие результаты:

1. Кобальт интенсивно накопляется в сердечной ткани из диеты, богатой этим элементом. Высокая концентрация кобальта в диете не влечет за собой повышения кобальта в циркулирующей крови.

2. Уровень свободных сердечных протеинов не изменялся при интоксикации кобальтом.

3. Концентрация свободных аминокислот специфическим образом значительно изменялась у экспериментальных животных. Уровень большинства аминокислот снижался, а у некоторых повышался.

## СЛИЗИСТАЯ ПРОТЕОЛИТИЧЕСКАЯ АКТИВНОСТЬ В ТОНКОЙ КИШКЕ СОСУЩИХ КРЫС

К. БАИНТНЕР и Ш. ЮХАС

В просвете и слизистой оболочке тонкой кишки новорожденных и сосущих крыс наблюдалась большая протеолитическая активность. Разделив в длину тонкую кишку на 5 частей, в 1-ой и 2-ой частях, в которых клетки не имеют вакуолизации, можно наблюдать наименьшую протеолитическую активность, а самую большую — в 4-ой части, где супрануклеарные вакуолы клеток эпителия наиболее ярко выражены. В слизистой оболочке можно определить по крайней мере четыре типа такой протеолитической активности, которая имеется и в панкреасе, и исходит, вероятно, из него. Неясно, что иммуноглобулины, усвоенные с молоком, каким образом избегают пищеварения в тонкой кишке сосущей крысы.

## ДЕЙСТВИЕ ЛИПИДОВ ПОЧЕЧНОГО СОСКА И ПРОСТАГЛАНДИНА $E_2$ НА ПРОДУКЦИЮ ГОРМОНОВ НАДПОЧЕЧНИКОВ

А. ШПЭТ, Б. ШАРКАДИ, Ж. ИНТЁДИ, А. КЁРНЕР и Й. САНТО

Действие липидов почечного соска крысы на продукцию стероидов надпочечников таких крыс, которые получили дексаметазон, было исследовано in vitro. Под действием липидов, экстрагированных из гомогенизата соска, продукция как альдостерона, так и кортикостерона значительно повышалась.

В другой части опытов срезы соска инкубировались при разных условиях и исследовалось действие экстрагированных из инкубационной жидкости липидов на кору надпочечников. Стероидогенный эффект наблюдался, если сосок инкубировался в такой жидкости, в которой состав ионов приближался к ион-составу крысы, находящейся в умеренном диуретическом состоянии.

Под влиянием аутентичного простагландина Е (3 М) продукция альдостерона немного, но значительно, а продукция кортикостерона в большой мере увеличивались.

## КОМПЕНЗАТОРНАЯ ГИПЕРТРОФИЯ НАДПОЧЕЧНИКА, ЯИЧНИКА И ЩИТОВИДНОЙ ЖЕЛЕЗЫ У ОЖИРЕВШИХ ПОСЛЕ НАРУШЕНИЯ ВЕНТРОМЕДИАЛЬНОГО ГИПОТАЛАМУСА КРЫС

Л. ХАЛМИ, Ч. НЯКАШ и Э. ЭНДРЁЦИ

Компензаторная гипертрофия надпочечника, яичника и щитовидной железы была изучена на самках крыс, имеющих нарушение медиального гипоталамуса на уровне вентро-медиального ядра. Одностороннее удаление периферических эндокринных органов и электролитическое нарушение мозга производились одновременно. Принятие пищи и воды и вес тела значительно повышались после нарушения гипоталамуса. Сравнивая степень компензаторной гипертрофии у крыс с ложной операцией и у крыс, показывающих характерное ожирение после нарушения гипоталамуса не наблюдалось дифференции. Вес гипофиза слегка снижался у ожиревших крыс.

Данные показывают, что функция гипофиза, контролированная «feedback» механизмом периферических гормонов не нарушалась у крыс с гипоталамическим ожирением.

## ДЕЙСТВИЕ ГОНАДОТРОПНЫХ ГОРМОНОВ НА УГАСАНИЕ ОБОРОНИТЕЛЬНОГО РЕФЛЕКСА И НА ДВИГАТЕЛЬНУЮ АКТИВНОСТЬ У КРЫС

Г. ТЕЛЕГДИ и Г. РОЖАХЕДЬИ

На крысах исследовалось влияние человеческого хорионгонадотропного гормона (HCG) и сывороточного гонадотропина жеребой кобылы (PMS) на угасание оборонительного условного рефлекса, межсигнальную деятельность в течение угасания и общую подвижность.

Установлено, что HCG ускоряет угасание оборонительного условного рефлекса, уменьшает межсигнальную деятельность в течение угасания и снижает свободную двигательную активность животных.

Примененная доза PMS оказалась неэффективной на вышеуказанные параметры поведения крыс.

## ДАЛЬНЕЙШИЕ ДАННЫЕ О ВЛИЯНИИ ХОРИОНГОНАДОТРОПНОГО ГОРМОНА ЧЕЛОВЕКА НА ОБОРОНИТЕЛЬНУЮ УСЛОВНОРЕФЛЕКТОРНУЮ И ДВИГАТЕЛЬНУЮ АКТИВНОСТИ КРЫС

Г. ТЕЛЕГДИ, Г. РОЖАХЕДЬИ и К. ЛИШШАК

Авторы исследовали на крысах влияние человеческого хорионгонадотропного гормона (HCG) на выработку и угасание оборонительного условного рефлекса, на межсигнальную деятельность в течение выработки и угасания рефлекса и на общую подвижность животных.

Установлено, что HCG не оказывал влияния на выработку оборонительного условного рефлекса и на межсигнальную деятельность животных в течение выработки рефлекса. В то же время HCG, вводимый ежедневно с начала выработки рефлекса, ускорял угасание, уменьшал межсигнальную деятельность в течение угасания и снижал свободную двигательную активность животных. Надпочечники или гонады не играют роли в вышеупомянутых эффектах HCG.

Вместе инактивированием HCG, что означает потерю гонадотропного влияния, исчезает также и его действие на поведение.

## СВЯЗЬ ТОКА И НАПРЯЖЕНИЯ НА СОМАМЕМБРАНЕ ГИГАНТСКОГО НЕЙРОНА LIMNAEA STAGNALIS

И. С. МАГУРА, И. КИШ и О. А. КРЫСТАЛ

Авторы исследовали возбудительные процессы мембраны гигантских нервных клеток центральной нервной системы Lymnaea stagnalis с помощью метода фиксации напряжении (voltage clamp). Ионные потоки вообще не отличались от тех, которые раньше наблюдались на других объектах. В течение фиксации напряжения, продолжающегося более 1 сек-ы, наблюдались ритмически повторяющиеся сигналы, накладывающиеся на поток, направляющиеся наружу. Эти сигналы, возможно, возникают с тех удаленных участков мембраны, на которые voltage clamp не влияет.

При помещении препарата в среду, свободную от Na, у большинства нейронов ток, направляющийся в начале вовнутрь, прекращается. Для исчезновения потоков, происходящих от удаленных участок мембраны, меньше времени необходимо. При добавлении в среду изотопа Ca не восстанавливается ток, направляющийся вовнутрь.

Исследовалось влияние предварительной деполяризации и гиперполяризации, а также влияние TEA на ионные потоки. Поляризация оказывает значительное влияние на ток, направляющийся как вовнутрь, так и наружу, а TEA влияет главным образом на ток, направляющийся наружу.

Из характеристики Volt-Amper был определен эквилибриум-потенциал Na и K. Первый не различается значительно от максимума потенциала действия, второй отрицательнее на 23—25 mV, чем потенциал покоя.

## ВЛИЯНИЕ ТЕОФИЛИНА НА СТЕРОИДОГЕНЕТИЧЕСКОЕ И ТРОФИЧЕСКОЕ ДЕЙСТВИЯ АКТГ in vivo

Й. МАРТОН, Э. ШТАРК, К. МИХАЙ и Б. ВАРГА

У гипофизектомированных крыс однократное введение теофилина повышало как базальный, так и повышенный под влиянием АКТГ уровень кортикостерона в сыворотке крови. Длительное введение теофилина усиливало вызванную АКТГ инволюцию зобной железы, но при влиянии АКТГ не оказывало воздействия на вес надпочечников. Синергизм теофилина и АКТГ — дальнейшее доказательство, что аденозин 3', 5',-циклический монофосфат является внутриклеточным медиатором стероидогенетического влияния АКТГ.

## ДЕЙСТВИЕ ДЛИТЕЛЬНОГО ЛЕЧЕНИЯ ДОПЕРИДОЛОМ НА ФУНКЦИЮ СИСТЕМЫ ГИПОФИЗ-КОРЫ НАДПОЧЕЧНИКОВ У КРЫС

Л. ДЕБРЕЦЕНИ, Л. ЧОНКА-ТАКАЧ, П. ХОРВАТ и Б. ЧЕТЕ

Действие дроперидола на функцию системы гипофиз-коры надпочечников изучалось на крысах. Вещество вводилось в течение 30-и дней, потом измерялась тотальная продукция кортикостероидов, продукция кортикостерона и альдостерона in vitro. Результаты показали, что дроперидол значительно увеличивал тотальную продукцию кортикоидов и кортикостерона, а секреция альдостерона заметно не изменялась.

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# *Physiologia*

## EFFECT OF DENERVATION ON THE PHOSPHOLIPID CLASSES IN TONIC AND TETANIC MUSCLES

By

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(Received April 26, 1971)

1. The amount of phosphatidylcholine increases in denervated tetanic muscle. In denervated tonic muscle the changes are not significant. In the contralateral innervated muscles the amount of phosphatidylcholine decreased.

2. Sphingomyelin increases in tonic and tetanic muscles both on the denervated and the contralateral side. The increase in sphingomyelin is more pronounced in tetanic and denervated muscles.

3. Phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine increase in both denervated tonic and denervated tetanic muscles. The increase is more marked in the tetanic muscle. In the contralateral muscles the amount of phosphatidylethanolamine and phosphatidylinositol decreases.

The changes in the total phospholipid content of muscles during their neurogenic atrophy have been studied by several authors (ARTOM 1941; FRIEDLANDER et al. 1941; DREYFUS and SHAPIRA 1948; HUMOLLER et al. 1952; HINES and THOMSON 1956; GRAFF et al. 1965a, b, c) but in muscles with different functions the individual phosphatides have not been investigated comparatively under pathological conditions.

In a previous work (HEINER et al. 1970) it has been shown that on the effect of denervation the amount and the qualitative composition of total non-choline phosphatides and choline phosphatides change in a different and characteristic way in tonic and tetanic muscles with different biological function. In the present study a detailed analysis has been performed in order to examine the changes in individual phospholipids in the course of denervation muscle atrophy.

### Methods

Adult rabbits kept on a normal diet were used. The muscles were inactivated by unilateral section of the sciatic nerve at the trochanteric level. The semimembranous muscle was used as a tetanic and the soleus muscle as a tonic one. Changes following denervation were studied at intervals of 2 to 6 weeks.

After removing the connective tissue, the muscles were homogenized in methanol in a glass homogenizer. Lipids were extracted with chloroform–methanol (2 : 1 v/v) at room temperature. Separation of phospholipids into two fractions, viz. choline phosphatides [phos-

phatidylcholine (PC), lysophosphatidylcholine (lyso PC), sphingomyelin] and "cephalins" [phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS)] was performed, as described earlier (HEINER and DOMONKOS 1970).

From part of the separated phospholipid fractions the lipid-P content was determined according to FISKE and SUBBAROW (1925). From other parts of these fractions the individual P-lipids were separated by silicagel thin layer chromatography (Kieselgel G. Stahl, Merck). The solvent system for the choline phosphatides was chloroform–methanol–water (65 : 25 : 4 v/v), for the non-choline phosphatides chloroform–methanol–25% ammonium hydroxide (65 : 25 : 4 v/v). In the case of non-choline phosphatides, in order to secure a better separation, the chromatographic procedure was repeated with the same solvent system. The separated lipids were visualized by iodine. All the lipid spots were collected quantitatively and the phosphorus was determined by a modification of BARTLETT's method (1959). A lipid sample containing a minimum of 0.2 $\mu$g atomic P was ashed with 1.2 ml of 10 $N$ $H_2SO_4$; 2—3 drops of 30% $H_2O_2$ were added and ashing was continued for one hour at 160° C. Then 2.7 ml of distilled water, 0.8 ml of 5% ammonium molybdate and 0.2 ml of Fiske and SubbaRow reagent were added. The mixture was kept in a water bath at 100° C for 7 minutes. The silicagel was removed by centrifugation and optical density was read at 800 m$\mu$ in a spectrophotometer. The optical density of the single lipid spots has been converted into a percentage of total extinction obtained for all the spots. On the basis of the percentage distribution, the quantity of phospholipid components was calculated from the total phosphorus content of the choline and non-choline phosphatide fractions.

## Results

Table I shows the changes of PC, lyso PC and sphingomyelin in the tonic muscle occurring under the effect of denervation. The PC content of the denervated tonic muscle in the examined time interval did not change markedly, merely a slight decrease was observed after the fifth week. On the opposite

**Table I**

*Effect of denervation on the amount of choline phosphatides in tonic muscle* ($\mu$ atoms P/g muscle, wet weight)

| Weeks following denervation | | PC | Lyso PC | Sphingomyelin |
|---|---|---|---|---|
| 2 | D | 4.95±0.48 (4)* | 0.07±0.02 (4) | 0.40±0.02 (4) |
| | I | 4.59±0.57 (4) | 0.08±0.01 (4) | 0.37±0.04 (4) |
| 3 | D | 4.98±0.56 (4) | 0.20±0.03 (4) | 0.53±0.06 (4) |
| | I | 4.15±0.17 (4) | 0.10±0.02 (4) | 0.38±0.03 (4) |
| 4 | D | 14.98±0.41 (5) | 0.24±0.05 (4) | 0.60±0.05 (6) |
| | I | 3.84±0.19 (5) | 0.20±0.02 (4) | 0.42±0.06 (6) |
| 5 | D | 4.98±0.40 (6) | 0.15±0.03 (6) | 0.69±0.10 (7) |
| | I | 4.13±0.55 (6) | 0.14±0.03 (7) | 0.44±0.05 (7) |
| 6 | D | 4.67±0.39 (6) | 0.13±0.03 (6) | 0.65±0.07 (6) |
| | I | 3.97±0.71 (6) | 0.13±0.03 (5) | 0.34±0.06 (6) |

PC: phosphatidylcholine
Lyso PC: lysophosphatidylcholine
D: denervated muscle
I: innervated, contralateral muscle
* Mean ± standard deviation. Number of experiments in brackets

innervated side, however, the amount of PC definitely decreased still the fourth week, and then a slow, moderate increase occurred.

The amount of lyso PC following denervation slightly increased both on the denervated and the contralateral sides. The rise was the most expressed in the fourth week and was somewhat greater in the denervated muscle than in the innervated one.

The amount of sphingomyelin pronouncedly rose in both the denervated and the contralateral muscles up to the fifth week, thereafter it decreased. This increase was, however, much more expressed in the denervated muscle.

Table II shows the PC, lyso PC and sphingomyelin content in the tetanic muscle. After denervation the amount of PC increased considerably up to the fifth week and decreased slightly in the sixth week. In contrast, in the contralateral innervated muscle the amount of PC decreased pronouncedly until the fourth week, to increase somewhat thereafter.

Following denervation, lyso PC showed an increase up to the fourth week in both the denervated and the innervated muscles. In the fifth week a decrease was observed on both sides and in the sixth week the amount of lyso PC reached its initial value.

The sphingomyelin content of the tetanic muscle after denervation showed a similar change as that of the tonic muscle. The amount of sphingomyelin of the tetanic muscles increased on both sides; this increase was much

**Table II**

*Effect of denervation on the amount of choline phosphatides in tetanic muscle* ($\mu$ atoms P/g muscle, wet weight)

| Weeks following denervation | | PC | Lyso PC | Sphingomyelin |
|---|---|---|---|---|
| 2 | D | 5.43±0.30 (4)* | 0.18±0.01 (4) | 0.41±0.03 (4) |
| | I | 4.65±0.36 (4) | 0.17±0.03 (4) | 0.33±0.02 (4) |
| 3 | D | 6.01±0.50 (4) | 0.16±0.03 (4) | 0.55±0.04 (4) |
| | I | 4.25±0.18 (4) | 0.16±0.02 (4) | 0.41±0.04 (4) |
| 4 | D | 6.16±0.63 (6) | 0.30±0.02 (5) | 0.66±0.08 (6) |
| | I | 3.46±0.34 (6) | 0.28±0.01 (6) | 0.49±0.06 (6) |
| 5 | D | 6.77±0.69 (7) | 0.28±0.08 (7) | 0.71±0.07 (7) |
| | I | 3.60±0.46 (7) | 0.24±0.06 (6) | 0.44±0.09 (7) |
| 6 | D | 6.47±0.77 (6) | 0.18±0.04 (6) | 0.73±0.06 (6) |
| | I | 3.98±0.52 (6) | 0.18±0.04 (6) | 0.40±0.06 (6) |

PC: phosphatidylcholine
Lyso PC: lysophosphatidylcholine
D: denervated muscle
I: innervated, contralateral muscle
* Mean ± standard deviation. Number of experiments in brackets

**Table III**

*Effect of denervation on the amount of non-choline phosphatides in tonic muscle* ($\mu$ atoms P/g muscle, wet weight)

| Weeks following denervation | | PE | PI | PS |
|---|---|---|---|---|
| 2 | D | 2.46±0.20 (4)* | 0.61±0.02 (4) | 0.12±0.03 (4) |
| | I | 2.75±0.29 (4) | 0.64±0.04 (4) | 0.09±0.01 (4) |
| 3 | D | 2.65±0.40 (4) | 0.64±0.05 (4) | 0.13±0.01 (4) |
| | I | 2.73±0.25 (4) | 0.63±0.07 (4) | 0.08±0.01 (4) |
| 4 | D | 2.92±0.24 (5) | 0.67±0.03 (5) | 0.15±0.02 (5) |
| | I | 2.46±0.44 (5) | 0.56±0.07 (5) | 0.10±0.02 (5) |
| 5 | D | 2.85±0.25 (7) | 0.73±0.05 (7) | 0.14±0.02 (7) |
| | I | 2.52±0.27 (7) | 0.54±0.03 (6) | 0.08±0.02 (7) |
| 6 | D | 2.71±0.20 (6) | 0.57±0.08 (6) | 0.08±0.02 (5) |
| | I | 2.69±0.36 (6) | 0.50±0.08 (6) | 0.07±0.01 (6) |

PE: phosphatidylethanolamine
PI: phosphatidylinositol
PS: phosphatidylserine
D: denervated muscle
I: innervated, contralateral muscle
* Mean ± standard deviation. Number of experiments in brackets

**Table IV**

*Effect of denervation on the amount of non-choline phosphatides in tetanic muscle* ($\mu$ atoms P/g muscle, wet weight)

| Weeks following denervation | | PE | PI | PS |
|---|---|---|---|---|
| 2 | D | 1.78±0.29 (4)* | 0.72±0.04 (4) | 0.05±0.01 (4) |
| | I | 1.47±0.20 (4) | 0.69±0.06 (4) | 0.06±0.01 (4) |
| 3 | D | 2.55±0.40 (4) | 0.73±0.04 (4) | 0.13±0.02 (4) |
| | I | 1.35±0.33 (4) | 0.67±0.02 (4) | 0.12±0.03 (4) |
| 4 | D | 2.46±0.28 (5) | 0.76±0.05 (5) | 0.11±0.02 (5) |
| | I | 1.15±0.35 (5) | 0.52±0.05 (5) | 0.07±0.01 (5) |
| 5 | D | 2.35±0.10 (7) | 0.78±0.06 (7) | 0.11±0.03 (7) |
| | I | 1.45±0.39 (7) | 0.56±0.07 (7) | 0.09±0.02 (7) |
| 6 | D | 2.27±0.14 (6) | 0.63±0.05 (6) | 0.07±0.01 (6) |
| | I | 1.52±0.29 (6) | 0.44±0.03 (6) | 0.06±0.02 (6) |

PE: phosphatidylethanolamine
PI: phosphatidylinositol
PS: phosphatidylserine
D: denervated muscle
I: innervated, contralateral muscle
* Mean ± standard deviation. Number of experiments in brackets

more expressed in the denervated muscle and could be observed even at the sixth week.

Table III shows the amount of PE, PI and PS in the tonic muscle. After denervation the amount of all the three phosphatides exhibited a moderate rise up to the fourth to fifth week. At the same time, in the contralateral muscle PE and PI decreased, while in the amount of PS there was no change.

Table IV summarizes the effect of denervation on the PE, PI and PS content of the tetanic muscle. The amount of PE in the denervated muscle increased. This increase was the most expressed during the third and fourth weeks. However, on the innervated side there was a small decrease; this was most expressed during the fourth week. The amount of PI slightly increased in the denervated muscle and definitely decreased in the contralateral muscle. The amount of PS following denervation increased on both sides and reached a maximum in the third week.

## Discussion

It has been shown previously (HEINER et al. 1970) that following denervation, the quantity and the qualitative composition of choline and non-choline phosphatides did not change to the same extent in the different muscles. In the present work it has been examined what kind of changes of the individual phospholipids (PE, PI, PS, PC, lyso PC and sphingomyelin) are taking place in each of the two phospholipid (choline and non-choline phosphatide) fractions.

Up to now the investigations into the effect of denervation on lipid metabolism were always made in a certain muscle without comparing the results with those obtained in other muscles (GRAFF et al. 1965a, b, c). Our examinations have proved that findings obtained for a certain muscle alone does not allow for general conclusions holding true for the whole of skeletal muscles. The tetanic, fast muscles with locomotor function fail to respond to pathological conditions in the same way as the tonic slow ones with static functions do. The results suggest that under the effect of nerve dissection the phospholipid metabolism of muscles with locomotor function reacts more sensitively than that of muscles with a static function. It has been also established that a unilateral nerve dissection affects the lipid composition of the contralateral muscles as well (HEINER et al. 1970).

When discussing the results, it must be taken into account that it is not only the changes occurring on the denervated side which are responsible for the differences appearing between the muscles of the denervated and innervated sides. As it is indicated by the results, the changes occurring in the contralateral muscles following unilateral nerve dissection, cannot be neglected.

Among the changes of the different phosphatides, those of the choline phosphatides are the most striking. Following nerve dissection, the amount of PC increases in the denervated tetanic muscles for some weeks. At the same time, the content in these phosphatides temporarily decreases in the contralateral muscles. The most pronounced differences between the innervated and denervated muscles can be observed during the fourth and fifth week. This is true for both kinds of muscle, but the differences are of smaller extent in the tonic, than in the tetanic muscles.

The alterations of lipid metabolism in the denervated and the contralateral, innervated muscles are best demonstrated by the changes in the lyso PC content, which under the effect of denervation, increases in the muscles, especially during the fourth week. This fact, together with the above mentioned results, indicates that phospholipid metabolism increases temporarily for some weeks in both kinds of muscle. However, in the denervated muscles an anabolism, while in the innervated muscles owing to the unilateral overstrain, a catabolism predominates.

The alterations of lipid metabolism occurring in muscles after unilateral nerve dissection are proved also by the changes in the sphingomyelin content. This increases not only in the denervated tetanic muscle, but in the innervated muscle too, although in the latter muscle the increase is small. The situation is similar in the tonic muscle, except that the changes are slighter. The alterations in sphingomyelin content found in the present study were in a fairly good agreement with our earlier results (HEINER et al. 1970) in that, under the effect of denervation, the fatty aldehyde content of the phosphatides increased in both the denervated and the innervated muscles. It is known, however, that fatty aldehyde is one of the precursors in the biosynthesis of sphingomyelin (ANSELL and HAWTHORNE 1964).

Among the changes of the non-choline phosphatides that of the PE was the most striking. This was the phosphatide mainly responsible for the changes observed in the total lipid-P content of the "cephalin"-fraction following denervation (HEINER et al. 1970). The changes in the amount of this phosphatide were smaller than those of the choline-phosphatides and more expressed in the tetanic than in the tonic muscles. On the effect of denervation the changes in PI occurring in both kinds of muscle on the denervated and the contralateral side alike, were similar to those displayed by the PE. The changes in PS were small and not characteristic for the two kinds of muscle.

As already stated (HEINER et al. 1970), the changes observed cannot be explained by some change in the muscle cell phase following denervation. The unidirectional changes of the non-collagenous protein content of the denervated muscles cannot be correlated with the multiplex changes of the different phosphatides.

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# THERMOREGULATORY ROLE OF PERIAORTIC BROWN ADIPOSE TISSUE IN RATS ACCLIMATED TO DIFFERENT AMBIENT TEMPERATURES

By

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In adult rats, acclimated to cold or kept at room temperature, cold exposure resulted in a temperature increase of the blood in the thoracic aorta just above the diaphragma as compared to the temperature in the aortic arch. It has been concluded that heat is being transferred from the periaortic brown adipose tissue to the blood in the thoracic segment of the aorta. In the warm-acclimated rat, atrophy of periaortic brown adipose tissue could account for the marked reduction or even absence of measurable heat transfer to aortic blood. In the absence of periaortic brown adipose tissue (guinea pigs, young rabbits) no temperature difference developed in response to cold.

Since SMITH (1961) suggested the thermogenetic role of brown adipose tissue, it has been proved to represent an important source of non-shivering thermogenesis in hibernators (SMITH and HOCK 1963; SMALLEY and DRYER 1963; SMITH and HORWITZ 1969), cold-adapted rats (SMITH 1961; SMITH 1964; SMITH and ROBERTS 1964), non-cold-adapted rats (DONHOFFER et al. 1964; DONHOFFER and SZELÉNYI 1965), warm-adapted rats (DONHOFFER and SZELÉNYI 1967) and in the new-borns and adults of a number of species (DAWKINS and HULL 1964; BRÜCK and WÜNNENBERG 1965; DAWKINS and SCOPES 1965; DONHOFFER and SZELÉNYI 1965; HULL and SEGALL 1965; AHERNE and HULL 1966; HEIM and HULL 1966a; HEIM et al. 1968; TARKKONEN and JULKU 1968). This tissue was generally held to heat the blood returning to the heart (SMITH 1963; SMITH and ROBERTS 1964; SMITH and HORWITZ 1969) or to be primarily of local importance (BRÜCK and WÜNNENBERG 1966b; TARKKONEN and JULKU 1968; HIMMS-HAGEN 1969). The interscapular part of brown adipose tissue was generally considered a representative part of the whole and the data obtained from temperature (DAWKINS and HULL 1964; BRÜCK and WÜNNENBERG 1966a; HEIM and HULL 1966a, b) and/or blood flow measurements (EVONUK and HANNON 1963; HEIM and HULL 1966a; KUROSHIMA et al. 1967; JANSKY and HART 1968) were extrapolated to the total brown adipose tissue (HEIM and HULL 1966a; IMAI et al. 1968). It has, however, been shown earlier that brown adipose tissue slices obtained from different areas exhibited different oxygen consumption in vitro, the largest being observed in slices originating from intrathoracic regions (SMITH and ROBERTS 1964), and that cold adaptation caused a more intensive proliferation of brown adipose tissue in the intrathoracic than in other regions (JANSKY

et al. 1969). Besides, warm-adaption resulted in a remarkable atrophy of periaortic brown adipose tissue (KELLERMAYER and SZÉKELY, unpublished observations).

In rats kept at room temperature, intrathoracic brown adipose tissue envelops the thoracic aorta in an amount roughly similar to that of interscapular brown fat (KELLERMAYER and SZÉKELY, unpublished data; JANSKY et al. 1969). In a preliminary communication SZÉKELY et al. (1970) suggested that periaortic brown adipose tissue transferred heat to the blood of the aorta in rats kept at room temperature.

To gain further insight into the role of periaortic brown adipose tissue, the experiments have been extended to rats acclimated to different ambient temperatures.

## Methods

Adult albino rats of both sexes of the Institute's colony were used. The animals were acclimated for 4—6 weeks to ambient temperatures of 4—8°C (5 rats, 265 ± 19 g, "cold-acclimated" group), to 30°C (5 rats, 275 ± 19 g, "warm-acclimated" group), or to room temperature (20—23°C, 10 rats, 260 ± 7 g, "control" group).

Two copper-constantan thermocouples 35 mm apart forming a probe were introduced under hexobarbital anaesthesia through the femoral artery into the aorta so that one of them came to lie in the aortic arch and the distal point a few mm above the diaphragma. Other thermocouples were placed into the colon, the interscapular brown fat, and, in some cases, into the liver. The observations were started on completely alert animals 6—12 hours after the operation had been performed. In some cases urethan was applied intraperitoneally in a dose of 0.3—0.4 g/kg body weight, to prevent restlessness. Oxygen consumption was measured with a modified Kipp-Noyons diaferometer (DONHOFFER et al. 1958) and a BD 1 type Kipp micrograph was used for precise (0.01°C) temperature recording.

Student's two tailed *t*-test was used for statistical evaluation.

## Results

Fig. 1 shows the response of a cold-acclimated rat to cold exposure. At an ambient temperature of 30°C there was no difference between the temperature measured in the aortic arch and 35 mm distally in the thoracic aorta. At an ambient temperature of 10°C, however, simultaneously with the increase in oxygen consumption of the animal and a sharp increase in interscapular brown fat temperature, blood temperature in the thoracic aorta began to exceed that in the aortic arch. After re-transfer to 30°C the former equilibrium was restored. Fig. 2 demonstrates that even small alterations in interscapular brown fat thermogenesis were accompanied by similar changes in the temperature difference between the two points in the thoracic aorta ($\Delta$T).

In other cases a small temperature difference existed between the two points already in a thermoneutral environment, and increased in the cold (Fig. 3).

Warming the blood in the thoracic aorta represents heat transfer from the periaortic tissues to the blood. The amount of heat transferred can be

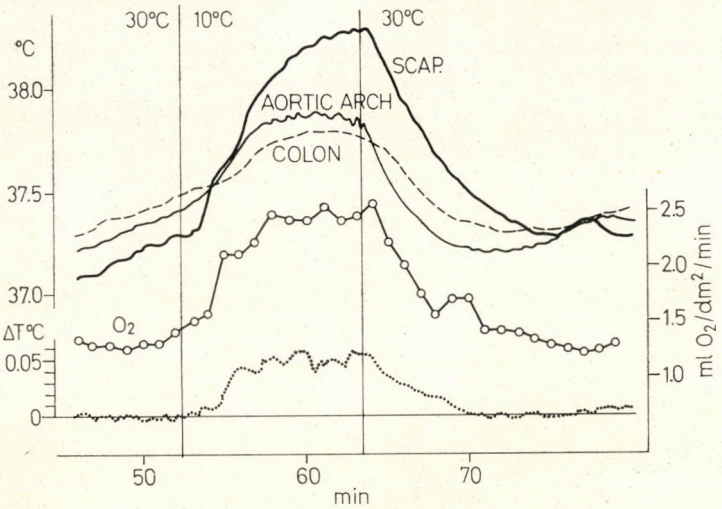


*Fig. 1.* Effect of cold exposure (10°C) on a 245 g cold-acclimated female rat. Temperature of interscapular brown adipose tissue (——); colon (— — —); blood in the aortic arch (———); oxygen consumption (o—o—o). Dotted line represents temperature difference ($\Delta T$) between thoracic aorta and aortic arch. The abscissa indicates the time elapsed since the measurements had been started

calculated from the $\Delta T$ and the blood flow through the thoracic aorta. The cardiac output of a rat weighing 280 g is about 80 ml/min (POPOVIC and KENT 1964), and it may be assumed that about half of the cardiac output flows through the thoracic aorta. During cold exposure, cardiac output increases by about 50% (JANSKY and HART 1968). In the case of the rat demonstrated

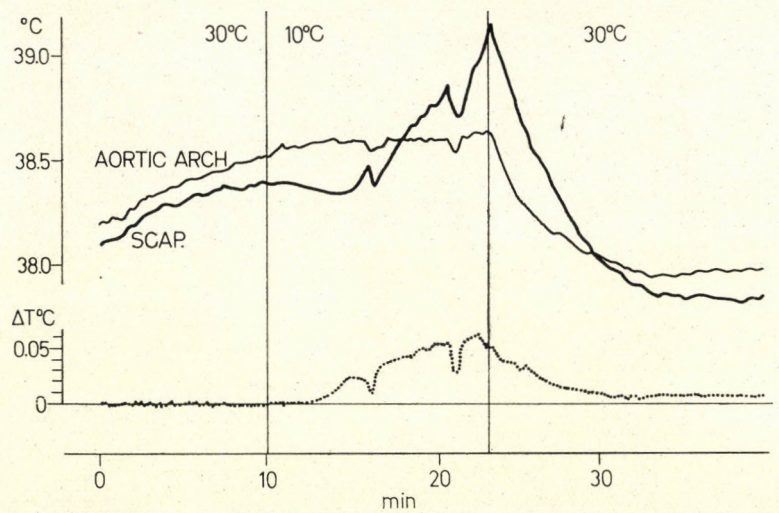


*Fig. 2.* Effect of exposure to an environmental temperature of 10°C on a 320 g male rat. Symbols as in Fig. 1

on Fig. 3, 40 ml/min and 60 ml/min blood was assumed to flow through the thoracic aorta in the thermoneutral and the cold environments, respectively. Accordingly, taking 1.0 as the specific heat of blood, a transfer of 0.8 cal/min was necessary to produce a $\Delta T$ of 0.02°C at an environmental temperature of 30°C, whereas in the cold, a transfer of 3.6 cal/min would have been necessary. This means that the heat transferred would have had to increase

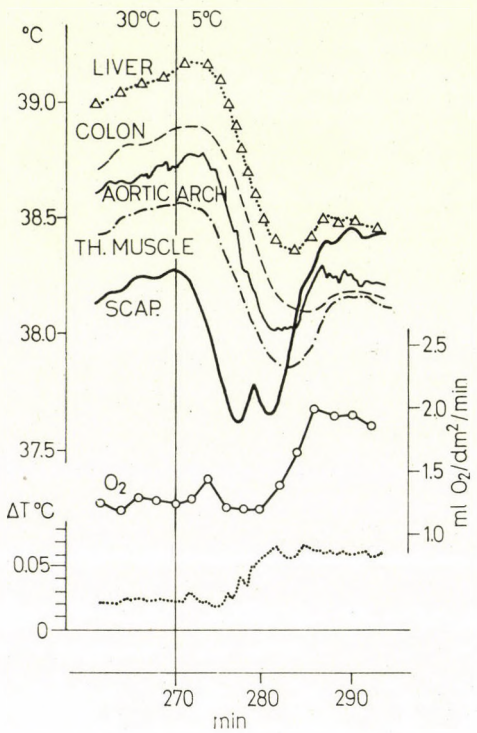


*Fig. 3.* Effect of exposure to 5°C ambient temperature on temperature of liver (△..△..△), colon (— — —), dorsal thoracic muscle (— · — · —), interscapular brown fat (———) and blood at aortic arch (———), and on $\Delta T$ (· · · · ·)

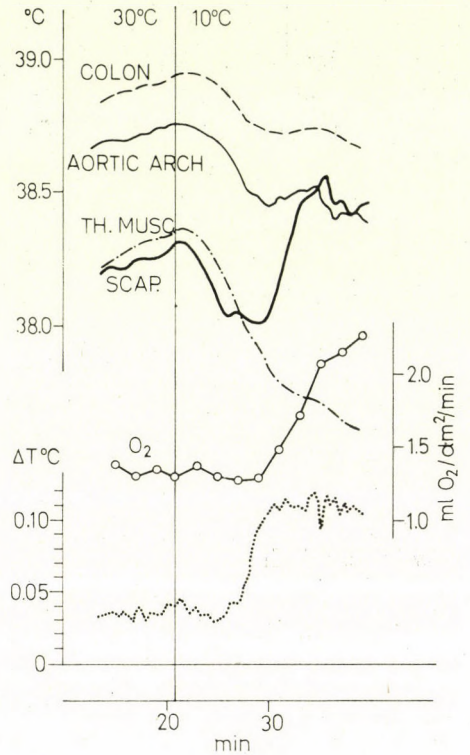


*Fig. 4.* Effect of cold exposure (10°C) on a 310 g male rat acclimated to room temperature. Symbols as in Figs 1 and 2

by 2.8 cal/min. The thermoregulatory increase in total heat production during cold exposure, as calculated from oxygen consumption, was 18.3 cal/min, i.e. 15.3% of the total thermoregulatory heat production had to serve to warm the blood of the thoracic aorta by 0.04°C.

The increase in $\Delta T$ in Fig. 3 appeared at 277 min, at the moment when interscapular brown fat temperature had just ceased to decrease, i.e. at the onset of brown fat thermogenesis. Temperatures at other measuring points

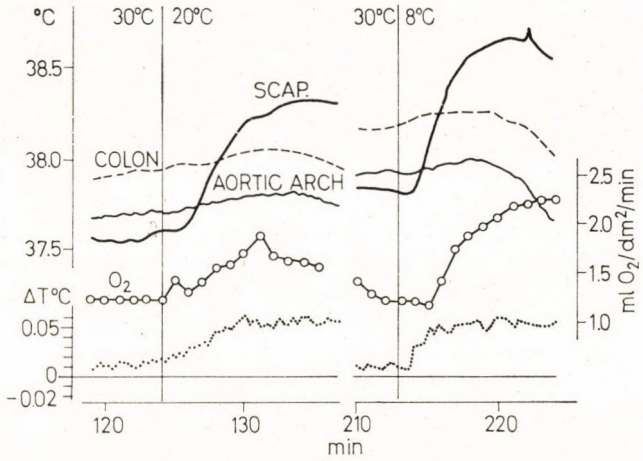


*Fig. 5.* Two details from an experiment on a 260 g male warm-acclimated rat. Symbols as in Figs 1 and 2

(even in the dorsal muscle beneath the brown fat) decreased further until interscapular brown fat temperature had begun to increase sharply (282—283 min) and $\Delta T$ in the aorta had reached its maximum. Taking into consideration the time-lag of the experimental setup (DONHOFFER et al. 1958), the increase in oxygen consumption coincided with the onset of brown fat thermogenesis and the increase in $\Delta T$.

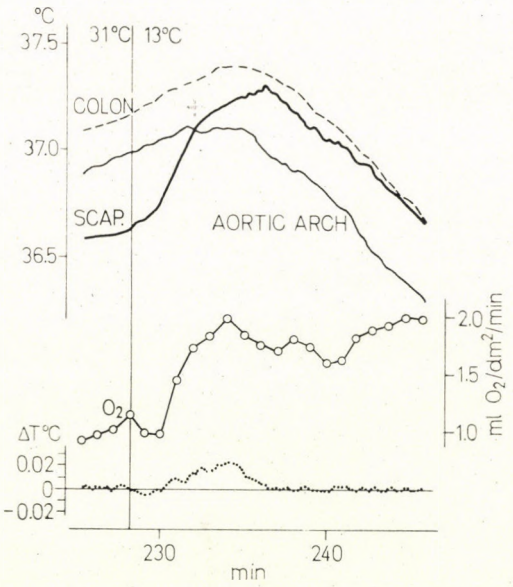


*Fig. 6.* Exposure to an environmental temperature of 13°C of a warm-acclimated rat (260 g ♀) caused a short and small increase of $\Delta T$ which disappeared in a few minutes. Symbols as in the former figures

**Table I**

| No. of rat | No. of cold exposures | Thermoregulatory changes of: $\Delta T$ (range) °C | $\Delta H_{transf}$, cal/min | $\Delta H_{prod}$, cal/min | Transferred fraction of $H_{prod}$, per cent | |
|---|---|---|---|---|---|---|
| 1. | 6 | +0.03 (0.01 to 0.05) | +2.4 | +18.2 | 13.2 | |
| 2. | 5 | +0.04 (0.02 to 0.06) | +2.1 | +23.9 | 8.8 | Cold- |
| 3. | 5 | +0.03 (0.02 to 0.05) | +1.8 | +22.9 | 7.9 | acclimated |
| 4. | 5 | +0.03 (0.03 to 0.04) | +2.0 | +21.7 | 9.2 | group |
| 5. | 6 | +0.08 (0.05 to 0.11) | +4.0 | +18.9 | 21.2 | |
| 1. | 3 | +0.05 (0.04 to 0.05) | +2.2 | +14.2 | 15.4 | |
| 2. | 5 | +0.04 (0.01 to 0.07) | +1.9 | +25.9 | 7.3 | |
| 3. | 2 | +0.03 (0.02 to 0.04) | +1.2 | +20.9 | 5.7 | |
| 4. | 5 | +0.01 (+0 to 0.02) | +0.4 | +20.3 | 1.9 | |
| 5. | 4 | +0.09 (0.08 to 0.10) | +5.0 | +22.7 | 22.0 | Control |
| 6. | 3 | −0.01 (−0.03 to +0.01) | +0.1 | +29.1 | 0.3 | group |
| 7. | 5 | +0.05 (0.03 to 0.08) | +2.8 | +21.3 | 13.1 | |
| 8. | 3 | +0.09 (0.08 to 0.11) | +4.8 | +22.6 | 21.2 | |
| 9. | 7 | +0.03 (0.02 to 0.04) | +1.8 | +19.3 | 9.3 | |
| 10. | 4 | +0.01 (−0.01 to +0.01) | +0.6 | +24.9 | 2.4 | |
| 1. | 3 | ±0 (±0) | −0.3 | +16.7 | −1.8 | |
| 2. | 1 | +0.04 | +1.6 | +12.0 | 13.3 | Warm- |
| 3. | 4 | −0.01 (−0.02 to +0.01) | −0.1 | +13.1 | −0.8 | acclimated |
| 4. | 5 | +0.04 (0.03 to 0.04) | +1.8 | +12.5 | 14.4 | group |
| 5. | 4 | ±0 (−0.02 to +0.01) | +0.3 | + 8.1 | 3.7 | |

T = temperature difference between the two points in the aorta
$H_{transf}$ = heat transferred to the blood of the aorta
$H_{prod}$ = total thermoregulatory heat production of the animal

Similar reactions were observed in rats kept at room temperature (Fig. 4).

Some warm-acclimated animals responded to cold exposure like the cold-acclimated rat (Fig. 5); in the majority of the experiments the temperature of aortic blood failed, however, to increase, whereas oxygen consumption and interscapular brown fat temperature increased in every instance. In the experiment demonstrated in Fig. 6, after cold exposure a small increase of $\Delta T$ was seen and even this disappeared within a few minutes, albeit interscapular brown fat temperature and oxygen consumption continued to increase.

Fig. 5 demonstrates that in warm-acclimated rats exposure to more severe cold failed to cause a greater increase of $\Delta T$ than that induced by exposure to less severe cold (18—20°C), therefore the latter temperature was

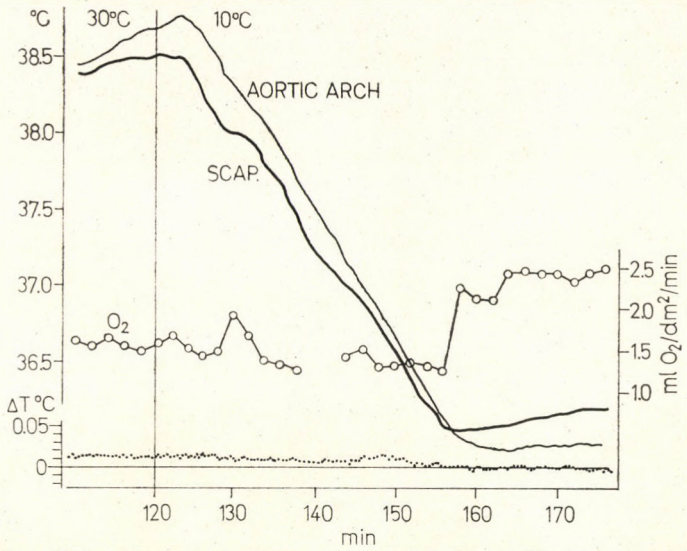


*Fig. 7.* The cold-acclimated guinea pig (700 g ♀) responded to cold exposure with an increase in thermogenesis in its interscapular adipose tissue. $\Delta$T showed a slight decrease instead of an increase. Symbols are similar as before

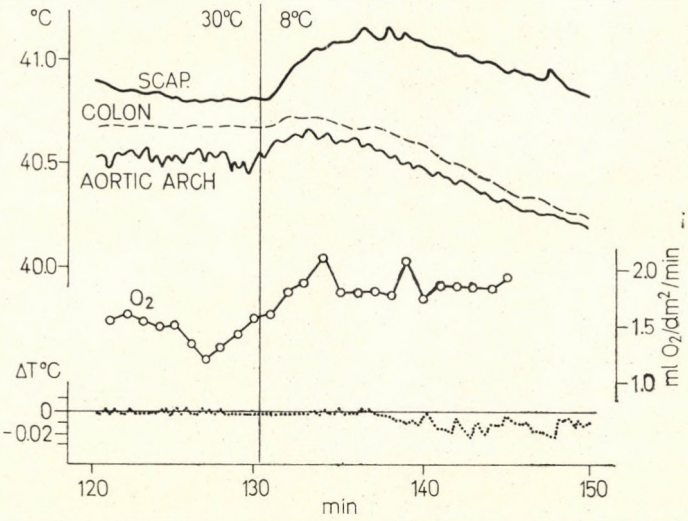


*Fig. 8.* The young rabbit (590 g) responded to cold exposure with no increase in $\Delta$T. For details see text. Symbols as in Figs 1—7

used in experiments on warm-acclimated rats to avoid a serious fall in body temperature.

Table I demonstrates, (a) the thermoregulatory change of $\Delta$T; (b) the heat transferred to the blood in the aorta ($\Delta H_{transf}$); (c) total thermoregulatory heat production ($\Delta H_{prod}$) and, (d) the fraction of total thermoregulatory heat production transferred to aortic blood.

In contrast to the rat, in adult cold-acclimated guinea pigs and in young rabbits the temperature in the thoracic aorta never rose above that in the aortic arch, while interscapular brown adipose tissue temperature exceeded core temperature (Figs 7 and 8).

## Discussion

The close parallelism between heat production by the interscapular brown fat and the warming of the blood in the thoracic aorta suggested that the periaortic brown adipose tissue was responsible for the phenomenon. It appeared possible, however, that the liver, as an important heat producing organ in the vicinity of the thoracic aorta, was involved in it. Although the temperature of the liver exceeded that of the aortic arch by about 0.45°C in a thermoneutral environment, in the cold environment the difference was reduced to about 0.25°C (Fig. 3). Moreover, liver temperature in some instances decreased somewhat below the arterial temperature, and continued to decrease in the cold well after the increase of brown fat thermogenesis and the increase in $\Delta T$. It is therefore obvious that in the cold, the liver could not have been the cause of any temperature increase at the distal point of the thoracic aorta.

The absence of the phenomenon in guinea pigs and rabbits finds its explanation in the absence of periaortic brown adipose tissue. The fact that in the rabbit and the guinea pig thermogenesis increased markedly in the interscapular brown adipose tissue, demonstrates that the heat produced by it is not responsible for the heating of the blood in the aorta.

The results obtained in warm-acclimated animals may be explained by the reduced amount of periaortic brown adipose tissue. Its considerably smaller amount in warm-acclimated animals accounts for the observation that the cold environment did not cause a statistically significant increase in the temperature difference in the thoracic aorta, while the difference was significant in the cold-acclimated group (Fig. 9).

The heat taken up by the blood of the aorta must have exceeded the calculated value (Table I) because brown adipose tissue is not confined to the investigated 35 mm segment but extends along the whole aorta. It is at the same time obvious that only a fraction of the heat produced by the periaortic brown adipose tissue can be transferred to the blood of the aorta; the rest is warming the other adjacent tissues. It has to be emphasized that the employed calculation could have resulted in not more than a rough approximation, since it depends on the correctness of the assumed cardiac output and the amount of blood flowing between the two measuring points. Nevertheless, with a little optimism one may assume that the calculated figures are not too far from actual values.

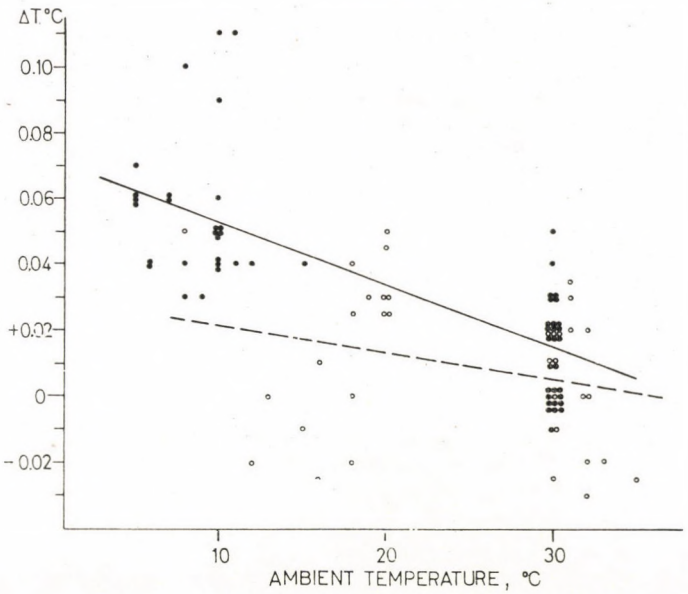


*Fig. 9.* Temperature difference between the two points in the thoracic aorta in cold-acclimated (dots) and in warm-acclimated (circles) rats at different ambient temperatures. The continuous line is the regression line for cold-acclimated animals ($y = -0.0019x + 0.0717$; $r = -0.697$; $p < 0.001$). The interrupted line represents the regression line for warm-acclimated rats ($y = -0.0008x + 0.0292$; $r = -0.267$; $0.1 < p < 0.2$)

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# THE EFFECT OF HYPOXIA, HYPERCAPNIA, β-ADRENERGIC BLOCKADE AND ANAESTHESIA ON HEAT PRODUCTION BY PERIAORTIC BROWN ADIPOSE TISSUE

By

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In cold-acclimated rats cold exposure caused an increase in the temperature of the blood in the thoracic aorta as compared to that in the aortic arch. Reduction or abolition of thermoregulatory heat production by hypoxia, hypercapnia, propranolol or hexobarbital reduced, or abolished the phenomenon in parallel with the thermogenetic function of interscapular brown fat. It is concluded that, in the rat, periaortic brown fat transfers heat to the blood of the aorta in the course of thermoregulatory heat production.

From the investigations of Smith (1961), Smith and Roberts (1964) and others (Donhoffer et al. 1964; Donhoffer and Szelényi 1967), cold exposure is well-known to induce a marked increase in thermogenesis in the interscapular brown adipose tissue in the rat as well as in a number of other species (reviewed by Smith and Horwitz 1969).

If perivascular brown adipose tissue were activated it would be expected to heat the blood flowing in the vascular bed. It was suggested that, in the rat, heat was being transferred during cold exposure from periaortic brown adipose tissue to the blood of the aorta (Székely et al. 1970).

In addition to activating brown fat thermogenesis by exposure to cold, it seemed worth-while to test whether stimuli or agents affecting thermoregulatory heat production without a change in ambient temperature (hypoxia, hypercapnia, propranolol, anaesthesia) exerted a similar effect on heat transfer from the periaortic brown adipose tissue to the blood in the aorta.

## Methods

Cold-acclimated adult rats of both sexes from the Institute's colony were used.

The methods were similar to those applied previously (Székely et al. 1971). An intraperitoneal cannula, the aortic probe containing the thermocouples, and the thermocouple in the interscapular brown fat were inserted under hexobarbital anaesthesia 6—12 hours before the experiments. The experiments were done on completely alert animals except of course those dealing with the effect of hexobarbital. Propranolol and hexobarbital were administered in the course of the observations intraperitoneally through the cannula inserted 6—12 hours earlier.

Hypoxia and hypercapnia were produced by attaching to the inlet of the measuring apparatus double walled plastic bags containing 12% $O_2$ in $N_2$ and 5% $CO_2$ in air, respectively (Szegvári et al. 1963; Várnai et al. 1970).

## Results

Fig. 1 demonstrates the effect of hypercapnia in a cold-acclimated rat. Prior to breathing 5% $CO_2$, brown fat thermogenesis and heat transfer to aortic blood were enhanced by exposure to cold. During 5% $CO_2$ breathing, brown fat temperature fell below colonic temperature, indicating a cessation or a marked reduction of brown fat thermogenesis. Simultaneously, the difference between aortic arch and distal thoracic aortic temperature ($\Delta$T) de-

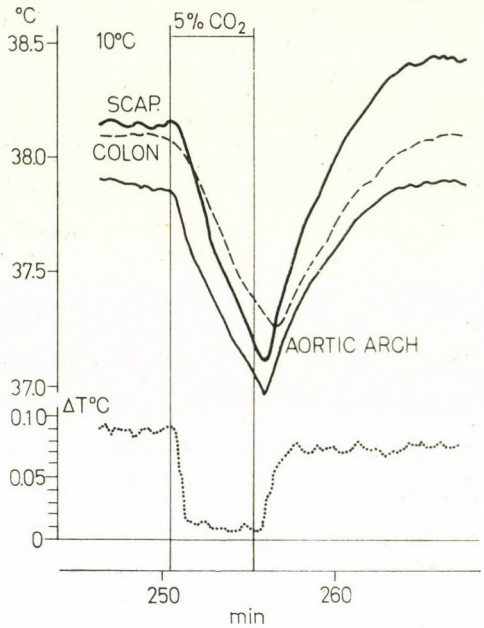


*Fig. 1.* Effect of $CO_2$ breathing in a cold-adapted rat during cold exposure on the temperature of interscapular brown fat (———), colon (— — —) and aortic arch (———). The dotted line represents the temperature difference between the two measuring points in the thoracic aorta. For details, see text

creased from +0.09°C to about +0.01°C. After termination of $CO_2$ exposure, $\Delta$T increased simultaneously with the increase in interscapular brown adipose tissue temperature.

Fig. 2 demonstrates the reproducibility of the effect in the course of the same experiment and, in addition, serves to compare the effects of breathing 5% $CO_2$ and of 12% $O_2$. Interscapular brown fat temperature fell to a lesser extent in response to breathing 12% $O_2$ than during breathing 5% $CO_2$. Similarly, heat transfer to aortic blood was reduced to a lesser extent in response to hypoxia than during hypercapnia, which completely abolished heat transfer from periaortic brown adipose tissue.

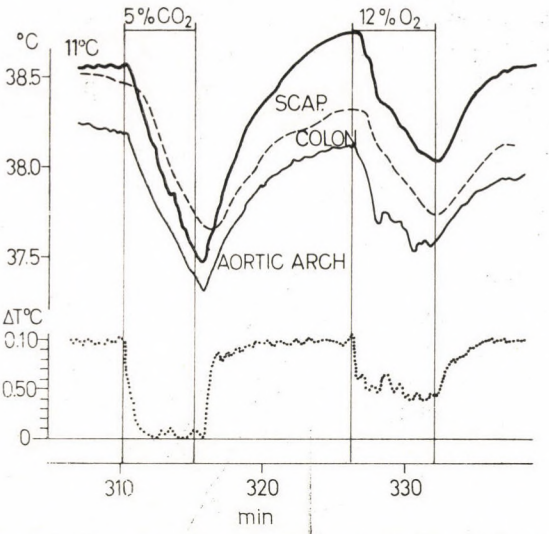


*Fig. 2.* Effects of repeated $CO_2$ exposure and of hypoxia in the cold in the same experiment as Fig. 1. Symbols are identical with those in Fig. 1. For details, see text

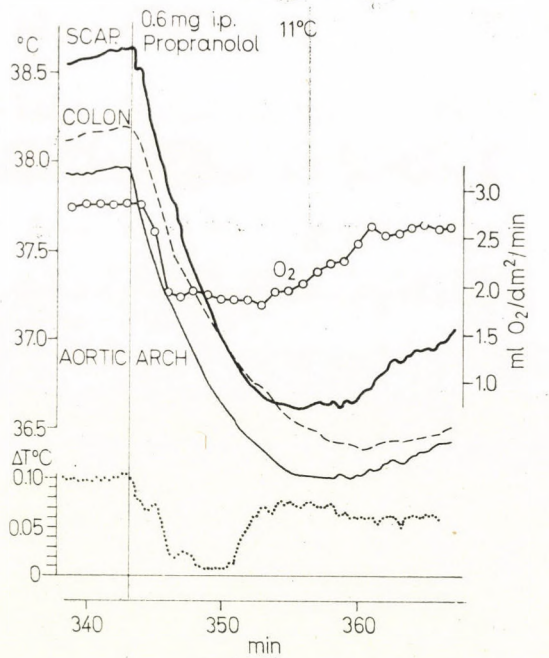


*Fig. 3.* Effect of 2.5 mg/kg intraperitoneal propranolol on temperature at the same sites as in earlier figures, and on oxygen consumption (o — o — o), during cold exposure. For details see text

Fig. 3 shows the effect of $\beta$-adrenergic blockade by 2.5 mg/kg propranolol. Before applying the drug, oxygen consumption was high, interscapular brown fat temperature exceeded colonic temperature, and a considerable amount of heat had to be transferred to the blood in the aorta. Propranolol decreased oxygen consumption and body temperature, brown fat temperature fell to a greater extent than did colonic temperature and $\Delta$T decreased

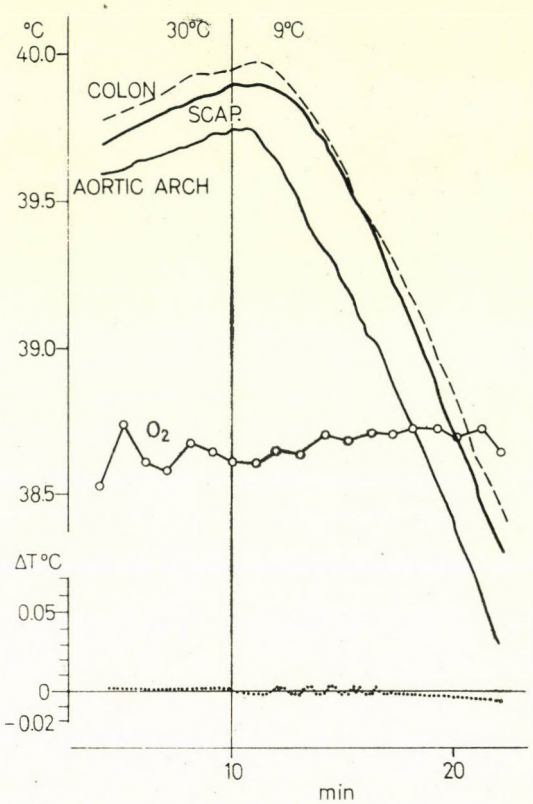


*Fig. 4.* Effect of cold exposure on a 250 g rat after 75 mg hexobarbital had been administered intraperitoneally 4 hours before "0" min. Symbols are identical with those in former figures. The animal was under the full effect of hexobarbital

gradually to zero, indicating that no heat was being transferred from the periaortic brown adipose tissue. As the first sign of recovery, $\Delta$T increased and interscapular brown fat temperature ceased to fall. Oxygen consumption, taking into consideration the time-lag of recording (DONHOFFER et al. 1958), increased simultaneously. Colonic temperature ceased to decrease only after interscapular brown fat temperature had exceeded core temperature.

Under hexobarbital anaesthesia, thermoregulatory heat production was abolished (Fig. 4): cold exposure failed to cause an increase in oxygen con-

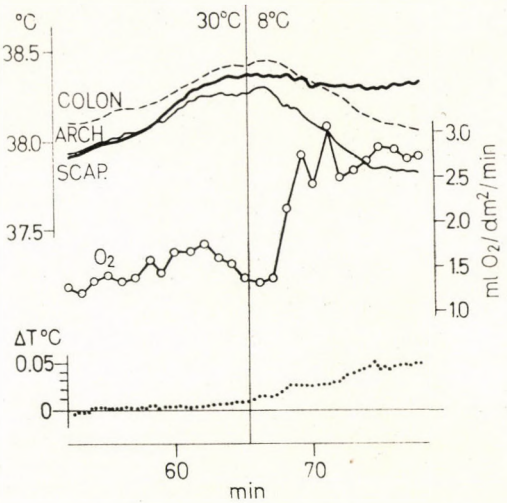


*Fig. 5.* The same animal 45 min later, showing some uncoordinated movements

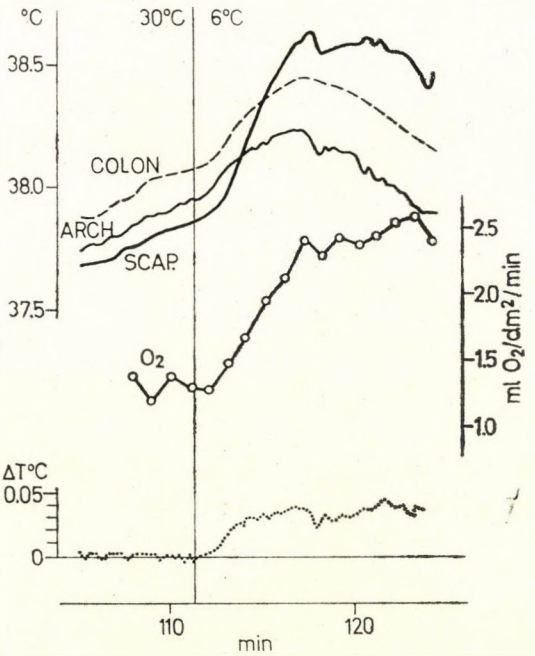


*Fig. 6.* The same animal after another 45 min, apparently recovered from the effect of hexobarbital

sumption, no heat was being transferred from brown adipose tissue to aortic blood, and interscapular brown fat temperature fell in parallel with colonic and aortic temperature. In the course of recovery from the effect of hexobarbital, the rat again responded to cold exposure with an increase in heat production. Simultaneously, interscapular brown fat temperature rose above colonic temperature and the temperature difference between the two aortic thermocouples ($\Delta$T) reappeared (Figs 5 and 6).

## Discussion

Any temperature change of the blood in the aorta indicates heat transfer through the wall of the aorta. Earlier experiments led to the conclusion that, in the rat, the rise in blood temperature between the aortic arch and the distal thoracic aorta in response to cold exposure was due to heat transferred from the periaortic brown adipose tissue. This conclusion was supported by the absence of the phenomenon in such species which have no brown fat around the aorta (guinea pig, rabbit). This observation evidently excluded interscapular brown fat as the cause of the phenomenon, since interscapular brown fat thermogenesis persisted in the guinea pig and rabbit.

The present experiments also support the role of periaortic brown adipose tissue. Hypoxia (Lintzel 1931; Hill 1959; Szegvári et al. 1961, 1963; Moore and Underwood 1962; Blatteis 1964) as well as hypercapnia (Stupfel 1960a, b; Bullard and Crise 1961; Szegvári and Várnai 1962; Szegvári et al. 1963) are well-known to reduce oxygen consumption and body temperature at ambient temperatures below the thermoneutral zone. Hypoxia was shown to reduce heat production also in brown adipose tissue (Dawkins and Hull 1964; Heim and Hull 1966a; Szelényi and Donhoffer 1968). Both hypoxia and hypercapnia strongly reduced thermogenesis in the interscapular brown fat, and reduced or abolished the temperature difference ($\Delta$T) in the aorta (Figs 1 and 2).

Propranolol in large doses is known to block the cold-induced increase in heat production (Webster and Hays 1968), and to result in a marked decrease of brown fat thermogenesis (Heim and Hull 1966b). Heat transfer from periaortic tissues to the blood changed in close parallelism with brown fat thermogenesis under the effect of the drug as well as during recovery from it. It is most unlikely that anything else than brown fat tissue could have been responsible for the heat transfer to the blood in the aorta.

Hexobarbital in the applied dosage abolished thermoregulatory heat production, and also the temperature difference between the measuring points in the aorta. During recovery the difference was re-established parallel with the metabolic response to cold.

The observation that during hypoxia and hypercapnia as well as under the effect of propranolol or hexobarbital the temperature difference between the aortic arch and the distal measuring point in the aorta was abolished, furnished further evidence of the role of periaortic brown adipose tissue in heating the aortic blood. The same applies to the gradual reappearance of $\Delta T$ in the course of recovery from the above effects.

Brown fat was generally supposed to heat the blood returning to the heart and the intrathoracic viscera (SMITH and HORWITZ 1969) or to be primarily of local importance (BRÜCK and WÜNNENBERG 1966). The observations presented here support the conclusion that, in the rat, heat is being transferred from the periaortic brown adipose tissue to the aortic blood stream.

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# MUSCLE ACTIVITIES OF PARTIALLY INNERVATED LIMBS DURING LOCOMOTION IN AMBYSTOMA

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Muscle potentials were recorded simultaneously from 8 muscles of intact and partially innervated forelimbs in freely moving Ambystoma. The partial innervation involved limbs supplied only by the 3rd, the caudal part of the 3rd, the 4th, the cranial part of the 4th, the caudal part of the 4th, and the 5th ventral root, respectively. The activities of functionally opposing muscle groups were extensively overlapping in myochronograms recorded from intact limbs during locomotion. Myochronograms of limbs innervated only by the 3rd, or by the caudal half of the 3rd, ventral root showed a predominantly protraction activity, but some of the muscles were active also during retraction. Myochronograms of limbs innervated by the 4th ventral root alone closely resembled the normal muscle activity pattern. This pattern seemed to be composed of the myochronograms recorded from limbs which were supplied only by the cranial part, and the caudal part, of the 4th ventral root, respectively. When the 5th ventral root supplied the limb, retraction activities prevailed in the myochronograms, though protraction activities were also recorded. In general, the active periods of muscles were shorter in the partially innervated than in the intact limbs. The activity patterns recorded in the different experimental groups indicated that the innervation of 5 muscles originated from all three segments, and that of 3 muscles from two segments. The total length of the active period of a muscle was composed of shorter active periods arising from different places of the three segments during a walking step. From the temporal distribution of the short active periods it has been concluded that each segment, or parts of them, generated a cyclic output pattern.

Limb muscles disclose complicated interactions which involve co-contractions of different degrees of antagonistic muscle groups in locomotion (ALTENBURGER 1937; ENGBERG 1964, TOMITA 1967). The action potentials recorded from eight forelimb muscles of freely moving salamanders showed that the muscles investigated co-contracted almost simultaneously during both the protraction (swing) and the retraction (stance) phase of the walking step (SZÉKELY et al. 1969). This kind of muscle interaction is generally regarded as an important factor in the stabilization of the limb and grading the movement, and a number of proprioceptive, postural and supraspinal mechanisms is supposed to be involved in the activities. This is, however, contradicted, at least in the salamander, by the observation that the muscle activity pattern does not change following limb deafferentation, though irregularities in placing the limb are apparent (SZÉKELY et al. 1969).

The spinal structure controlling the delicate time sequence of muscle activities is unknown. Transplantation experiments performed on urodele embryos indicated that the limb moving section of the spinal cord alone

has the capacity to move the limb in a co-ordinated manner (DETWILER 1936; WEISS 1941; SZÉKELY 1963). This section consists of three segments in most urodeles and, as shown by NICHOLAS and BARRON (1935), the stimulation of any of the spinal nerves emerging from these segments evokes contraction of almost all of the forelimb muscles in Ambystoma. Similar results have been reported by the present authors (SZÉKELY and CZÉH 1967) in that on systematic stimulation by microelectrodes of these three segments, muscle contractions were observed under the dissecting microscope. According to these findings, protractor muscles are generally represented cranially from the retractor muscles, but the topographical relations of individual limb muscles are inaccurately reflected in their spinal representation. From these results the idea has been put forward that the inaccurate somatotopic representation of limb muscles may conceal a functional arrangement of motor neurones in the sense that irrespective of their functional denomination and topographical situation, muscles which are active in a certain phase of stepping, are innervated from the same sites of the spinal cord.

To test this supposition, it seemed interesting to record muscle potentials from limbs innervated by a single segment of the limb moving section of the spinal cord. Since the majority of the limb muscles are represented in each of the three brachial cord segments in Ambystoma, it was expected that the activity pattern recorded simultaneously from 8 muscles will provide information concerning the functional organization of motor neurones.

## Methods

The experiments were performed on 10 *Triturus cristatus* and 14 *Ambystoma mexicanum*. In Triturus, innervation of the forelimb originates largely from the 3rd and 4th spinal segments, to which the 5th segment contributes with a small side branch only. In Ambystoma, the brachial plexus is built up from the nerves of all the three segments. Since this latter type of innervation was more suitable for the present purpose, the results have been based mainly on data obtained from Ambystoma. Before recording, the spinal nerves were cut in six different combinations so that together with the control animals they could be divided into 7 groups according to the innervation left intact (Fig. 1). Group 1 comprised two intact animals for the normal myochronogram. In Group 2 the 4th and 5th spinal nerves were severed, and the limb was innervated solely by the 3rd nerve (3 animals). In some cases the 3rd and 4th ventral root originated with two rootlets, and part of the motor fibres comprising the ventral root could be left intact. In Group 3 all nerves were severed except the caudal part of the 3rd ventral root (1 animal). In Group 4 the limb was innervated by the 4th spinal nerve alone (3 animals). In Group 5 and Group 6, each consisting of a single animal, the limb was moved by the cranial part and caudal part of the 4th spinal nerve, respectively. In Group 7, only the 5th spinal nerve was left intact (3 animals). In one animal of Groups 2, 4 and 7 the nerves were deafferented and the recording was repeated on the next day. Since the pattern did not differ significantly from that recorded previously, further deafferentation experiments have not been performed. In further 3 animals selected from the same groups, first the normal pattern was recorded, then, with the electrodes left in their original position, the nerves were cut and the recording was repeated. The results of these "control" experiments did not differ from those of the rest.

For recording, the following 8 muscles were used: *acromialis* (shoulder protractor), *dorsalis scapulae* (shoulder elevator), *latissimus dorsi* (shoulder retractor), *pectoralis* (shoulder

depressor), *brachialis* (arm flexor), *extensor ulnae* (arm extensor), *flexor digitorum* (forearm flexor) and *extensor digitorum* (forearm extensor). The insertion of electrodes into the muscles and the recordings from freely moving animals were made as described previously (SZÉKELY et al. 1969). The recording apparatus was a Hellige electroencephalograph combined with a set of high input impedance preamplifiers with RC circuits. In 3 animals the recordings were made with the Bell-Howell oscillograph using type 7—363 galvanometers and a set of appropriate preamplifiers (Electrophysiological Instruments Ltd., Midlothian). The high frequency range of the galvanometers (0—1000 H) permitted to record the activity of individual muscle fibres and to compare these results with the interferograms recorded with the EEG apparatus. No difference in time pattern was found between the two types of recording.

A few obvious limitations of the method have been described earlier (SZÉKELY et al. 1969). The most relevant of these is that the pattern of the myogram of individual muscles was somewhat influenced by the position of the electrodes within the muscles. These varia-

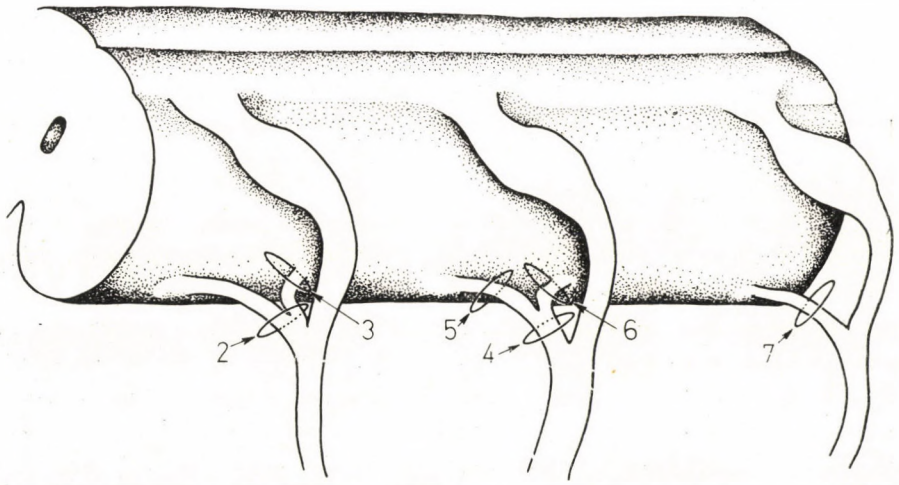


*Fig. 1.* Schematic representation of the brachial section of the spinal cord with the three (3rd, 4th and 5th) spinal nerves constituting the brachial plexus. Numbers pointing to the encircled ventral roots indicate which of the nerves supplies the limb in the respective experimental groups

tions of the myogram will be described together with the results for Group 1. Also, there are individual variations in the length of the three segments, consequently in the number of motor fibres of the three ventral roots. Therefore, when studying the activity of a single segment, or of part of it, variations may be expected to occur from one animal to another. In view of the small number of animals, especially in Groups 3, 5 and 6, we could not obtain data sufficient for a reliable estimation of whether or not these variations had influenced the final results. A few obvious variations will be pointed out, and they suggest that a larger number of animals would have furnished more exact results, though the basic pattern of activity of the individual segments would not have been altered essentially.

## Results

The movement of the limb in the act of walking has been analyzed in detail elsewhere (WEISS 1941; SZÉKELY et al. 1969), therefore only a few pertinent details will be repeated here. The stepping movement can be divided

into two phases: protraction and retraction. During protraction the elbow is slightly bent and the wrist, from a dorsi-flexed position at the end of retraction, becomes volar-flexed and with gradual extension reaches the ground in a completely extended position. During retraction the elbow on which the animal seems to balance its weight is bent in about a right angle, and the wrist assumes a dorsi-flexed position as the body is pushed forward. These two phases, which constitute the cycle of a walking step, will be distinguished in the myochronograms. If the limb is innervated by the 3rd segment, it performs only an incomplete protraction, in that the animal lifts the operated limb and drops it from a position perpendicular to the body. No change can be seen in the movement when the innervation arises from the 4th segment. A limb innervated by the 5th segment is extended almost parallel to the body, and visible movements occur only at the wrist.

*Group 1*

Fig. 2 shows the muscle activity pattern of an intact animal. The phases of protraction and retraction (arrows) are reconstructed from the activity of *dorsalis scapulae* and *extensor digitorum* which disclose the most stable activity coinciding with protraction, as has been shown previously (SZÉKELY et al. 1969) by means of step-marker used to indicate when the foot was on and off the ground. The close similarity of the present myochronogram with that obtained in Triturus renders a detailed description superfluous. However, a few details may be pointed out. The activity of *acromialis* appears consistently in protraction, and it usually goes over to the beginning of retraction. An activity in the middle of retraction can occasionally be observed (as in the case represented), but its presence seems to depend on the position of the electrodes within the muscle. The activity of *dorsalis scapulae* is one of the most stable parts of the pattern, although, depending on the electrode's position, this muscle may also show a slight activity during retraction. Owing to its slenderness, *latissimus dorsi* is the muscle most difficult for recording. The activity obtained here is usually preceded by an activity of lower amplitude, starting in the middle third of retraction (cf. Fig. 9). The activity of *pectoralis* may be regarded as characteristic; its short activity during protraction may be absent if the electrodes are implanted into the posterior edge of the muscle. Of the arm muscles, *brachialis* shows a short protraction activity which continues, sometimes with a short interruption, into retraction. This latter activity starts with a high amplitude which gradually decreases towards the end. *Extensor ulnae* arises with three heads, and its activity pattern is again slightly dependent on the electrode's position. With the electrodes in its middle part (head I), the muscle displays a retraction activity which ends with a high amplitude and lasts longer than that of *brachialis*. There

seems to be a reciprocity in the amplitude height of the two arm muscles, but this difference was not consistent enough for being appreciated within the reliability of the method. Unfrequently, *extensor ulnae*, too, showed a short protraction activity. The patterns of the forearm muscles are rather stable. They seem to depend the least on the position of the electrodes. A reciprocal

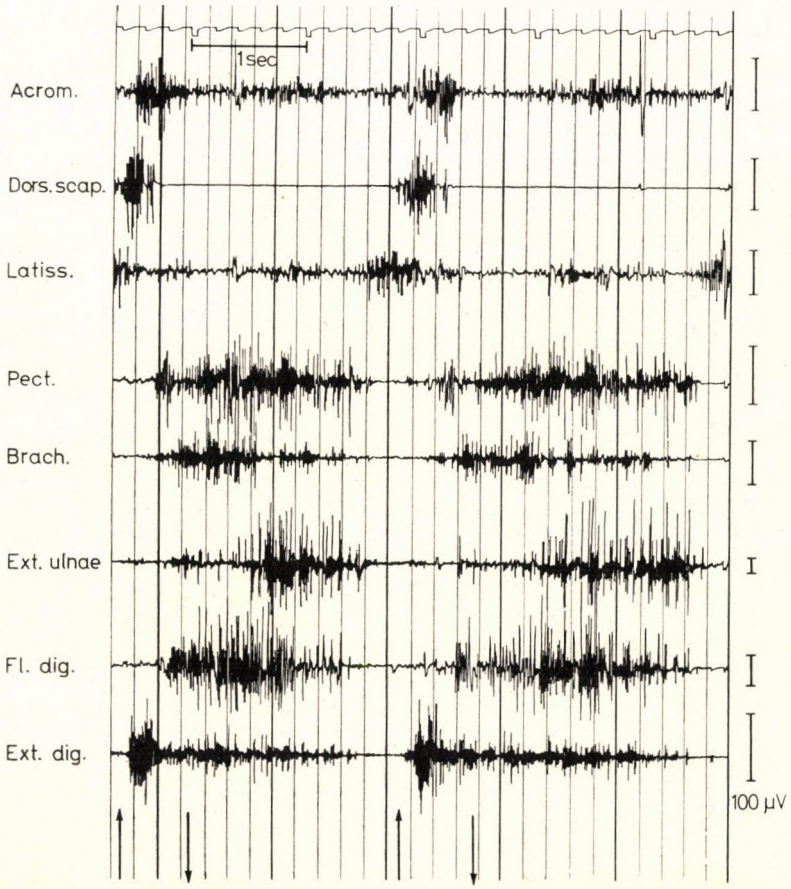


*Fig. 2.* Normal myochronogram of the eight muscles investigated. Upward pointing arrow indicate when the limb is lifted, downward pointing arrows indicate when the limb is put down. The first line is the time scale, on the right voltage calibrations are shown

relationship could be seen in the amplitude of their activities, *extensor digitorum* showing a higher amplitude during protraction, and *flexor digitorum* during retraction. The protraction activity of the latter muscle may sometimes be absent. The ratio of the length of protraction to the length of retraction was approximately 1 : 3 in most of the cases.

*Group 2*

After nerve transection the interferograms became thinner, a number of activities was absent, and the recording required a higher gain. These changes are shown in Fig. 3 which represents the myochronogram of a limb innervated by the 3rd segment. While the amplitude of muscle activities was subject to individual variations, their timing showed fairly constantly the effect of

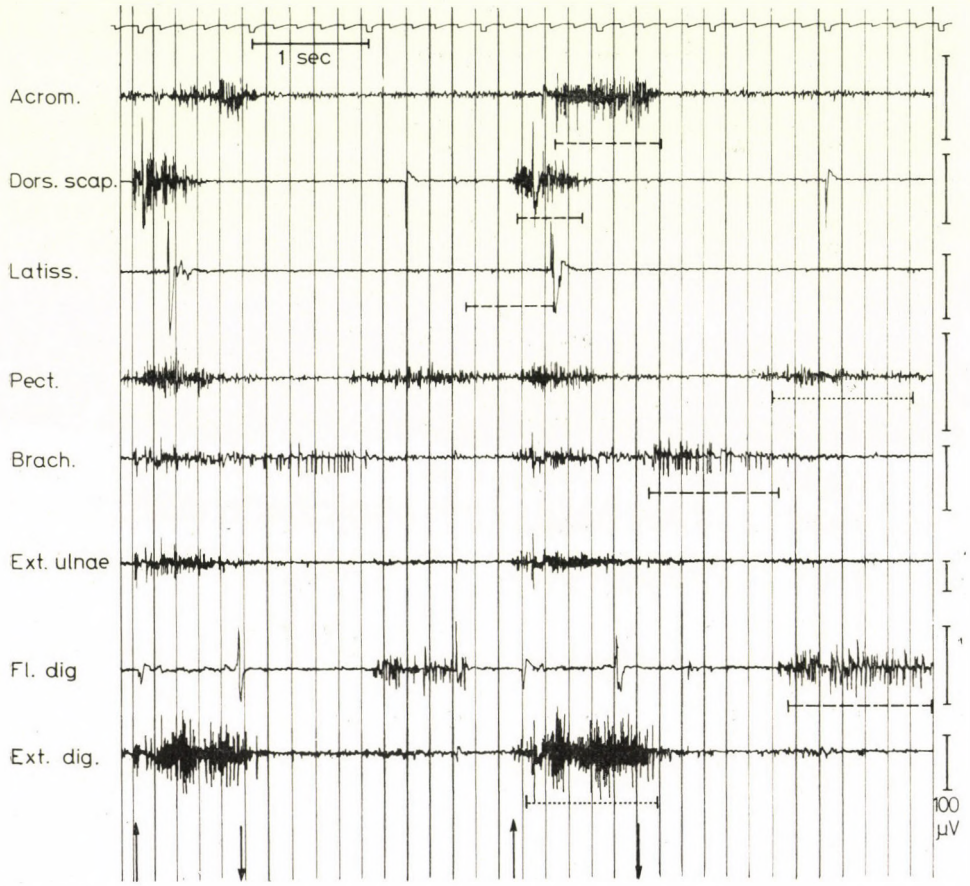


*Fig. 3.* Myochronogram of a limb innervated by the 3rd spinal nerve. Broken lines under the activities recorded on the 3rd, 2nd, 1st, 5th and 7th channels indicate the cyclic activity of the corresponding muscles (see text). *Latissimus* activity has been obtained from another myochronogram of this experimental group. Dotted lines indicate simultaneous activities

nerve transection from one animal to another. It is mainly these latter changes which we shall consider.

If one compares Figs 2 and 3, the timing of the activity of *acromialis* and *dorsalis scapulae* appears to be normal (during retraction *acromialis*

is not consistently active). Owing to electrode failure, in the case represented no activity has been recorded from *latissimus dorsi;* in the other two animals this muscle disclosed a slight activity during, and immediately preceding, protraction. *Pectoralis* is active in protraction and in the second half of retraction. In another animal there was also a short activity in the first half of retraction, the two active periods having been separated from each other. The arm muscles are active in protraction and *brachialis* shows an activity also in the first part of retraction. This latter pattern was seen only in this animal. Of the forearm muscles, *flexor digitorum* is active during the second half of retraction, and the extensor muscle during protraction.

The myochronogram clearly demonstrates the dominance of protraction activities. The time span of protraction is increased at the expense of retraction, showing a ratio of 1 : 2. Within the extended period of protraction the activity of muscles is longer than in intact animals, and muscles which normally seldom disclose a protraction activity (e.g. *extensor ulnae*), are also contracting. The prevalence of protraction is somewhat reduced when the animal is walking slower. This suggests that some of the protraction activities may be attributed to labyrinthine reflexes, since at higher speed the animal has a bad limp. The retraction activities of the four muscles are obviously too weak to bring about visible motion of the limb.

*Group 3*

If the limb is innervated by the caudal part of the 3rd segment, the only movement it performs is a slight elevation. Such a myochronogram is shown in Fig. 4; in it the activity of four muscles can be observed. The activity of *Acromialis* is shorter than in the former myochronograms, *pectoralis* is active in the first part of retraction, and *extensor ulnae* at the end of retraction. *Extensor digitorum* shows the usual activity during protraction, and this goes over into retraction with a lower amplitude. At higher walking speed a slight activity can be recorded also from *pectoralis* and *extensor ulnae* during protraction.

*Group 4*

As the movement of limbs innervated by the 4th segment does not differ appreciably from the normal movement, the myochronograms recorded from such limbs are also very close to the normal pattern (Fig. 5). The slight deviations are as follows. *Acromialis* starts a little later. *Dorsalis scapulae* (indicated with bars) and *latissimus dorsi* show a normal pattern; perhaps the *latissimus*' activity ends somewhat sooner than in the normal pattern.

*Pectoralis* is active in the second part of retraction; in another animal its activity began, however, sooner. The arm and forearm muscles show a normal pattern with the exception of a gap between the protraction and retraction activities of the forearm muscles. This gap was much less conspicuous in the

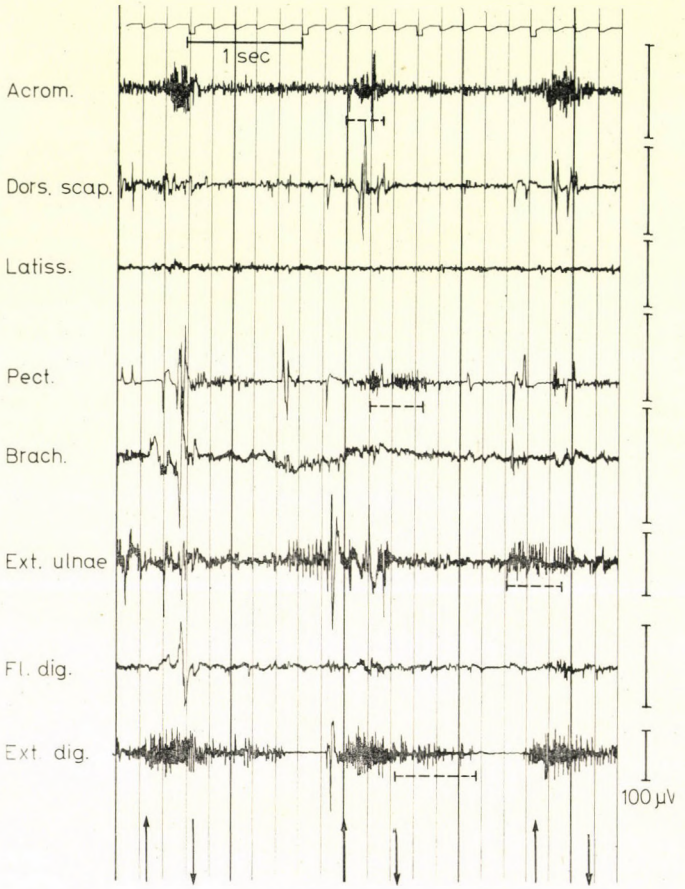


*Fig. 4.* Myochronogram of a limb innervated by the caudal part of the 3rd ventral root. Broken lines under the activities recorded on the 1st, 4th, 8th and 6th channels indicate the cyclic activity of the corresponding muscles

other two animals. It may be noted that the wider gap between protraction and retraction in this record is somewhat artificial, since the animal made a short interruption between the two phases of the walking step. The ratio of the length of protraction to that of retraction is also close to normal.

*Groups 5 and 6*

Myochronograms recorded from limbs innervated by the cranial or caudal part of the 4th segment may be considered together. Figs 6 and 7 show extensive overlaps in the active periods of individual muscles in the

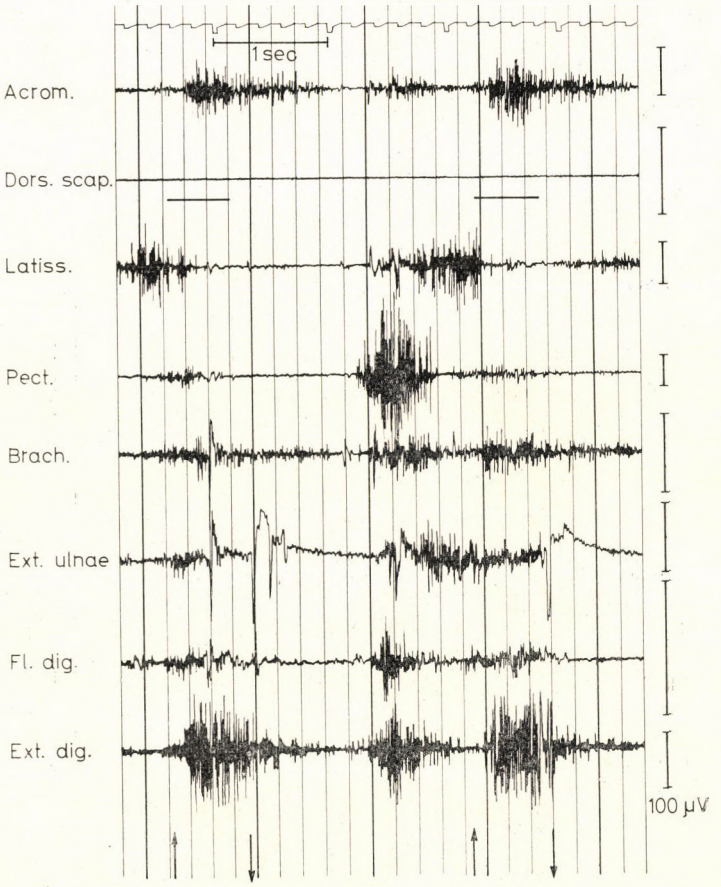


*Fig. 5.* Myochronogram of a deafferented limb innervated by the 4th spinal nerve. The activity pattern is the same as in the other animals in which the sensory innervation of the 4th segment was intact. The second channel is silent, the horizontal bar indicates the activity of *dorsalis scapulae* as obtained from another myochronogram of this group

two myochronograms, but it is also apparent that some of the activities starts earlier, or ends later, in one myochronogram than in the other. Concerning the details, *acromialis* and *dorsalis scapulae* are active almost with the same timing as in animals with the whole 4th spinal nerve left intact. *Dorsalis scapulae* appears in Fig. 6 with much higher amplitude and for a longer period

of time than in Fig. 7, and the reverse is true for *acromialis*. In addition to protraction, *latissimus* shows also a retraction activity in Fig. 7. *Pectoralis* is active for a short while at the beginning and at the end of retraction in Fig. 6, while in Fig. 7 its activity is limited to the middle part of retraction. *Brachialis* activity is relatively pronounced in protraction and confined to

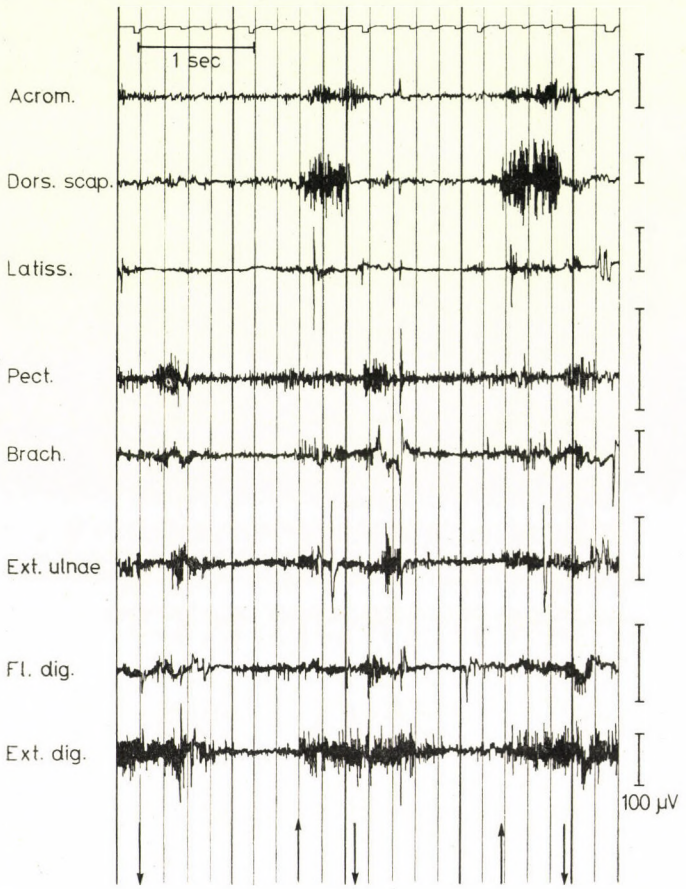


*Fig. 6.* Myochronogram of a limb innervated by the cranial part of the 4th ventral root

the second part of retraction in Fig. 6, whereas in Fig. 7 this muscle is active in the first part of retraction. *Extensor ulnae* shows some protraction activity in Fig. 6, its retraction activity starts sooner in the same myochronogram and ends later in Fig. 7. The first part of the retraction activity of *flexor digitorum* appears in Fig. 6, while the second part seems to arise more from the caudal part of the 4th segment. Of the protraction activity of *extensor digitorum*

only the last part is represented in Fig. 7. The retraction activity seems to be divided between the two myochronograms, the first part appears in Fig. 6, and the second of this activity seems to be transferred to Fig. 7.

Unfortunately, one animal only was used for each of these two records and we have no information about the possible individual variations, though

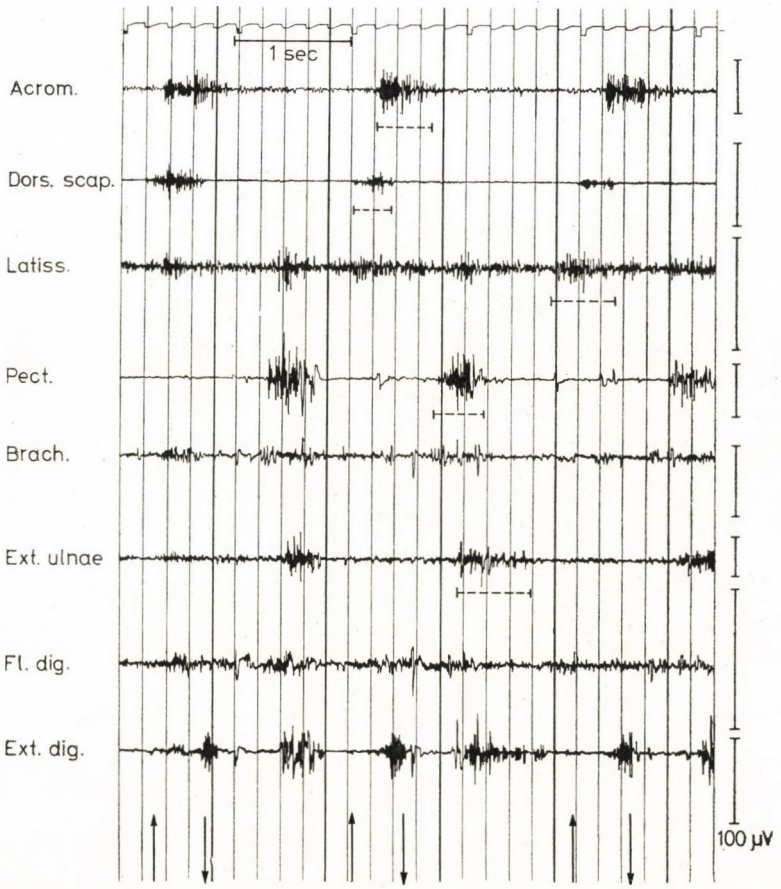


*Fig. 7.* Myochronogram of a limb innervated by the caudal part of the 4th ventral root. Broken lines under the 2nd, 1st, 4th, 6th and 3rd channels indicate the cyclic activity of the corresponding muscles

a few comments may be made on the results. If one compares Figs 6 and 7 with Fig. 5, with the exception of the comparatively large gap between the protraction and retraction activities of *pectoralis* and of the forearm muscles, the activities of individual muscles appear to complement each other to give rise to the pattern seen in Fig. 5. It has, however, been mentioned that this gap was much less conspicuous in the other two animals. On the other hand,

if one compares Fig. 4 with Fig. 6, an early retraction activity of *pectoralis* and *extensor digitorum* is apparent in both myochronograms. This suggests that motor neurones responsible for these activities may be situated at the border of the 3rd and 4th segments and, depending on individual variations, their axons leave the cord either through the caudal rootlet of the 3rd, or the cranial rootlet of the 4th, root.

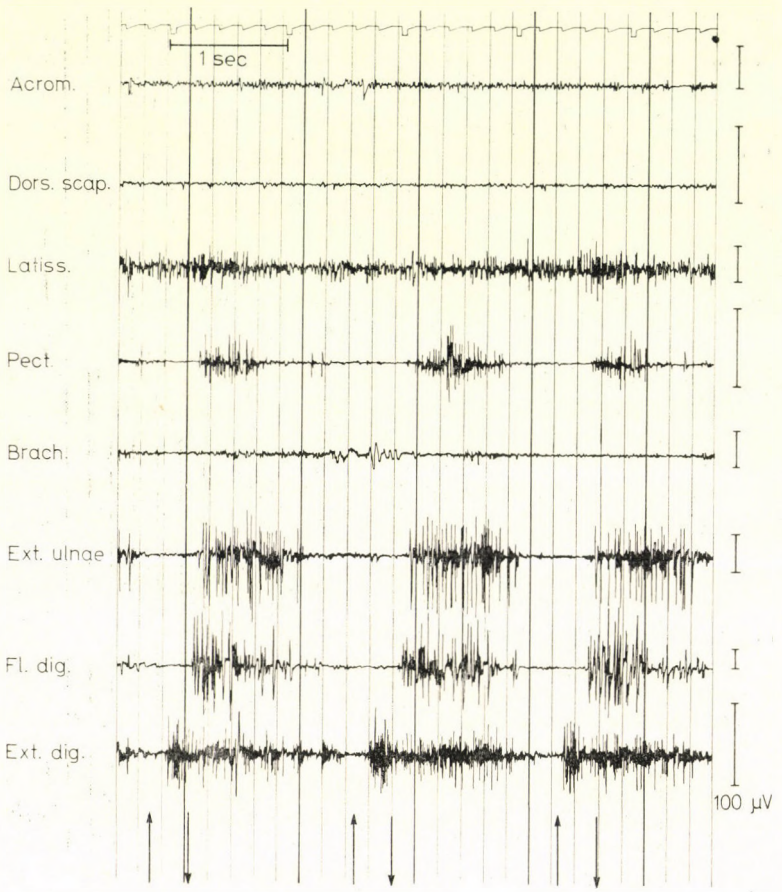


*Fig. 8.* Myochronogram of a limb innervated by the 5th spinal nerve. Channels 1, 2 and 5 are silent, these muscles are not represented in the 5th segment

*Group 7*

A strong prevalence of retraction activity can be seen in myochronograms recorded from limbs which are innervated by 5th segment (Fig. 8). Channels 1, 2 and 5, for *acromialis, dorsalis scapulae* and *brachialis,* are silent. This is in accordance with our previous finding (SZÉKELY and CZÉH

1967) that these muscles could not be driven from the 5th segment with electrical stimuli. Channel 3 is very noisy in the presented myogram and unfortunately little information was obtained about this muscle from either of the other two animals. As far as one can make out from this record, *latissimus* is active both in protraction and retraction, it ends, however, somewhat sooner than in normal patterns. The four other muscles are normally timed in retraction, the protraction activity of *Flexor digitorum* is absent, and that of *extensor digitorum* starts later than in the intact animal. In one of the animals the protraction activity of this latter muscle was also absent. The ratio of the length of protraction to that of retraction is approximately 1 : 4.

## Discussion

The first feature which appears in the myochronograms is the extensive co-contraction of functionally opposing muscles. This interaction of antagonistic muscle groups has been discussed earlier (SZÉKELY et al. 1969), and from the finding that it did not change following deafferentation of the limb, we have concluded that the pattern of muscle activities of walking is laid down in the structure of the spinal cord in newts.

Another feature of the results is that, in addition to the strong protraction activity, the 3rd segment controls also a weak retraction activity, in the 5th segment the retraction activity prevails, and the two activities are well balanced in the 4th segment. Regarding the vigorous hand movements, protraction activity may have been more pronounced in the 5th segment, could we have recorded it from the small hand muscles which are innervated largely by this segment. These results wholly corroborate our previous findings of the localization of motor neurones innervating individual muscles: with the exception of a few protractor muscles, the whole limb is represented in every one of the three segments, but in different combinations in each segment (SZÉKELY and CZÉH 1967). It can also be seen that in the records taken from partially innervated limbs, the interferograms are thinner and the amplitude lower, indicating the activity of fewer muscle fibres than in the intact animal. Nevertheless, if one regards the activities which are the least influenced by the electrode position in intact animals (c.f. Fig. 9), within the time span of these activities shorter or longer active periods could always be recorded from the 8 muscles of partially innervated limbs, unless the muscle was absent from the innervating segment (e.g. *acromialis*, *dorsalis scapulae* and *brachialis* in Fig. 8). Thus, five of the eight muscles investigated receive trisegmental and the other three bisegmental innervation. The shortened activities recorded after nerve transection raise the question whether the shortening is due to shorter commands from a single segment of the spinal cord, or part of the muscle activity is beyond the detection of the electrodes. Owing to technical

difficulties, there is no direct way to decide between the two alternatives, but the following considerations are in favour of the assumption that the multisegmental innervation is evenly distributed in the muscle and although the electrodes pick up muscle potentials from a circumscribed area, the activity patterns recorded may be regarded as a measure of the output from the actually innervating part of the cord.

(i) The average cross-section of a muscle on the free extremity (disregarding shrinkage during the histological procedure) is about 0.6 sq.mm for the smallest *(brachialis)*, and 1.8 sq.mm for the largest muscle *(extensor ulnae)*. The size of a muscle fibre is around 20—40 $\mu$ in diameter, and there are some 1000 fibres per square millimeter. If one inserts a pair of electrodes, 50 $\mu$ in diameter, one would expect that they pick up the potentials of most of the muscle fibres. This, however, is not the case. A small muscle like *acromialis* occasionally shows a retraction activity if the electrodes are in a favourable position. The size of the muscle (700 $\mu$ in thickness) and the number of fibres (600) indicates that in the case of an eccentric electrode position the greatest possible distance between the electrodes and the most distant fibres cannot be more than 500 $\mu$. This permits a rough estimation that the electrodes record potentials from an area of about 300 $\mu$ in diameter, which contains some 150 muscle fibres, and this is not more than about one fourth of the muscle.

(ii) In a large broad muscle, such as *pectoralis*, which measures 13—15 mm in width, it is obvious that the electrode cannot sense the action potentials of all fibres, yet the muscle was never silent in the partially innervated limbs. In our previous work (SZÉKELY and CZÉH 1967) we found a sign of somatotopic representation of this muscle in the spinal cord. The anterior part of the muscle was represented in the cranial end, the posterior part in the caudal end of the brachial section of the cord, but the representation of different parts of the muscle extensively overlapped in other areas. In these areas distinct parts of the muscle could be activated by stimulation of the same or adjacent points of the spinal cord. The same applies to other large muscles (*latissimus*, *dorsalis scapulae*, *extensor ulnae*, forearm muscles). The observation indicates that the multisegmental innervation is evenly distributed in the muscle and whichever segment furnished the innervation, there were always a few active fibres around the electrodes.

(iii) The amphibian muscle has a multiterminal innervation, that is, each muscle fibre has more than one neural junction. In the frog the *sartorius* muscle is innervated by two spinal segments, and the nerves of the cranial segment occupy the proximal row, those of the caudal segment the distal row of termination sites in the muscle (MILEDI 1968). If in Ambystoma the multisegmental innervation is distributed similarly, then wide areas of the muscle could be innervated by small parts of the cord. In fact, this type of innervation may explain that the larger part of muscle activities appeared fairly consistently, independently of the positions of the electrodes. It could account also for the rather extensive interaction of antagonistic muscle groups, which is much more pronounced in urodeles than in mammals during locomotion (ALTENBURGER 1937; ENGBERG 1964; TOMITA 1967).

(iv) Finally, it has to be taken into consideration that limb movements other than walking may interfere with the attempt to make inferences from the muscle activity pattern to the nervous command of locomotion. However, the only kind of complex movement the forelimb of a urodele is capable of performing is the walking step which may slightly be modified by different reflexes. Deafferentation experiments in which the muscle activity pattern remained unchanged, suggest that when the animal is quietly walking over an even surface, an output pattern characteristic of the walking step can be expected from the spinal cord.

If the above reasonings are acceptable, then we arrive at the third feature worth considering of the results. This is that the total length of activity of individual muscles is made up of shorter or longer active periods arising from the different parts of the three limb moving segments. Fig. 9 represents how the normal activity pattern is composed of the activities of the three segments. The diagram demonstrates that every little part of the active period arises at least from two segments, and if we look at the details, there is no

apparent order in the contribution of the segments, or parts of them, to the establishment of the total length of a particular active period. In other words, the end of an active period may arise from the spinal cord more cranially than the beginning (e.g. *pectoralis*), or the first part of an activity may arise more cranially than its second part does (e.g. *extensor ulnae:* cf. Figs 3, 6

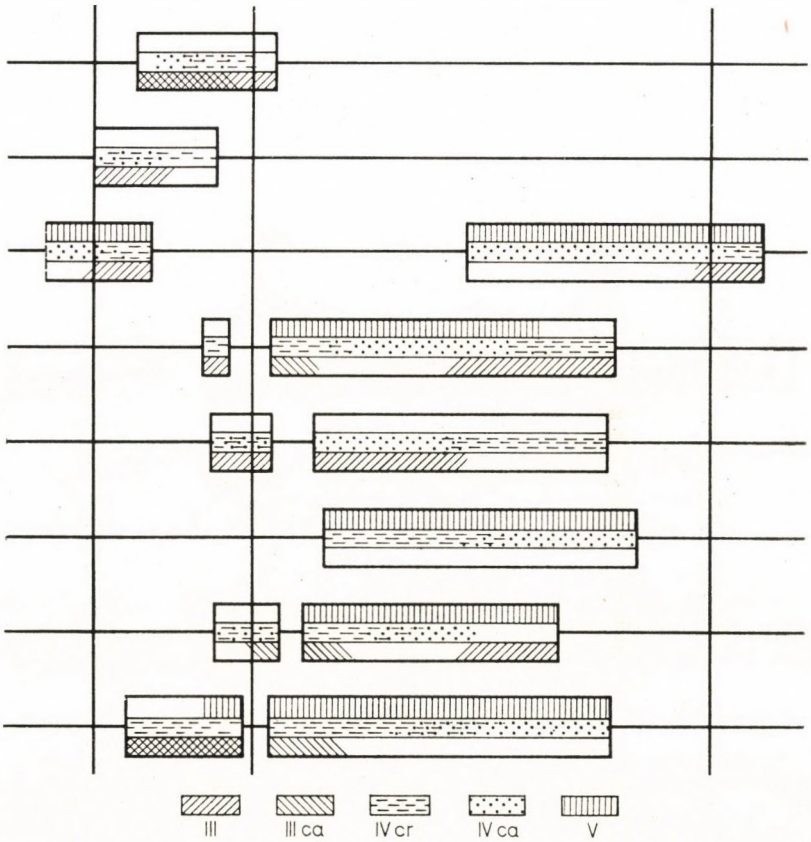


*Fig. 9.* Diagrammatic representation of the activities of the three segments placed in the muscle activities of an average step of an intact animal. Only activities appearing consistently are shown. Vertical lines separate the phase of protraction from that of retraction. The different patterns indicate the origin of the respective active periods. III: 3rd segment; III ca: caudal part of 3rd segment; IV cr: cranial part of 4th segment; IV ca: caudal part of 4th segment; V: 5th segment

and 7). There are considerable overlaps between the parts building up a total active period. Perhaps, as Figs 3 and 4 suggest, if we could record from limbs innervated by more and more smaller parts of the cord, the overlaps would be smaller, and the patterns of Fig. 9 indicating the origin of activity in the different parts of the cord, more and more variable. This assumption has two

bearings. First, the individual motor neurones innervating a particular muscle do not fire continuously during the whole active period of the muscle. They appear to deliver only short bursts which consecutively arrive in the muscle from the different parts of the cord resulting finally in the total active period of the muscle. Second, in each arbitrary small area (for example in one half of a segment) of the limb moving segments the different groups of motor neurones which innervate the different muscles, deliver their bursts with a time shift, or some of them simultaneously. This conclusion is perhaps beyond our experimental data and its meaning is not immediately apparent, but we arrive at a point where the conclusions drawn from our previous and present results meet again. Their agreement initiates even further steps of speculation into the functional organization of the spinal cord. We do it, however, with considerable reservation that on the basis of the pattern of eight muscles we try now to draw conclusions concerning the structure of that part of the spinal cord which actually controls the activity of more than 30 limb muscles.

Stimulating with a microelectrode in the spinal cord we have found (SZÉKELY and CZÉH 1967) that when a particular muscle was driven from an electrode track, then a set of other muscles could also be driven from other points of the same track. Then this muscle might be absent in the next track, and would appear again in the following one, but now together with a different set of muscles. This phenomenon kept repeating itself as we proceeded with the stimulation in the craniocaudal direction. With reference to this particular muscle, or to any other muscle for that matter, assemblies of motor neurones representing the limb muscles in different combinations could be discerned within the motor column. Looking now at Fig. 3 and considering only the activity of *latissimus*, *dorsalis scapulae*, *acromialis*, *brachialis* and *flexor digitorum* (underlined with broken lines), they are active in the order mentioned. (*Extensor digitorum* may be substituted for *acromialis* and the retraction activity of *pectoralis* for *flexor digitorum* in this order. The rest of activity is disregarded since it may be due to labyrinthine reflexes.) Similarly, in Fig. 4 *acromialis*, *pectoralis*, the retraction activity of *extensor digitorum* and *extensor ulnae* follow each other. Motor neurones which control the muscle activities underlined with broken lines, may be regarded as a neuronal assembly. The assembly in the 3rd segment comprises groups of motor neurones (Fig. 3) which represent all but one *(extensor ulnae)* of the 8 muscles, while the groups in the caudal part of the 3rd segment (Fig. 4) represent 4 muscles *(acromialis, pectoralis, extensor digitorum, extensor ulnae)*. As the sequence of muscle activities suggests, the groups of motor neurones representing individual muscles deliver consecutive bursts. (Simultaneous muscle activities indicate simultaneously firing groups of motor neurones: *acromialis—extensor digitorum* and *flexor digitorum—pectoralis* in Fig. 3.) This pattern of activity is cyclically repeated

during locomotion, it may, therefore, be called a cycle. Similar cycles can also be detected, though not so clearly, in Figs 6 and 7 (e.g. *dorsalis scapulae*, *acromialis*, *pectoralis*, *extensor ulnae* and *latissimus* in Fig. 7). The cyclic activity is obviously concealed in Figs 5 and 8, but if the 5th nerve could be divided, the 5th segment would perhaps have shown a similar cycle.* Were we able to record from more muscles of the limb innervated by smaller parts than one half of a segment, these cycles would probably be more prominent, and the simultaneous activities more rare. From this consideration it seems as if the motor column could be divided into neuronal assemblies of arbitrary extent, which represent a set of muscles, each in different combination. Groups of motor neurones representing individual muscles within these assemblies are interconnected by excitatory and inhibitory interneurones in such a manner that they are firing consecutively, giving rise to a cyclic activity. The assemblies are, furthermore, so intercoupled that the many small cycles they generate fit together and result in the single large cycle of the walking step.

This view presupposes the cyclic interconnections of neurones involved in the processing of the appropriate motor output for limb muscles, but we do not have any data to support this assumption. We are also aware of the fact that the arbitrary grouping of neurones could be made in a number of different ways on the basis of the presented experimental data. Therefore, we regard our proposition as a working hypothesis which is to be tested in further experiments. Nevertheless, the observation that the muscle activity of a deafferented limb proceeds according to a normal pattern, is easy to explain by assuming that the brachial spinal cord comprises a number of intercoupled cycle generators. Model studies on networks composed of cyclically interconnected artificial motor and inhibitory neurones, demonstrate the remarkable capacity of such structures to transform continuous input into a cyclic output pattern (Székely 1965, 1968; Kling and Székely 1968; Ádám 1968). The third paper of this series will show that the muscle activity pattern of a limb innervated by a completely isolated section of the brachial spinal cord, compares favourably with the normal pattern, a capacity which is also expected of such a structure.

Finally, the results allow a further assumption to be made. If one repeats Weiss' (1941) experiment of grafting a limb into the brachial region so that it becomes innervated by branches of the brachial plexus, and assumes that the nerves are preferentially connected with the appropriate muscles, then this limb should move in a co-ordinated manner and synchronously with the normal limb. Depending on the segment from which the limb is innervated,

* This is supported by a single experiment performed on a *Salamandra maculosa* in which the 5th nerve is somewhat thicker and subserves more muscles than in Triturus, though it is much smaller than in Ambystoma. In the muscle activity pattern of the limb innervated by the 5th nerve only, *latissimus*, *extensor digitorum* and *pectoralis* showed a similar cyclic activity.

the activity pattern of its muscles should resemble the activity of the innervating segment. Such a finding would exclude the necessity of assuming the "myotypical modulation" of motor neurones proposed by WEISS to explain the synchronous movements of the normal and supernumerary limbs. The next paper gives an account of this investigation.

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# MUSCLE ACTIVITIES RECORDED SIMULTANEOUSLY FROM NORMAL AND SUPERNUMERARY FORELIMBS IN AMBYSTOMA

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Supernumerary limbs grafted into the brachial region of axolotl larvae were innervated by the 3rd, 4th and 5th nerve, respectively. The grafts moved in a co-ordinated manner and synchronously with the normal limb during smooth locomotion; they often turned into a clonic rigidity if the host performed strained movements, or remained motionless when the animal moved slowly in water. Complete step-like movements of limbs were seen if the nerve supply came through the 4th nerve; retraction or protraction was performed to a limited extent if the limbs were innervated by the 3rd or 5th nerve. Muscle potential recordings from four pairs of homologous muscles of a limb supplied by the 3rd nerve revealed an irregular muscle activity pattern with very little co-contractions of homologous muscles. If the 4th nerve subserved the graft, muscle activity was close to the normal pattern and the homologous muscles contracted simultaneously, though irregular activities were frequently recorded. In myograms recorded from limbs innervated by the 5th nerve, the activity of the arm muscles was, as a rule, irregular, and the forearm muscles showed an activity pattern characteristic of the function of the 5th nerve. In the irregular patterns the activity of another muscle could frequently be recognized. It has been concluded that limb muscles were unable to "modulate" the function of the innervating spinal centre. On the basis of the present and previous results the assumption is put forward that any one of the nerves of the brachial plexus conveyed a rhythmic impulse pattern to which the supernumerary limbs responded with a co-ordinated step-like movement.

In an impressive series of experiments, WEISS (1937a, b, c, d) found that extra limbs grafted into the brachial region and innervated by branches of the brachial plexus, moved in a co-ordinated manner and in synchrony with the normal limb. This observation provided the basic results for the formulation of the theory of "myotypic modulation" of motor neurones. According to this theory, every individual muscle has a differential character, probably biochemical in nature, which is imparted to the innervating motor neurones through the axon. This makes the neuronal surface more receptive to a certain kind of synaptic ending than to another, and the motor neurones become so attached to the central motor apparatus that they fire at the time appropriate for the function of the innervated muscle. Hence the homologous muscles of the normal and of the grafted limb contract simultaneously and the two limbs move synchronously. The theory has been tested in a variety of experiments. It has been shown that whichever segment of the brachial cord, or whichever branch of the brachial plexus, gave rise to the innervation, the supernumerary limb moved in the same way. Various axial orientations of the grafted limb did not influence the co-ordinated and synchronous move-

ments, even if these movements obviously hindered the animal. From the normal functional restitution following nerve cross-unions in fish, to the synchronous movements of duplicated digits in man, all were ascribed to the myotypic modulation of motor neurones (WEISS 1941, 1955). In a series of experiments performed on kittens with the tibial and peroneal nerves cross-united, ECCLES et al. (1960) were able to show a redistribution of monosynaptic connections according to the scheme of myotypic modulation. However, in a later experiment the unsatisfying statistical significance of the data made the authors to regard their findings as not sufficiently conclusive to support the myotypic modulation hypothesis in its present form (ECCLES et al. 1962).

While electrophysiological investigations did not disprove its general validity, the theory became restricted to lower vertebrates. Nevertheless, the first doubt emerged from experiments performed on fish (SPERRY and DEUPRESS 1956). In their subsequent experiments performed on the external ocular muscles (SPERRY and ARORA 1965) and fin muscles (MARK 1965) of the cichlid fish, the authors have explicitly concluded that, following cross-union of nerves, the functional recovery of these muscles was due to an appropriate anatomical reconstitution of neuromuscular connections.

Working on the spinal cord of urodele (SZÉKELY 1963) and chicken (STRAZNICKY 1963) embryos it was found that movements of limbs innervated by heterotopic cord segments were characteristic of the innervating segments rather than of the anatomical construction of the limb. In other experiments performed on axolotls, it was found that most of the limb muscles were represented all over the brachial region of the spinal cord (SZÉKELY and CZÉH 1967), and a selective outgrowth of motor nerves into the limb muscles during ontogenesis was shown (STRAZNICKY and SZÉKELY 1967). On the basis of these results, assuming a rigid central motor apparatus and selective neuromuscular connections, we have questioned the validity of the myotypic modulation theory (SZÉKELY 1966, 1968). However, these data supplied only indirect evidence against the theory. By recording muscle activities from both normal and supernumerary limbs innervated by the same brachial plexus, the problem can be investigated in a more direct way. If our assumptions are correct, then the supernumerary limbs should yield a muscle activity pattern which is characteristic of the activity of the innervating segments in a way suggested in the preceding paper (SZÉKELY and CZÉH 1971).

## Methods

The experiments were performed on *Ambystoma mexicanum*. At the midlarval stage a forelimb excised from the left or right side of the donors together with a piece of the shoulder-blade, was transplanted close to the host's own forelimb on the same side. The brachial plexus originates from the 3rd, 4th and 5th spinal cord segments. The transplantations were made in the following manner. Through a small opening made on the skin, the 3rd, 4th or 5th spinal

nerve was dissected and severed from the brachial plexus as far distal as possible. The shoulder-blade of the graft was then fitted into the wound and spread underneath the skin. The cut spinal nerve was introduced through a hole made on the shoulderblade, and the distal end laid among the muscles. Thus WEISS' (1937a) original technique was exactly repeated to secure the best chance for the limb to become innervated by the 3rd, 4th or 5th spinal nerve. The animals were kept over a period of one year. Metamorphosis was induced by administering 25 mg of powdered thyroid (Thyranon, Organon) daily for a fortnight, then the animals were subjected to electromyographic investigations. In the animals which had metamorphosed, the 3rd nerve was introduced into the graft in 2 cases (Group 1), the 4th nerve in 9 cases (Group 2), and the 5th nerve in 7 cases (Group 3). In view of the poorly developed shoulder musculature of the graft, recordings were made only from the arm and forearm muscle. The following pairs of homologous muscles were used: normal (*N*) and transplanted (*T*) *brachialis*, *N* and *T extensor ulnae*, *N* and *T flexor digitorum*, *N* and *T extensor digitorum*. The recordings were made as described previously (SZÉKELY et al. 1969), and in a few cases they were repeated after deafferentation of both limbs and, like in former experiments (SZÉKELY and CZÉH 1971), no difference was found in the muscle activity pattern. The innervation of the limbs was finally checked by careful preparation under the dissecting microscope.

## Results

### *Behaviour*

The movement of the grafted limbs was regularly observed under the dissecting microscope while the larvae were small, and later with the naked eye in freely moving and restricted animals. Although we were biassed to find failures when the animals walked smoothly on even ground, we could not detect any major difference in the movement of the grafted limbs. There were only a few minor details which did not fit exactly into WEISS' (1937a) description. In Group 1, retraction of the grafted limb was smaller in extent than that of the normal limb. In Group 2 the protraction was smaller, and the elbow remained frequently in an extended position during this phase of the step. In one case, as the normal limb had finished retraction and began to move forward, the grafted limb still continued its retraction movement and followed the normal limb into protraction with an appreciable lag. In this animal retraction of the normal limb was of smaller extent, and therefore shorter in duration than usual. In Group 3, the grafted limbs were held, very characteristically, in an extended position parallel to the body. In 5 animals the shoulder and elbow were motionless, and only the wrist and digits performed flexions and extensions coinciding with the movement of the normal limb. Occasionally, small abductions of the shoulder could be observed coinciding with protraction of the normal limb. On an uneven surface, irregularities were obvious in the movements of grafted limbs. The synchronous movement had become deranged, and the stiffened graft paddled irregularly at the shoulder, or stood motionless, as the animal made efforts to overcome an obstacle. A similar phenomenon was observed in restricted animals. In an effort to escape, it performed vigorous but regular movements with the four normal limbs, and the graft remained motionless in a clonic rigidity, perpendicular to the body.

The synchronous movement often failed to come about in reflexes evoked by slight mechanical stimuli. A gentle touch applied to the hand of the normal limb, evoked a flexion or a slow walking step, but only of the normal limb, while the graft remained motionless. To similar stimuli of the graft both limbs responded with a synchronous movement, but quite frequently it was the normal limb only which gave the response. Separate reflexes of the graft could be evoked only in animals of Group 3. During swimming when the animal made occasional movements with the normal limb, the supernumerary limb usually remained motionless.

From these observations the general impression was obtained that the co-ordinated and synchronous movement of the graft was confined to the case of smooth locomotion. When the animal moved vigorously, or the movement was too quiet, the graft produced irregular movements, or remained motionless. The results of muscle recordings will be described separately for each group.

*Group 1*

In view of the dissection findings of the two animals one had to be neglected in further considerations. In this case the introduced 3rd nerve slipped out from the graft and found its way back to the brachial plexus. The extra limb was innervated from many uncontrollable sites, and the muscle activity pattern was more like in the next group.

In the second animal the grafted limb was innervated solely by the 3rd nerve which gave a tiny branch also to the normal limb. The muscle activity pattern is shown in Fig. 1. The recording from *T extensor ulnae* is noisy but still demonstrates that, with the exception of *N brachialis*, the rest of the muscles disclosed an irregular activity pattern. Protraction activity is much too enhanced in *T brachialis*, and the muscle is silent in retraction. The activity of *N extensor ulnae* resembles that of *Dorsalis scapulae*, and it is obvious that the two arm extensors do not run together. Three of the forearm muscles, *N flexor digitorum*, *T flexor digitorum* and *N extensor digitorum*, disclosed more or less the same activity pattern. The crenation of the baselines indicate a slight retraction activity which can better be seen in other parts of the record made at higher gain. The activity of *T extensor digitorum* is rather similar to that of *flexor digitorum* of an intact animal and, in fact, it contracts simultaneously with *N brachialis*.

If one compares this myogram with that obtained from partially innervated limbs (cf. Fig. 3 of SZÉKELY and CZÉH 1971), our expectation that the grafted limb will show the activity of segment 3, has not been fulfilled completely. There is a clear prevalence of protraction activity but the protraction prevalence of some of the normal muscles and the strong

retraction of *T extensor digitorum* is disturbing. On the other hand, the synchronous activity of the homologous pairs is shown only by the forearm flexors.

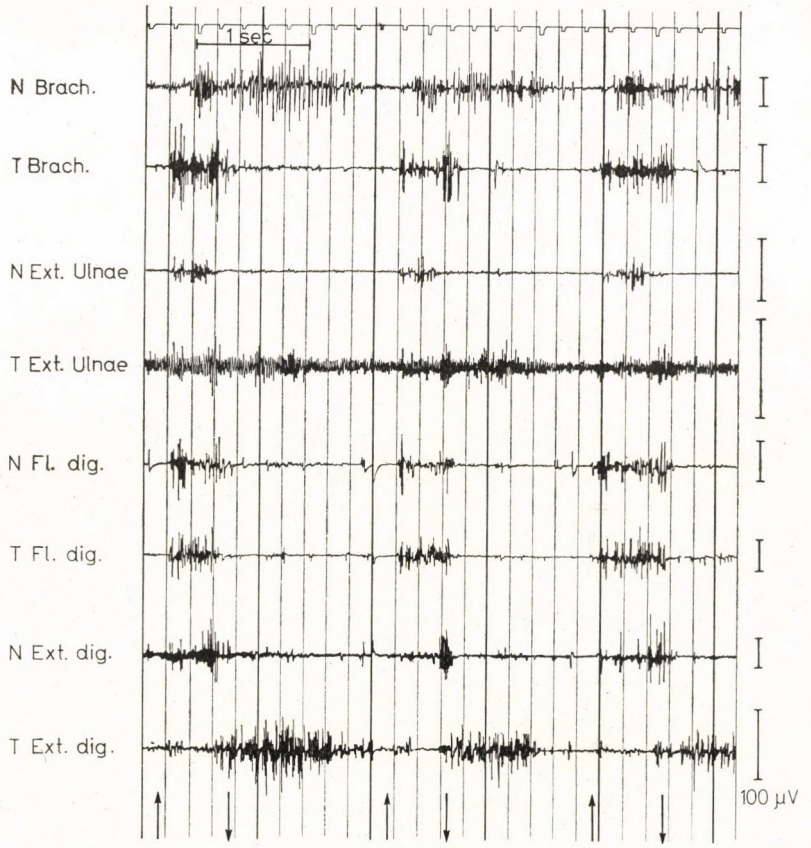


*Fig. 1.* Myograms recorded from four pairs of homologous muscles of normal and supernumerary limbs. The supernumerary limb was innervated by the 3rd spinal nerve which sent a small branch to the normal limb as well. Arrows indicate the protraction and retration phases of the normal limb

*Group 2*

According to dissection findings the grafted limb was innervated by the 4th nerve in 2 of the 9 animals. In these two cases the innervating nerve gave a small branch to the normal limb as well. In three animals the 5th nerve contributed with a small branch to the graft's innervation, and in the rest both limbs received mixed innervation from two or three segments.

Despite the various sources of nerve supply, the myograms obtained from the 9 animals are comparable with the normal activity pattern, and the homologous pairs, though with exceptions, contract simultaneously. The

myogram shown in Fig. 2 represents a case in which the graft is moved solely by the 4th nerve, and the synchronous activities of the homologous muscles strongly support WEISS' assumption. The myogram needs little comment. The muscle activities show a normal pattern, and the homologous muscles

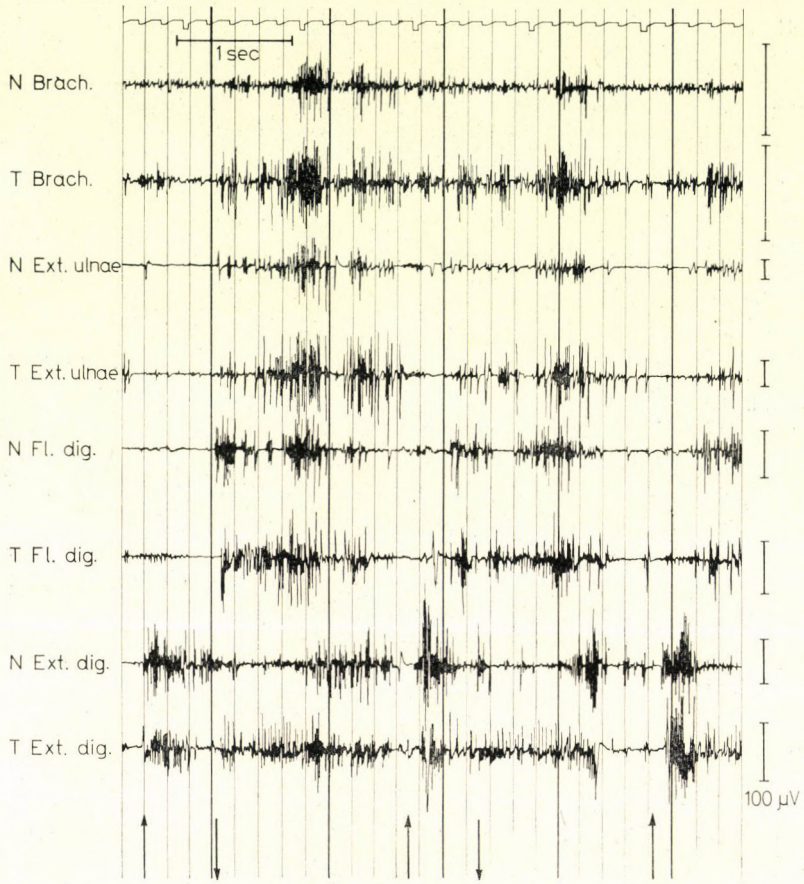


*Fig. 2.* Myograms of four pairs of homologous muscles. The supernumerary limb was innervated by the 4th spinal nerve which contributed also to the innervation of the normal limb

appear to contract simultaneously. However, there are some deviations which are definitely within the reliability of the technique. *T brachialis* discloses a rather long active period which goes over almost uninterruptedly into the next protraction phase, a pattern never seen in a normal myogram and closely resembling that of *latissimus*. More or less the same applies to *T extensor ulnae*, which is better seen in other parts of the records. As to synchrony, the activity of these two muscles is the most synchronous in the whole myogram.

The rest of the animals showed similar myograms, though with less regularity and with less spectacular co-contractions of the homologous muscle pairs. Irregularities were most frequent in the activity pattern of the arm muscles, and always in that of the two *brachialis*. These irregularities consisted

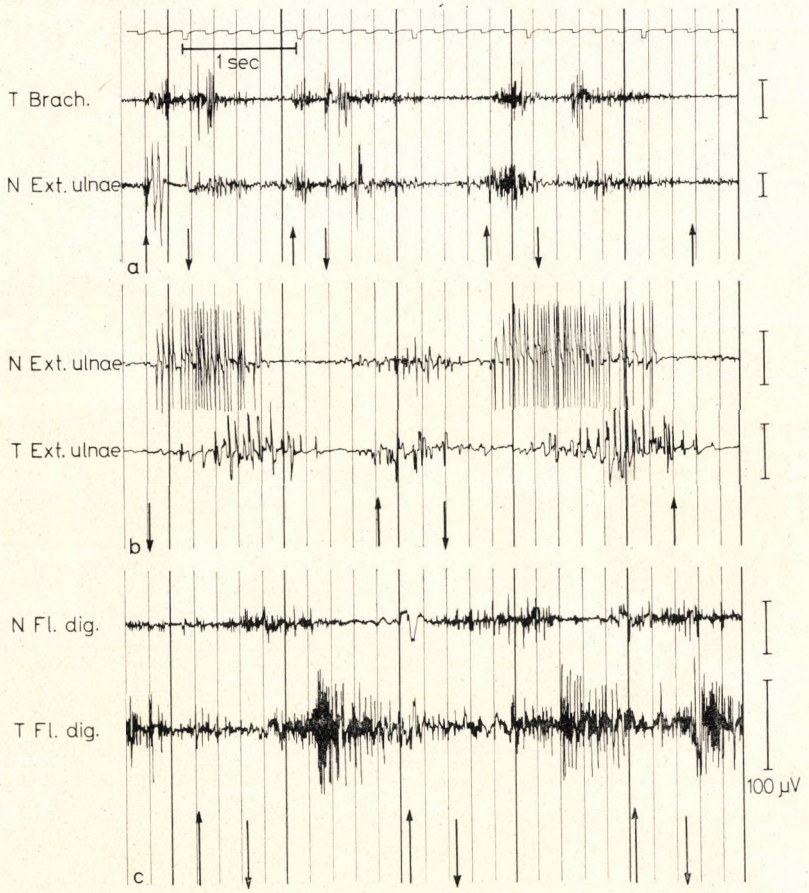


*Fig. 3.* Samples of myograms taken from three different records of Group 2 illustrate irregular activity patterns. a: *T brachialis* and *N extensor ulnae* showed the best co-contraction in the record. In addition to the 4th nerve, the supernumerary limb received a tiny branch from the 5th nerve. b: The two *extensor ulnae* contract with a time shift during retraction. Note the synchronized low frequency activity of *N extensor ulnae*. The supernumerary limb received a mixed innervation from the 3rd and 4th nerves. c: Alternating activities of *N* and *T flexor digitorum*. The supernumerary limb was innervated by the 4th nerve alone

of abnormally elongated active periods, or the retraction activity was divided into two separate parts not matching the normal activity pattern of the muscle. Figs 3b and c demonstrate separate contractions of *N* and *T extensor ulnae*, and of *N* and *T flexor digitorum*. In some instances the interferogram of a muscle consisted of highly synchronized low frequency activities in which

the spike-like potentials followed each other irregularly with relatively long intervals (Fig. 3b). In a number of cases, heterologous muscles (like *T brachialis—N extensor ulnae, T extensor ulnae—N flexor digitorum*) showed the best co-contractions in the myogram (Fig. 3a). Curiously enough, the least regular myogram was obtained from the second animal in which the graft was innervated solely by the 4th nerve.

To summarize the results of this group, in addition to practically normal activities, a number of irregular patterns were recorded from both limbs. In some of the irregularities one could recognize the activity pattern of another muscle, or the pattern included the activity of more than one muscle.

*Group 3*

In view of the considerable length of the 5th nerve, this type of operation was easy to perform and this group yielded the most consistent results. In 5 animals the graft was supplied by the 5th nerve alone, and in 2 animals the 4th nerve contributed with a very small branch to the innervation.

Fig. 4 shows the myogram recorded from a limb with pure 5th innervation. Regarding first the muscles of the normal limb, the ram muscles show a normal activity pattern with the exception of a short burst at the end of the retraction activity of *N brachialis*. In the forearm the timing of muscle contractions is as it should be, but the retraction activity of *N flexor digitorum* is rather poor. In the transplanted limb, *T brachialis* is active for a shorter period than is its normal mate, and its interferogram contains a synchronized low frequency component. *T extensor ulnae* contracts almost uninterruptedly throughout the walking cycle. The forearm muscles are active with a timing which is characteristic of the activity of the 5th segment. It may be relevant that the sum of the activities of the normal and transplanted forearm muscles gives a normal activity pattern (cf. Fig. 2 of SZÉKELY and CZÉH, 1971).

A similar completion of activities of the normal and transplanted forearm muscles is seen in three other recordings. Apparently, the grafted muscles disclose the activity of the 5th segment which together with that of the normal muscles makes up the normal pattern. To the arm muscles, notably to *extensor ulnae*, this applies only in two cases. In the rest of the animals the arm muscle showed different irregular patterns. In one animal in the activity of *T extensor ulnae* the pattern of *latissimus* could clearly be recognized. In another case the *T brachialis* and *T extensor ulnae* contracted in complete synchrony. The irregular activities of arm muscles might explain that elbow movement was observed only in two animals. In one of these animals the activity of *T brachialis* was considerably stronger than that of *T extensor ulnae*, and the myogram of the second animal is shown in Fig. 5.

The activity pattern in this myogram is atypical, showing irregularities in both the normal and the grafted muscles. Most striking is the typical *extensor digitorum* activity pattern of *N extensor ulnae*. This pattern is more or less followed by that of *T brachialis*. The pattern of both the normal and

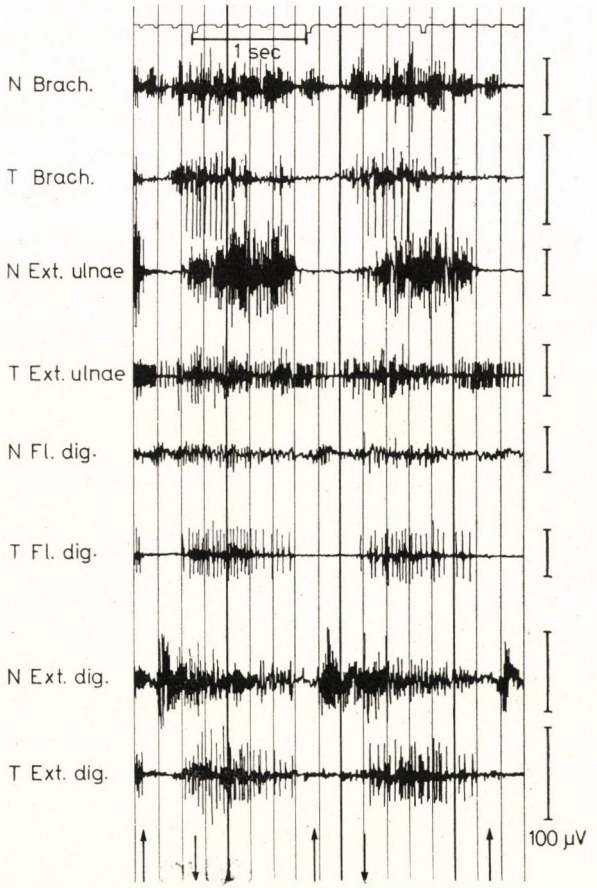


*Fig. 4.* Myograms of four pairs of homologous muscles. The supernumerary limb was innervated by the 5th nerve. The limb was motionless at the elbow

the transplanted *extensor digitorum* is more like what is normally obtained from *extensor ulnae*. The activity of the rest of the muscles is difficult to characterize. First we thought that the muscles were monitored in a wrong order, but careful tests proved that the order was correct. Innervation of the graft was also atypical; the 5th nerve sent a relatively small branch into the graft, its greater part joined the normal plexus. This small branch fused with the nervus brachialis, and this latter nerve could be followed proximally into a scar tissue where nerves could no more be traced. Apparently, nerves injured

at the operation converged towards the graft and the innervation became contaminated from uncontrollable sources. The normal plexus may have been deranged also at operation, and this could have accounted for the atypical activity of *N extensor ulnae*.

The results of this group most unambiguously support our assumption that the grafted limb yields the activity of the innervating segment.

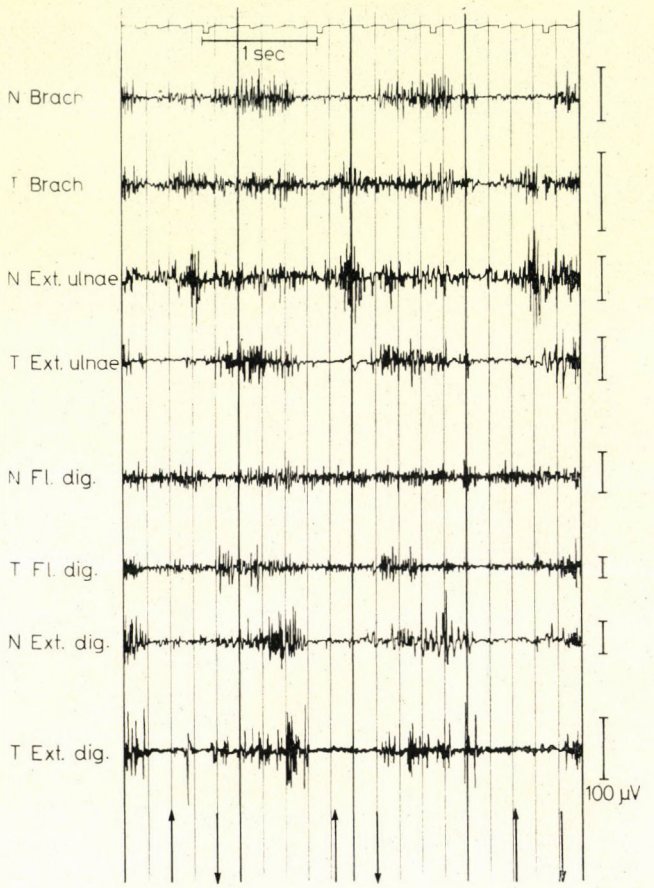


*Fig. 5.* Myograms of four pairs of homologous muscles. In addition to a branch from the 5th nerve, the supernumerary limb probably received innervation from uncontrollable sources. The elbow was also participating in the movement

## Discussion

As mentioned above, the behavioural observations have corroborated WEISS' description in that the two limbs moved together during walking. The observed minor deviations from synchrony, and the separate movements

of the two limbs evoked by mechanical stimuli, are in themselves not conclusive enough to abandon the concept of myotypic modulation. The normal myochronogram (SZÉKELY et al. 1969; SZÉKELY and CZÉH 1971) which shows extensive co-contractions of limb muscles, indicates that looking for synchronous or asynchronous activities of homologous muscles may easily lead to erroneous conclusions. There is no way to tell whether the synchronous activity of a homologous pair of muscles is due to myotypic modulation, or the motor neurones of the grafted muscle just happen to fire exactly in time with those of the normal muscle. Nevertheless, the occurrence of asynchronous activities of homologous muscles, or of synchronous activities of heterologous muscles, was sufficiently frequent to indicate a high rate of "mismodulation". Our former results obtained from partially innervated limbs suggest another way to investigate the question. With the presumption of selective neuromuscular reconnections, the assumption was that the grafted limb must yield a muscle activity pattern characteristic of the function of the innervating segment. This expectation was, however, fulfilled only partly by the results for Group 1, and somewhat better by those for Groups 2 and 3. Two factors may be involved in the differences between the expected and the recorded results. First, the reinnervation was not as selective as it has been presumed. This was in fact the case, and it will be discussed later. Second, we investigated the acticity of arm and forearm muscles which are represented to the least extent in the 3rd segment. Therefore, limb muscles with 4th, or 5th, innervation have had a greater chance to receive the appropriate supply and, consequently, to yield the expected results. This is supported by the finding that activity patterns that we called irregular, occurred in Group 1 and in (the arm muscles of) Group 3 more frequently than in Group 2. However, a number of irregularities were found in Group 2 as well (Fig. 3), and the collection of irregular patterns in Fig. 5 alone would be sufficient to throw a serious doubt on the myotypical modulation. Furthermore, in the irregular activity patterns, as opposed to normal patterns, the activity of another muscle could often be recognized. These observations together with our previous data mentioned in the introduction, strongly advocate the assumption that the innervated periphery is unable to alter the function of the respective spinal centres. Therefore, we propose to discuss the synchronous movements of normal and grafted limbs on a different basis, wholly ignoring the myotypic modulation.

Data obtained from these and other experiments suggest that (1) central and (2) peripheral components must be considered to account for the synchronous movement of the two limbs.

(1) The synchronous movement comes about only if the supernumerary limb receives its innervation from the brachial segments of the spinal cord. Previous experiments revealed a number of properties of this part of the cord.

These, and only these, segments are capable of moving the limb in a co-ordinated manner (DETWILER 1936; WEISS 1955; SZÉKELY 1963). Motor neurones of individual muscles are so distributed that nearly all limb muscles receive innervation from each of the three brachial segments (NICHOLAS and BARRON 1934; SZÉKELY and CZÉH 1967). Each of these segments, or parts of them delivers a rhythmic output which is a cycle in the sense that it starts with protraction and ends with retraction (SZÉKELY and CZÉH 1971). On the basis of these findings it is reasonable to assume that the grafted limb innervated by any nerve of these segments receives rhythmic impulses which contain the commands for both protraction and retraction activities.

(2) The peripheral components are manifold. They include, above all, the problem of selective reinnervation of muscles. Actually, it was one of our premises, and it was assumed mainly on the basis of SPERRY and AROA's (1965) and MARK's (1965) findings mentioned in the introduction. In a recent paper MARK (1969) has pointed out the multiterminal innervation of muscle fibres in amphibians and fishes to account for the selective innervation which occurs in these lower vertebrates but does not in mammals. He assumes that muscles are preferentially forming connections with nerves with which they had been connected before. The multiterminal innervation permits some sort of competition for sites of nerve terminations, the right nerves outnumber the wrong nerves and take the command over the muscle.

The multiterminal muscle innervation, which is also multisegmental in Ambystoma, would indeed provide ample opportunities for a competitive selectivity of this kind. For a better definition of selectivity, we have to answer the question whether it implies that every one of the empty sites of terminations is hooked up by the very same nerve which normally makes contact (i) with that particular subneural apparatus, (ii) with that particular muscle, or (iii) the nerve may be of whatever origin, but functionally matches that particular muscle. By functional matching is meant that the firing period of the nerve partly or wholly coincides with the normal active period of the muscle. In the present experiments, from the normal activity patterns of the grafted muscles the question could not be answered unequivocally. The first alternative, viz. that selection would occur at the level of individual subneural apparatus, can perhaps be excluded, but the second and third alternatives might have been equally at work in the restoration of the normal activity pattern. In the best case, therefore, we may speak of a normal functional restoration, but we are left in complete uncertainty concerning the mechanism of restoration. The results contradict rather than support the premise that the kind of selective reinnervation hypothesized by MARK (1969) accounted for the co-ordinated and synchronous movement.

Yet, the grafted limb moves in a co-ordinated manner, which implies a certain kind of distribution of muscle strength during walking. The recording

experiments do not furnish reliable information about muscle strength, though one intuitively assumes that during protraction the strength of protractor muscles has to overcome that of the retractors, and the reverse is true during retraction. Therefore, a certain degree of distribution of nerves conveying protraction and retraction commands is required. If this does not happen, the limb, or part of it, remains motionless, like the elbow of 5 animals in Group 3. WEISS regarded such phenomena as exceptional failures of modulation, but what really happened was that the activity of the extensor muscle unmistakably exceeded that of the flexor muscle (Fig. 4). Similar incoordinations of muscle strength could not be shown as convincingly in Group 2, nevertheless the behavioural observations revealed a number of irregularities which indicated that the distribution of muscle strength was not as good as could have been expected in the case of selective reinnervation. The movement of the grafted limb was much too weak, certainly inapt for propelling the body. As a rule, the extent of movements became greatly reduced, or the limb remained motionless when loaded with the fine recording wires, though we could record potentials from the muscles. This indicates that passive motions (elasticity of the skin and muscles, the weight of the limb, the tugging effect of the normal limb) might have contributed to the occurrence of co-ordinated limb movements. The clonic posture of the grafted limb of a restricted animal, while the normal limb moved regularly, clearly demonstrated how easily the muscle co-ordination collapsed when the animal made an effort to escape. In fact, co-ordinated and synchronous movements were observed only during smooth locomotion of the animal over even ground. In many cases the slightest disturbance brought about obvious inco-ordinations and the appearance of the co-ordinated movement was kept within narrow limits. It is difficult to escape the impression that the distribution of muscle strength, and the ingrowth of nerves conveying protraction and retraction commands, happened on a statistical basis.

There were, however, complete movements and normal activity patterns which indicated that the statistical distribution of the different ingrowing nerves had somehow to be biassed. Such a bias might well be provided by the large scale of functional matching of different motor neurones, as suggested by the normal myochronogram. A second factor could be the mechanical guiding of the growing nerves which was so brilliantly demonstrated by WEISS (1955). This may account for PIATT's (1942) findings that regenerating nerves reconstitute their normal arborization pattern even if they grow into limbs previously deprived of innervation. Depending on their anatomical position, nerve fibres destined to innervate the ventral musculature have an easier access to the route of the nervus brachialis, and those of the dorsal musculature may find their way easier to the nervus extensorius. It has been shown on embryological material that the initial orientation of nerve sprouts may

determine the direction of growth (SZENTÁGOTHAI and SZÉKELY 1956). This would not be the kind of pathway selection or competitive reinnervation suggested by SPERRY and ARORA (1965) and MARK (1969), but rather a statistical preference of one direction to another by the growing nerves. If the anatomical derangement of the nerves is too great, the directional ingrowth can happen in the opposite way, of which we have provided ample evidence (cf. Fig. 5). Thirdly, with respect to the establishment of co-ordinated movements, the anatomical structure of the limb must not be overlooked. Owing to its construction, the only complex movement the limb is capable of performing is the walking step. If the limb once starts moving in response to rhythmic impulses, it will produce a co-ordinated step-like movement.

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# ACTIVITY OF SPINAL CORD FRAGMENTS AND LIMBS DEPLANTED IN THE DORSAL FIN OF URODELE LARVAE

By

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Fragments of the spinal cord and forelimbs were deplanted into a common tunnel of the dorsal fin of urodele larvae. In some cases a sensory ganglion was also introduced with the spinal cord fragment. Whenever neural connections developed between the spinal cord and limb deplant, spontaneous movements and reflex responses of the limb were observed. If the neural tissue deplants originated from the thoracic section of the spinal cord, the spontaneous movement consisted of irregular twitches of the limb, while if the spinal cord tissue was taken from the brachial region, co-ordinated step-like movements were observed. Myograms recorded from the arm and forearm muscles of limbs displaying co-ordinated movements compared favourably with normal myograms. As revealed by histological investigations, the structural integrity of the brachial spinal cord deplants was indispensable for the development of co-ordinated limb movements. It has been concluded that the pattern of limb movement is programmed in the structure of the brachial spinal cord segments which are capable of moving the limb in a co-ordinated manner without receiving rhythmic afferentation and without any supraspinal control.

WEISS (1950) has developed the technique of deplanting fragments of nervous tissue and limbs into the dorsal fin of urodele larvae. The gelatinous tissue of the fin serves as a culture chamber in which the deplants grow and differentiate in relatively indifferent surroundings. Under these conditions neural connections develop between the deplanted nervous centre and limb which is capable of performing different kinds of movement independently of the host's own nervous system. This kind of model nervous system has been used by a number of authors to study the development and morphology of the deplants (WEISS 1950; SZÉKELY and SZENTÁGOTHAI 1962; SNIDER et al. 1969), and the effect of different ions and drugs on the activity of the supernumerary limb (DESMEDT 1954; SNIDER et al. 1968). In the present work it has been used to investigate the functional capacity of deplanted spinal segments taken from different regions of the cord.

The work has been initiated by the long controversy between WEISS (1936) and GRAY (1950) whether or not the spinal cord is capable of controlling co-ordinated limb movements without receiving rhythmic afferent impulses. Relying on the observation that the toad deprived of spinal sensory innervation shows alternating limb movements characteristic of walking, WEISS maintains the central autonomy of motor patterns. In contrast, GRAY found that the integrity of the sensory and motor supply to at least a single spinal

segment is indispensable for co-ordinated limb movements in toads. According to his view, the ambulatory rhythm is dependent on a rhythmical excitation of peripheral sense organs, and the intact segment provides the rhythmic afferentation for the initiation and maintenance of co-ordination.

On the basis of our previous finding that the normal muscle activity pattern of walking remains unimpaired following deafferentation of both forelimbs in urodeles, we have concluded that the pattern of motility is somehow programmed in the structure of the spinal cord (SZÉKELY et al. 1969). In the light of GRAY's results, this conclusion has undoubtedly been anticipated, for in this experiment we could not exclude the role of rhythmic afferentation arising in other parts of the body. An adequate approach to the WEISS—GRAY controversy, and to find support for our assumption, seemed to be the investigation of the function of isolated spinal cord segments in model nervous system preparations. The present paper gives an account of these investigations.

## Methods

During the last few years several deplantation experiments were performed using larvae of *Pleurodeles waltii*, *Triturus cristatus* and *Ambystoma mexicanum*. Operations were carried out according to the method of WEISS (1950), by preparing a longitudinal canal in the dorsal fin of larvae and introducing the desired spinal cord segments with a fine glasspipette. The mouth of the canal was closed with the limb deplant. Two kinds of spinal cord deplants were used. In a group of animals the brachial section of the cord, 2—3 segments in length, was introduced into the dorsal fin. In some cases the insertion of a sensory ganglion taken from the 10th cranial nerve preceded the spinal cord deplant to secure the development of a reflex arc. In another group which contained only newt larvae, the spinal cord deplant, similar in length, was taken from the thoracic section of the cord and was introduced either with, or without, a sensory ganglion. Two to three weeks after deplantation the movements of the deplanted limbs were regularly observed and many of them recorded cinematographically. Around the onset of metamorphosis the larvae were fixed for histological investigation.

The present description is largely based on the study of 9 axolotl larvae which have attained a size sufficient for electromyographic investigation. Before recording, the animals were filmed and the myograms were compared with the movements of the limbs observed in the motion pictures.

## Results

Within 2—3 weeks after operation spontaneous movements of the supernumerary limbs developed in most of the cases with surviving spinal cord fragments. The movements appeared completely independent of the host's own movements, destruction of the spinal cord of the host by electrocoagulation did not produce any appreciable change in the activity of the supernumerary limb.

In addition to spontaneous movements, reflex activity could be observed in most preparations containing a sensory ganglion. Slight mechanical stimuli applied to the dorsal fin in the region of the ganglion deplant greatly enhanced the limb's spontaneous movement or initiated the movement if the limb

happened to be silent. Similar reflexes could be evoked from the region of the cord deplant, and in many cases from the supernumerary limb itself. Surprisingly enough, but as described earlier (WEISS 1950; SZÉKELY and SZENTÁGOTHAI 1962), this enhancement of limb motility could be attained also in preparations without a sensory ganglion. In these cases the reflexogenous zone was mostly confined to the region of the cord deplant, but in some preparations well-defined small areas of the supernumerary limb also proved sensitive. Destruction of the host's spinal cord did not influence this reflex activity. Histological investigation performed earlier, and in connection with the present work, clearly indicated the absence from these preparations of any sensory neurons including the Rohon—Bird cells. However, in every case of reflex activity the host's sensory fibres were incorporated in the deplanted cord fragments. In a previous paper (SZÉKELY and SZENTÁGOTHAI 1962) on the morphology of small cord deplants, the route of sensory fibres could be traced through the deplanted centre to the dorsal fin or the supernumerary limb in serial sections of Bodian specimens. In favourable cases it could clearly be seen that the traversing sensory fibres had come into close relation with the dendrites and perikarya of motor neurones, running for a while together with one or the other. This finding has led to the conclusion that such mere appositional contacts might have been effective in impulse transmission, and their activities were called pseudo-reflexes. Further details of the reflex activity and histology of these preparations have been described in a previous paper.

Of the spontaneous activities, two kinds of limb movement could be discerned. Preparations with spinal segments from the thoracic section of the cord showed exclusively movements which had no resemblance to natural limb movements of any larval stage of these animals. As described earlier (WEISS 1950; SZÉKELY and SZENTÁGOTHAI 1962), they were rather a series of irregular twitches of different muscles or muscle groups. Sometimes bursts of such twitches followed each other in quick sequence and fused for a short while into tetanus. Reflex activities usually brought the limb into clonic movements by increasing the frequency of the bursts. As a rule, the frequency of twitches and the occurrence of clonus could not be related to the presence or absence of the sensory ganglion.

In contrast, deplanted limbs of animals that received spinal segments from the brachial section of the cord, disclosed a different type of movements. The limbs were motionless for periods longer than in the former group, and when they once started moving there was a definite excursion at one, two or all three joints of the limb. In other words, these limbs performed a movement which might be described as a partial, or a complete walking step (Fig. 1). These step-like movements appeared either spontaneously, 3 to 5 steps in a series, or could be evoked by mechanical stimuli. The presence or absence

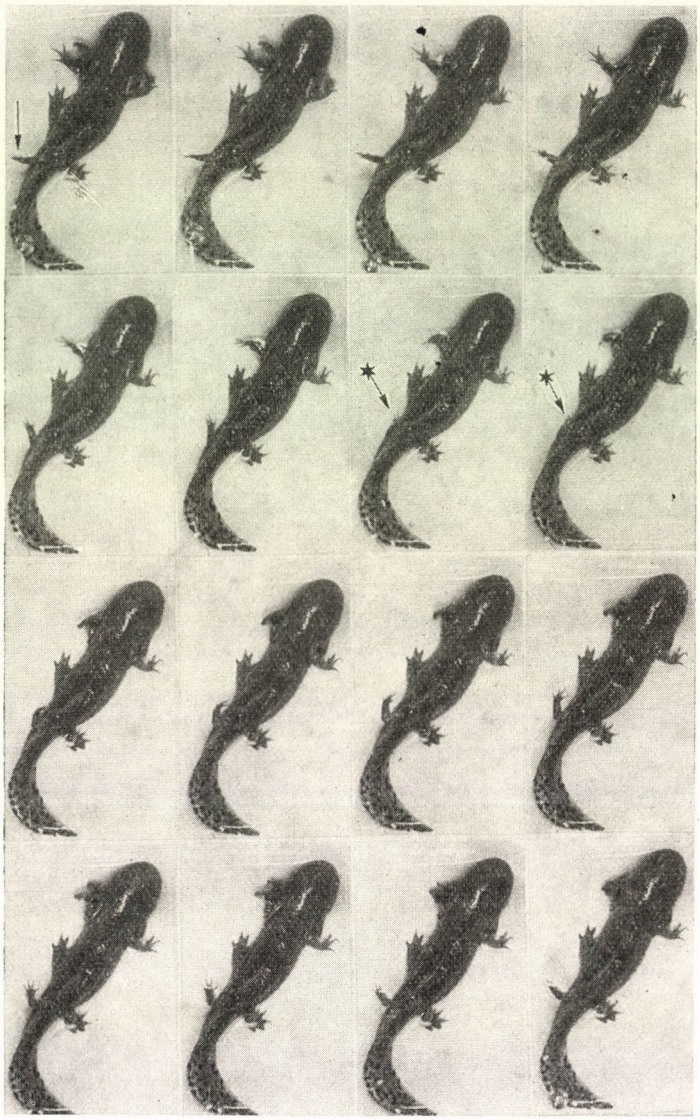

*Fig. 1.* Moving pictures illustrating the step-like movement of the deplanted limb (arrow). At arrows with asterisk the contours of the limb fades into the background. Recorded at a speed of 24 frames/sec. If the first picture is reckoned as the 1st frame, then the pictures were made from the following frames from left to right: 2, 29, 37, 40, 42, 44, 46, 48, 50, 52, 54, 57, 61, 64, 67. The limb movement starts with an extension of the forearm (first two pictures), and with a short break goes over into the stepping movement

of a sensory ganglion did not seem to influence the amplitude and the occurrence of the movements, nevertheless they could be evoked easier in preparations with sensory neurones.

The muscle activity pattern was recorded from the arm and forearm muscles of the supernumerary limbs of 9 adult axolotls, 5 of which showed complete steps. The common feature of the myograms was a long lasting activity of the muscles, with similar thickness and amplitude of the interferograms as in normal animals (SZÉKELY and CZÉH 1971). In some cases, timing of muscle activity closely resembled the normal pattern except for differences in amplitude, and the movements seen on the moving pictures

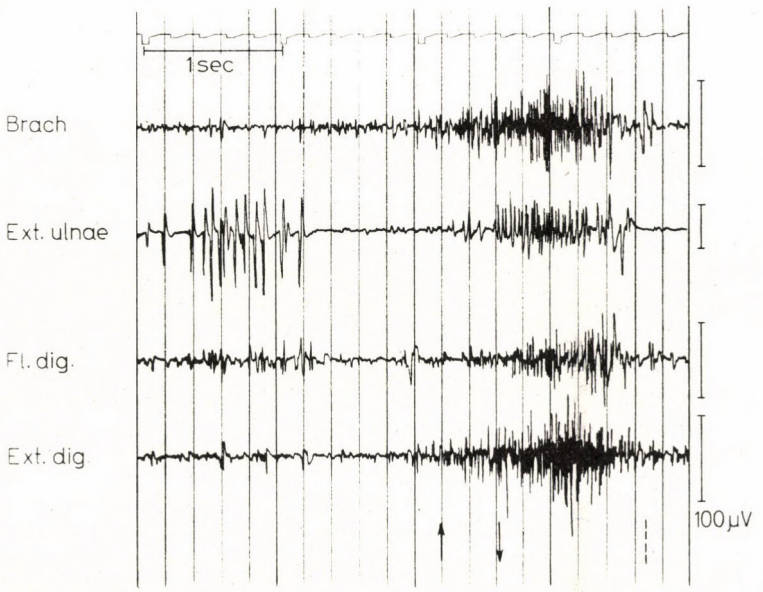


*Fig. 2.* Myogram recorded from four muscles of the deplanted limb shown in Fig. 1. The strong activity of *extensor ulnae* coincides with elbow extension seen in the first two pictures of Fig. 1. The upward pointing arrow indicating the beginning of protraction corresponds to the third picture. The vertical broken line indicates the assumed end of retraction

could be correlated with the activity of muscles (Figs 2, 3). In other cases, although a good walking step was seen during recording, muscle activity lasted uninterruptedly over several steps, and the movement could not be correlated with the myogram. The other 4 animals displayed only parts of a walking step; the step-like movement of the supernumerary limb was often interspersed with clonic twitches, and the fragment of the step ended usually in a clonic rigidity of the limb. In favourable cases such movements could be correlated with the myogram (Fig. 4).

As revealed by histological investigations, development of a complete step-like movement depended on the size and structural integrity of the spinal cord deplant. If only a small fragment of the cord survived and its structure had become deranged, movement of the supernumerary limb was

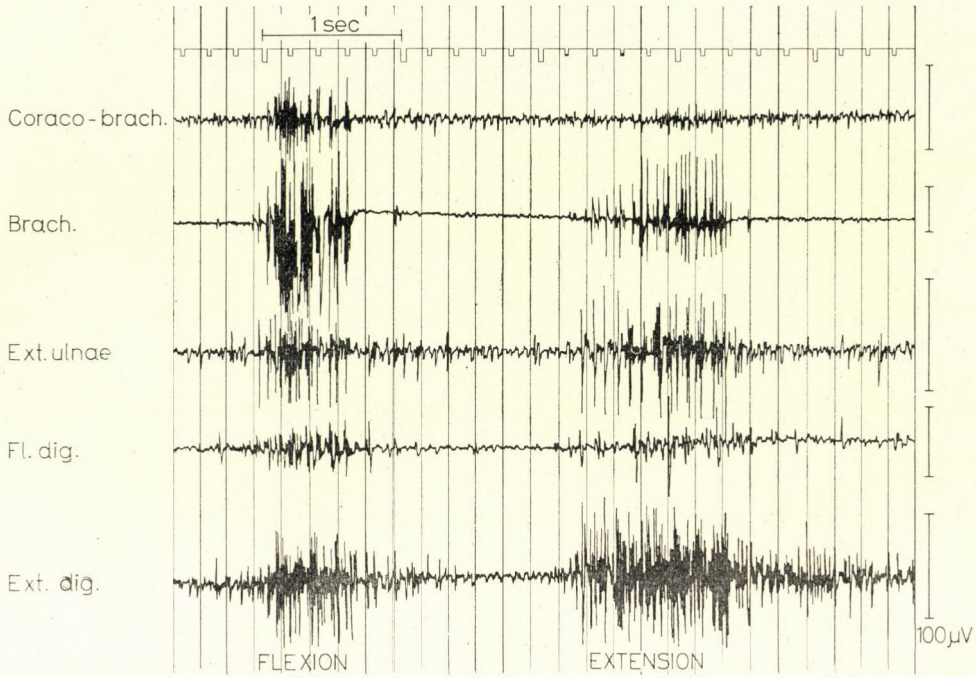


*Fig. 3.* Myograms of muscles of a deplanted limb. The step-like movement consisted of a flexion followed by elbow and wrist extension. According to its anatomical position, *coracobrachialis* may be regarded as a retractor muscle

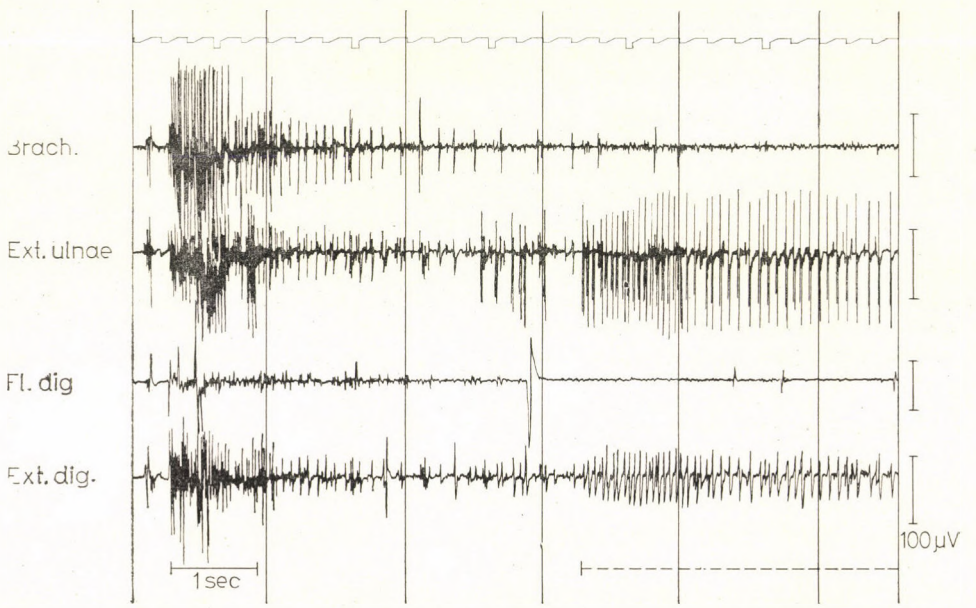


*Fig. 4.* Myogram of 4 muscles of a deplanted limb. The movements consisted of a strong extension of the elbow and wrist (solid line), which turned into clonic twitches lasting for several seconds (broken line)

not better than when the deplant was innervated by thoracic segments. Qualitatively speaking, if the outlines of the grey matter were recognized in histological sections, it could be regarded as necessary for controlling a step-like movement (Fig. 5). On the other hand, cord deplants originating from the thoracic region, irrespective of their structural integrity, were never able to elicit any coordinated limb movement.

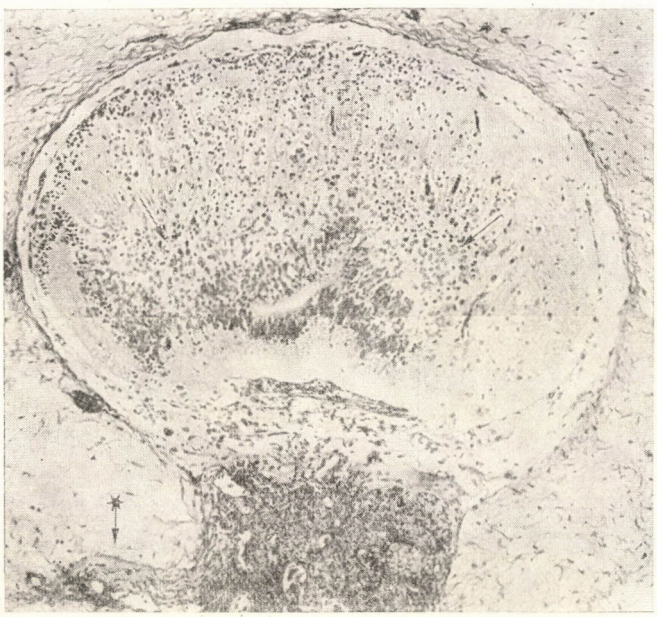

*Fig. 5.* Cross-section of a deplanted fragment of the spinal cord. Arrows point at the sites of the ventral columns. On the left, the ventral column is surrounded by a cluster of small cells, a protrusion of undifferentiated neurones from around the central canal. The dorsal part of the grey matter is underdeveloped. Arrow with asterisk points at the nerve which had invaded the deplanted limb

## Discussion

Since the experiment of TOWER (1937) performed in dogs it has been known that a spinal cord section isolated and deafferented in situ is completely silent. This seems to be valid also for the newt, as to its hind limbs innervated by lumbosacral segments isolated and deafferented in situ remain motionless under any circumstances (unpublished observation). It seems, therefore, that the kind of afferentation provided by the deplanted sensory ganglion, or the sensory fibres of the dorsal fin, is indispensable for keeping the cord deplant alive. To answer the question posed in the introduction, it is relevant to decide whether or not this afferentation has a rhythmic character. It

may be argued that afferent impulses arising from the limb deplant moving rhythmically, or from the dorsal fin pulled by the movements of the limb, are actually of rhythmic character, and this is which initiates and maintains the step-like movement. This is, however, not very likely. Rhythmic afferent impulses have certainly no role in the initiation of the movement. It frequently appears spontaneously in the otherwise motionless animal, or can be evoked with slight instantaneous touches which can hardly be regarded as rhythmic stimulation. As the limb moves, it may generate rhythmic afferent impulses, but in this case the rhythmic movement precedes the rhythmic afferentation. We could observe step-like movements of insensitive limbs, and if this function of the cord deplant were dependent on rhythmic afferent inflow, it would be left alone with the kind of rhythmicity arising only from the dorsal fin. It seems safe to exclude the rhythmic character of the afferent impulses as a prerequisite in the control of co-ordinated movements.

The difference in limb movement between the two groups of animals corroborates our previous finding in that spinal cord segments only from the brachial region are capable of controlling co-ordinated limb movements (SZÉKELY 1963). In the case of brachial cord deplants, the general character of movements, the sequence and extent of excursions at the joints, compares favourably with those of a normal limb; nevertheless the muscle strength is rather poor during these movements. In some cases the limb loaded with the electrodes moved to a smaller extent than before, indicating that even weight of the wires was sufficient to interfere with the movement. In other animals the comparison of the movement with the myograms clearly revealed that some parts of the movement were due to passive displacements at the joints. Thus, the movements of deplanted limbs resembled more closely that of supernumerary limbs grafted into the brachial region (CZÉH and SZÉKELY 1971), and the factors mentioned in this paper might have their share in the establishment of the step-like movement observed in the present experiment.

This reservation holds even more if one compares the myogram of deplanted limbs with that of normal limbs. It is obvious that a completely normal myogram cannot be expected. Even if all motor neurones of the muscles investigated had survived in the cord deplant, there is no guarantee that the motor fibres would find the appropriate muscles (CZÉH and SZÉKELY 1971). Despite this handicap there are only two deviations in the myogram shown in Fig. 2. First, the protraction activity of *extensor digitorum* is too small and not separated from the retraction activity, second, the retraction activity of the same muscle has a higher amplitude than that of *flexor digitorum*. Unquestionably, this is our best record. The myogram shown in Fig. 3 is not as encouraging; however, if one takes the "flexion" phase for protraction, the "extension" phase for retraction, it compares quite favourably with that of a supernumerary limb grafted into the brachial region. The common feature of myograms

recorded during co-ordinated movements is the lasting activity of the muscles and the thickness of the interferograms.

It could not be investigated in these preparations whether or not the long muscle activities are composed of small cycles generated in different parts of the deplanted centre, as assumed previously (SZÉKELY and CZÉH 1971.) There are, however, observations which suggest that in the preparations displaying co-ordinated movements the different excitatory and inhibitory activities of the deplanted neurones had to be distributed in a favourable way. Repeated mechanical stimuli easily resulted in clonic rigidity of the limb, and one had to wait for several minutes before a step-like movement appeared again. Sometimes the mere implantation of the electrodes was sufficient to bring the limb into tetanus and rendered the recording impossible. The myogram in Fig. 4 is also illuminating in this respect. After the cessation of the co-ordinated movement, muscle activity fades away, then two of the muscles start firing at a highly synchronized low frequency. In this case the excitation was focussed on these few motor neurones, or the rest was steadily inhibited. The requirement of structural integrity of the cord deplant speaks also for an appropriately balanced excitatory-inhibitory neural activity. The results together with these considerations strongly advocate the notion that the pattern of motility is programmed in the spinal cord's structure which is capable of moving the limb in a co-ordinated manner without receiving rhythmic afferentation and without any supraspinal control.

## ADDENDUM

Since the work has been submitted for publication, E. S. HARCOMBE and R. J. WYMAN reported an experiment (Diagonal locomotion in deafferented toads. J. Exp. Biol. **1970,** 53: 255—263), in which they found normal locomotion following total deafferentation in toads. After spinalization these animals were extremely lethargic.

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# THE ISOLATED SHELL-GLAND AS AN EXPERIMENTAL OBJECT

## TRANSMURAL POTENTIAL DIFFERENCE AND SHORT-CIRCUIT CURRENT IN THE HEN'S SURVIVING SHELL-GLAND

By

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A technique has been elaborated for the determination in vitro of the potential difference and short-circuit current of the hen's shell-gland together with some factors which may influence the membrane's electrical properties. When there were soft, brittle or hard eggs in the shell-gland, the transmural potential difference of the surviving membrane varied between 5—23 mV, in most cases between 5 and 6 mV. The mucosal side was negative to the serosal side. Short-circuit current density values were 10—26 $\mu$A/sq. cm.

One of the primary functions of the avain shell-gland is to deposit $CaCO_3$ to form the egg shell. In domestic fowl, up to 6 g of calcium carbonate is secreted in a 20 hour period but the period of active calcification is only about 14 hours (MONGIN 1968). It is clear that this organ performs a particularly intensive biological work.

The aim of this paper is to describe a method for studying the surviving shell-gland of laying hen and to report on the potential differences measured on this organ when mounted as a biological membrane. Experiments were carried out to clarify the effect of media with different ion compositions and of various gas mixtures bubbling through the bath on the transmural potential difference, as well as the response of the shell-gland to certain substrates and ions. Though our primary aim was to obtain data on the potential difference, the results of short-circuit current measurements are also included.

The method applied for studying the electrophysiological parameters of the shell-gland is similar to that developed and used in Ussing's laboratory for the study of frog skin and other biological membranes, but has so far not received sufficient attention. Only a single paper has been published recently on in vivo (LEONARD 1969) and another one on in vitro (HURWITZ et al. 1970) measured potential difference values of the organ.

## Methods

*Membrane preparation.* Seventy-five regularly laying and six non-laying White Leghorn G 51 hens were used. They were in the first year of laying. The condition of the reproductive organs, the location of the egg in the oviduct and the condition of the shell-membrane were palpated before killing.

The hens were decapitated, their abdominal cavity was opened and the location of the eggs noted. The shell-gland was excised and placed in a 39°C solution identical with the bath fluid, but without glucose and Na-succinate. A piece of the shell-gland was blotted with filter paper and immediately mounted in the apparatus; the exposed area in contact with the medium was 5 sq.cm.

The volume of bath fluid pipetted into the compartments was 10 ml on each side of the tissue. The incubation medium was vigorously circulated and aerated on both membrane surface by continuous flow usually of 95% $O_2$ + 5% $CO_2$. The temperature of all solutions was brought to 39°C prior to introduction into the chamber. Incubation at 39°C lasted for a period of about two hours.

*Apparatus for measuring the electrical parameters.* We have constructed an apparatus based partly on designs by USSING and ZERAHN (1951), FERREIRA et al. (1966), EDMONDS and MARIOTT (1968) and FORTE et al. (1967).

Description of the apparatus (Fig. 1). The solution chambers (5) are drilled into two halves of a plexiglass block, 4.5 × 6.0 × 11.0 cm (1). The shell-gland can be fitted as a membrane separating the bath fluids in the two chambers each of which can hold 10 or 20 ml of incubation medium. The membrane holders are important parts of the apparatus (3); they can be slid into the appropriate slots of the two plexiglass blocks. The size of the openings of the membrane holders can be varied to fit the dimensions of the tissue under investigation. The openings are sealed by gasket-rings embedded in the grooves round the openings. The openings of the membrane holders are surrounded by stainless steel pins (4). After the shell-gland has been mounted, the two half-blocks can be bolted together by means of two screws (2). A gentle tightening of the screws is sufficient to make the apparatus water-tight. Three ports are drilled into each chamber. These inlet ports are used to introduce the desired gas (10), the KCl-agar bridges for potential difference measurement (9) and the salt-bridges for measuring the short-circuit current (8). Incubation medium can also be added through these ports and the contents of the chamber can rapidly be exchanged through them. Each chamber is surrounded (11) by an independent electrically heated jacket (12).

*Measurement of the potential difference.* The bridges used for the measurement of transmural potential consisted of 17 cm long U shaped glass tubes 2 mm in diameter filled with 3.5 *N* KCl/3% agar. The tips of the bridges in the chambers were 1—2 mm from the shell-gland membrane. The other ends of the bridges reached into a trough filled with 3.5 *N* KCl solution (7) to establish contact with the saturated KCl-calomel electrodes (type Radelkis OP 810). At the beginning of the experiments the balance of the potential difference measuring electrodes was always checked before mounting the shell-gland tissue between the chambers to ensure a less than 1 mV asymmetry of the electrodes. In some case the junction potential in the chambers was measured at the end of the experiment, after removal of the shell-gland tissue.

Potential difference across the shell-gland tissue was measured with a precision mV-meter (type Radelkis OP 205, readings ±0.25 mV).

Immediately after having assembled the apparatus the potential difference generated between the mucosal and serosal surfaces of the shell-gland tissue was recorded. With the same bath fluid on both sides of the shell-gland tissue the mucosal side was always negative to the serosal side. During the experiments the potential difference was read at approximately 5 or 10-minute intervals. Mean potential difference was determined from the sum of successive readings, divided by the number of readings in 5 minutes; the results were expressed as mean ± S.E.

*Short-circuit current.* This is a current of appropriate direction which when passing through the tissue reduces the potential difference between its mucosal and serosal surfaces to zero. The current through the tissue flowed through the U tube filled with 3.5 *N* KCl/3% of agar (internal diameter of tube ends 2 mm) reaching into the incubation medium. Within the chambers the end of the bridges was about 2.5 cm from the tissue surface.

The other end of the bridges was fitted into the funnels (6) drilled into the plexiglass block and filled with AgCl-saturated 3.5 *N* KCl. Thick silver wire spirals coated with AgCl were immersed into the same funnels.

Voltage was supplied from a dry battery (10, Fig. 2) and by means of the potential divider (9, Fig. 2) the outer E.M.F. was adjusted gradually so that the potential difference across the shell-gland tissue, as read on the potentiometer, should drop to zero. This was obviously equal to the potential of the short-circuited shell-gland membrane.

The current flowing through the shell-gland membrane which reduced the potential difference to zero was read on the micro-ammeter (8, Fig. 2) every five minutes during the whole experiment to obtain a sufficiently accurate graphical integration of the total amount of electricity flowing through the shell-gland. Mean short-circuit current was calculated similarly to the mean potential difference. All results were expressed as mean ± S.E.

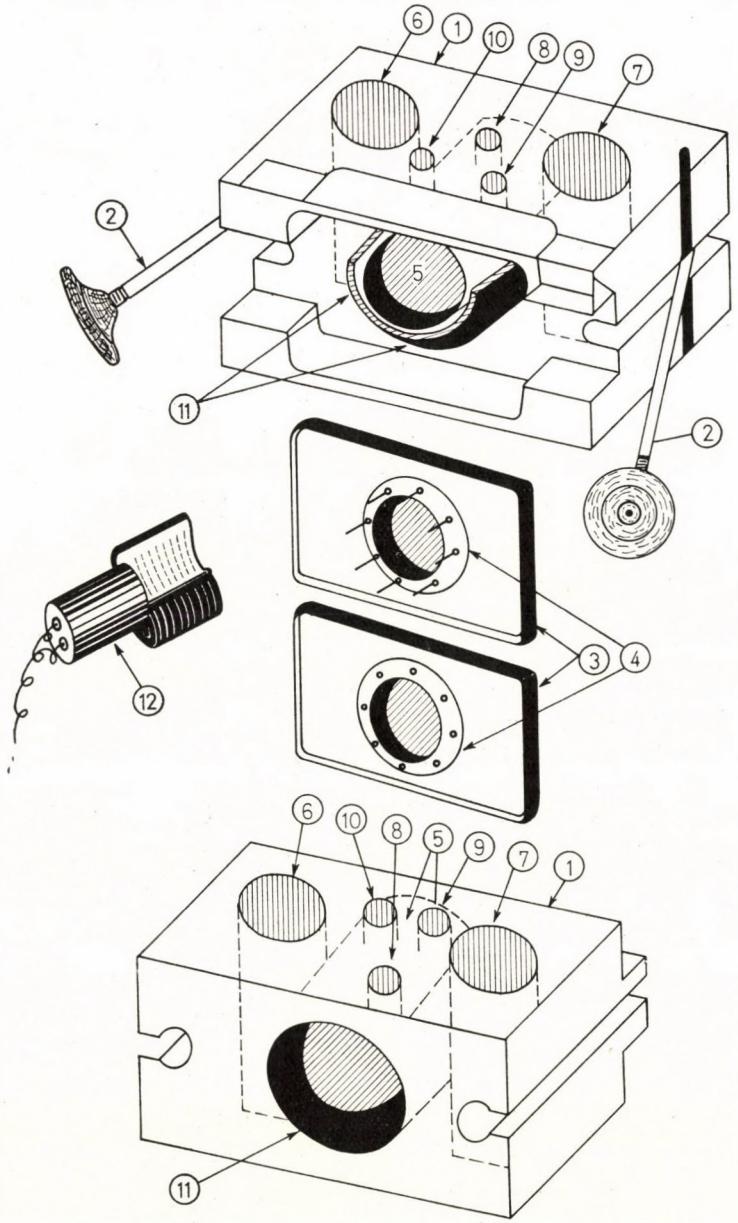


*Fig. 1.* Diagram of the apparatus

Resistance was calculated by dividing the natural transmembrane potential difference by the value for the short-circuit current.

*Solutions.* A series of stock solutions was prepared from analytical grade reagents appropriately mixed to obtain the desired compositions. Krebs—Ringer-bicarbonate and Krebs—Ringer-phosphate solutions were prepared from stock solutions immediately before use, the

rest were modifications of the basic solutions. Their composition was varied by replacing NaCl in the Krebs—Ringer-bicarbonate by an equivalent amount of KCl ("potassium"-Ringer), or by replacing NaCl in the Krebs—Ringer-sulphate solution by an equivalent amount of $Na_2SO_4$ ("sulphate"-Ringer). A similar Krebs—Ringer-bicarbonate solution was also prepared in which NaCl was replaced by Na-citrate ("citrate"-Ringer).

An incubation medium was prepared as described by EHRENSPECK et al. (1967; Ehrenspeck's solution) and "plumping" and "oviposition" medium on the basis of the composition of the shell-gland fluid as described by EL JACK and LAKE (1967).

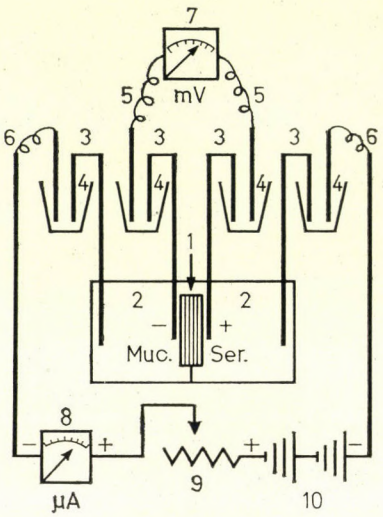


*Fig. 2.* Diagram of the apparatus used for the determination of potential difference and short-circuit current

## Results

With eggs in the shell-gland, the potential difference between the two surfaces of the membrane was usually 5—6 mV (range 5 to 23). The mucosal side was always negative to the serosal side (some special cases when this polarity was reversed will be described later).

Eighty surviving shell-glands were investigated; results may be classified as follows.

### *1. Potential difference responses in different media supplemented with glucose and succinate at concentrations of 16 and 10 mM, respectively*

*a)* mV values in bicarbonate-, phosphate- or sulphate-Krebs—Ringer baths. Table I sums up the potential difference values of active and inactive shell-glands. The data in the three vertical columns indicate that potential difference decreased least in phosphate-Ringer and most in sulphate-Ringer after one and two hours of incubation.

**Table I**

*Data for bicarbonate-, phosphate- and sulphate-Ringer solutions, each containing 16 mM of glucose and 10 mM of Na-succinate*

| Time (minutes) | $HCO_3^-$-Ringer | | $HPO_4^{=}$-Ringer | | $SO_4^{=}$-Ringer | |
|---|---|---|---|---|---|---|
| | egg in the uterus | | | | | |
| | mV | ±SE | mV | ±SE | mV | ±SE |
| 0 | 7.66 | 1.77 | 6.33 | 0.83 | 8.16 | 2.18 |
| 10 | 7.16 | 1.93 | 7.33 | 0.88 | 6.00 | 1.75 |
| 20 | 6.33 | 2.59 | 7.16 | 0.61 | 4.66 | 1.38 |
| 30 | 6.66 | 2.60 | 6.66 | 0.61 | 4.16 | 1.59 |
| 40 | 6.16 | 2.22 | 6.50 | 0.76 | 3.66 | 1.36 |
| 50 | 5.66 | 2.13 | 5.50 | 0.04 | 3.50 | 1.32 |
| 60 | 5.00 | 1.76 | 4.83 | 0.46 | 2.33 | 1.15 |
| 80 | 4.50 | 1.50 | 5.16 | 1.63 | 2.00 | 1.00 |
| 100 | 3.66 | 1.35 | 5.33 | 2.16 | 1.50 | 0.86 |
| 120 | 2.66 | 1.10 | 5.33 | 2.42 | 0.66 | 0.36 |
| | n = 3 | | n = 3 | | n = 3 | |
| | inactive uterus (n = 3 per medium) | | | | | |
| 0—120 | 0.5 | | 0.5 | | 0.5 | |

*b)* mV values obtained in various media changed in successive periods. In Table II are presented the potential difference values read every 10 minutes during 120-minute incubation. In experiment *1.a* media of the same composition were used for incubation. In this group in the first period (40 minutes) the medium was a NaCl solution which was then replaced by phosphate-Ringer (for 30 minutes) and in the last 40 minutes the bath was a bicarbonate-Ringer solution. The horizontal rows in Table II indicate the physiological state of the oviduct at the time of investigation. The most interesting data in this table are those summarizing the response of the active shell-gland with egg in it (in brackets). Data have not been selected according to the quality of the shell, as this aspect will receive special attention (Results *3*).

The potential difference is negligible when the egg is in the magnum or the oviduct is inactive.

*c)* mV values obtained in various media with special regard to citrate-Ringer. In one of the experimental groups the response of the shell-gland in bicarbonate-Ringer was compared to that in citrate-Ringer. It was found that in the latter bath the potential difference always increased as compared to the preceding period. On the left side of Fig. 3, the means in this group and their standard deviations are plotted. The graph continues on the right

side to demonstrate the results of a single experiment when the citrate effect has been detected after five periods of incubation always in some bath of different composition.

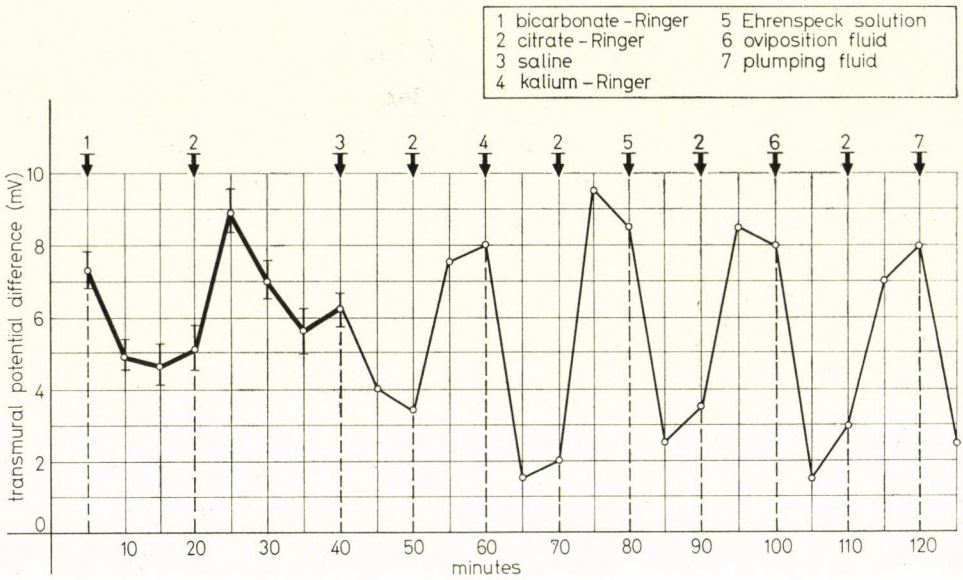


*Fig. 3.* Effect of different media on potential difference. Left: mean potential difference and ±SE for one group. Right: potential difference in a single experiment

**Table II**

*Data for saline, phosphate- and bicarbonate-Ringer solutions, each containing 16 mM of glucose and 10 mM of Na-succinate**

| Physiological state of oviduct or uterus | Saline | | | | | $HPO_4^{\prime\prime}$-Ringer | | | | $HCO_3^{\prime}$-Ringer | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | minutes | | | | | | | | | | | |
| | 0 | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 | 120 |
| | transmural potential difference (mV) | | | | | | | | | | | |
| inactive oviduct | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 1.5 | 1.5 | 1.0 | 1.5 | 1.5 | 1.5 | 1.0 |
| egg in magnum | 0 | 2.55 | 5.5 | 4.0 | 2.5 | 2.0 | 2.5 | 1.0 | 1.5 | 1.5 | 1.5 | 0.5 |
| egg in isthmus | 1.0 | 2.5 | 3.5 | 4.5 | 4.5 | 4.0 | 4.0 | 4.0 | 3.5 | 3.0 | 3.5 | 4.0 |
| egg in uterus | 8.6 | 7.0 | 5.2 | 4.4 | 3.9 | 3.6 | 3.3 | 2.9 | 2.5 | 2.1 | 3.9 | 4.1 |
| (n = 9) ±SE | 1.6 | 1.0 | 0.6 | 0.4 | 0.4 | 0.2 | 0.3 | 0.4 | 0.3 | 0.4 | 0.3 | 0.4 |
| immediately after laying | 6.0 | 5 7 | 4.8 | 4.1 | 3.2 | 4.2 | 4.2 | 3.7 | 3.0 | 4.5 | 5.0 | 4.5 |

* During the experiment the bath fluid was changed each in turn

*2. Effect of gas mixtures bubbled through the medium*

Attempts were made to clarify the requirements of aeration. The effect of $O_2$, $CO_2$, $N_2$, 95% $O_2$ + 5% $CO_2$, and 50% $O_2$ + 50% $CO_2$ mixtures on the potential difference was studied. The shell-gland is extremely sensitive to anoxia. As $O_2$ supply is suspended, potential difference immediately drops but rapidly regenerates on the receipt of more oxygen. The $O_2$ demand of

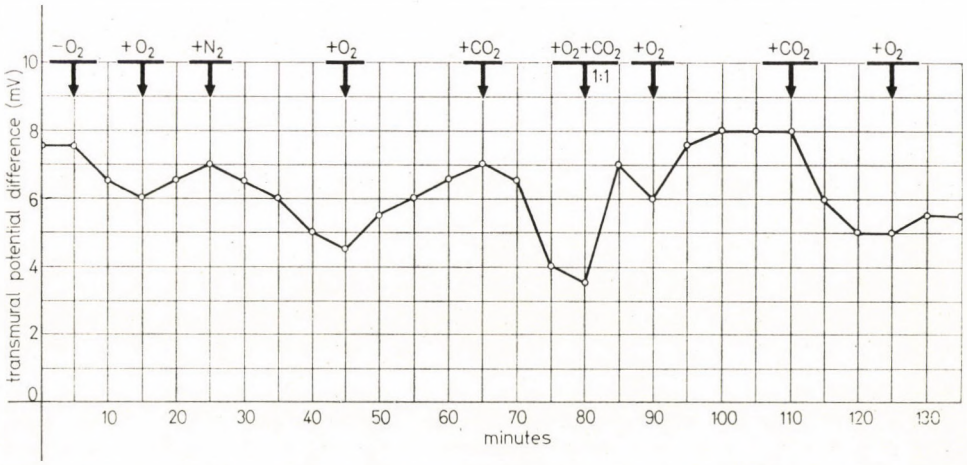


*Fig. 4.* Effect of $O_2$ deprivation on potential difference and changes in the phase of restitution. Gas composition was changed only on the mucosal side; the serosal side was exposed continuously to 95% $O_2$ + 5% $CO_2$

this tissue is satisfied even by a 50—50% $O_2$—$CO_2$ mixture. The responses are shown in Fig. 4. All this was valid only when the gas quality had been changed on the mucosal side. The same treatment on the serosal side caused no change in potential difference for 5—10 min. If the muscle layer had been removed before mounting the shell-gland into the chambers, the response was similar on the serosal side.

*3. The potential difference trend in different physiological states of the shell-gland*

It seemed interesting to assess the difference between the potential difference in the early and late phases of secretory activity. It was supposed that the shell-gland with brittle shelled egg will display the highest and the most constant potential difference since secretory activity is the highest in this phase. This assumption, however, proved incorrect. When several experiments were performed on the same day, it was found frequently that preparations from a pouch with soft or hard shell were more suitable for producing higher and relatively more constant potential difference values. Fig. 5 shows

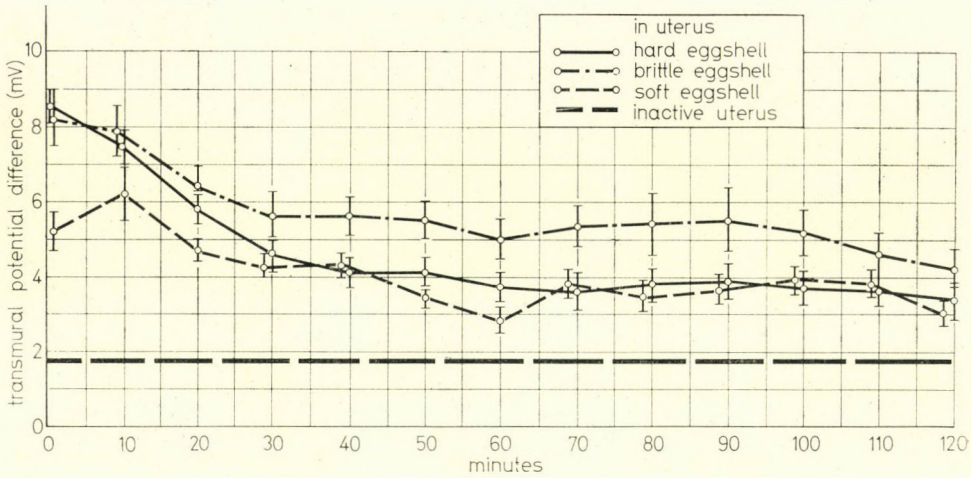


*Fig. 5.* Potential difference (±SE) in the periods of soft, brittle and hard shell formation

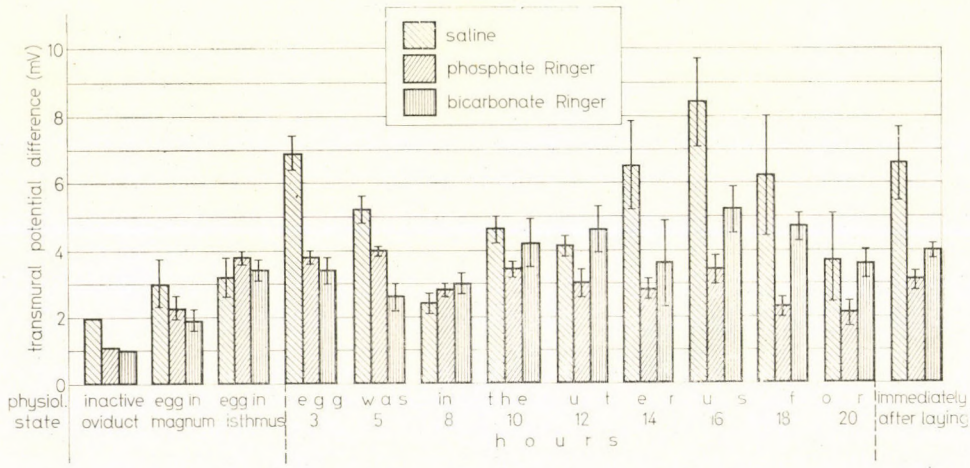


*Fig. 6.* Potential difference during the whole period of shell production measured at 2—3 hour intervals. Responses in three different media

a certain insignificant difference between the values of the three types of shell-gland and the top graph is considered to represent the group with brittle shells.

For the analysis of the relationship between transmural potential difference and the physiological state of the shell-gland, a series of experiments was carried out including measurements every 2 or 3 hours during the whole period of shell production. For this purpose, suitable laying hens were selected from a stock. Their shell-gland was incubated in three different media. Fig. 6

clearly shows that in none of the periods was transmural potential difference extraordinarily high. Therefore in our further investigations we saw no point in insisting on a definite secretory activity of the shell-gland.

*4. Simultaneous measurements of potential difference and short-circuit current*

*a)* Comparison of the effects of some substrates. For this experiment 6 surviving shall-glands were kept in bicarbonate-Ringer solution. After the first period (30 minutes) the bath was replaced by fresh medium, to check the spontaneous changes due to the effect of the fresh solution. In the 5th minute after the beginning of this second period of incubation, to two series 16 mM of glucose, to two other 10 mM of Na-succinate, and to the third 1 mM of Na-acetate were added to the bath. In the applied doses these substrates had no influence on the parameters. The data are summed up in Table III.

*b)* Response to citrate-Ringer. The effects of citrate-Ringer and of bicarbonate-Ringer on the shell-gland during incubation were compared with those noted in three other experiments using citrate-Ringer versus potassium-Ringer (without sodium). The responses unequivocally indicated that after

**Table III**

*Data for bicarbonate-Ringer, on the addition of 16 mM of glucose, 10 mM of Na-succinate, or 1 mM of acetate to the solution in the chamber*

| Minutes | mV | μA/sq. cm | Ohm · sq. cm | mV | μA/sq. cm | Ohm · sq. cm | mV | μA/sq. cm | Ohm · sq. cm |
|---|---|---|---|---|---|---|---|---|---|
| first medium | $HCO_3'$-Ringer | | | $HCO_3'$-Ringer | | | $HCO_2$-Ringer | | |
| 5 | 15.0 | 40 | 375 | 5.0 | 14 | 355 | 12.0 | 26 | 460 |
| 10 | 12.0 | 36 | 330 | 5.0 | 14 | 355 | 12.0 | 26 | 460 |
| 15 | 10.0 | 34 | 290 | 5.0 | 14 | 355 | 9.0 | 22 | 405 |
| 20 | 9.0 | 32 | 280 | 4.0 | 10 | 380 | 8.0 | 20 | 400 |
| 25 | 7.5 | 28 | 265 | 4.0 | 10 | 380 | 6.0 | 17 | 355 |
| fresh medium | $HCO_3'$-Ringer | | | $HCO_3'$-Ringer | | | $HCO_3'$-Ringer | | |
| 30 | 6.0 | 17 | 365 | 5.0 | 13 | 375 | 6.0 | 18 | 340 |
| 35 | 6.5 | 17 | 385 | 4.0 | 10 | 415 | 5.0 | 15 | 320 |
| 40 | 5.0 | 14 | 345 | 3.0 | 8 | 355 | 6.0 | 18 | 360 |
| supplements | 16 mM glucose | | | 10 mM Na-succinate | | | 1 mM Na-acetate | | |
| 45 | 6.0 | 13 | 405 | 2.0 | 8 | 235 | 5.0 | 15 | 320 |
| 50 | 5.0 | 13 | 340 | 2.0 | 6 | 330 | 6.0 | 17 | 355 |
| 55 | 4.0 | 10 | 380 | 2.0 | 6 | 330 | 5.0 | 16 | 300 |
| 60 | 4.0 | 9 | 430 | 2.0 | 6 | 330 | — | — | — |

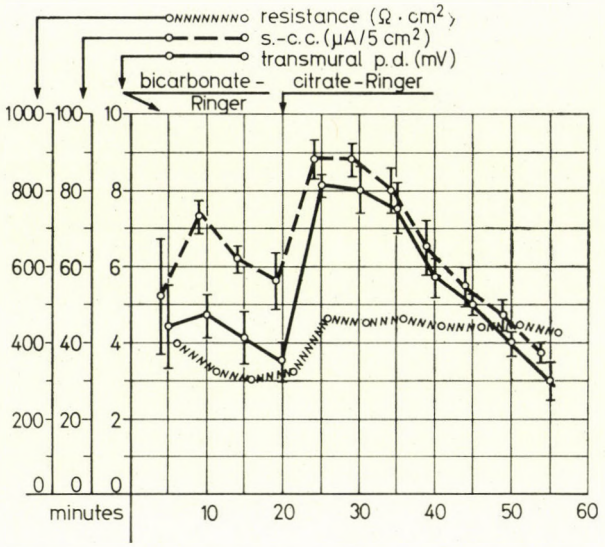


*Fig. 7.* Potential difference, short-circuit current and resistance vs. time. Bicarbonate-Ringer and Na-citrate-Ringer as bath fluids were subsequently placed in the chambers

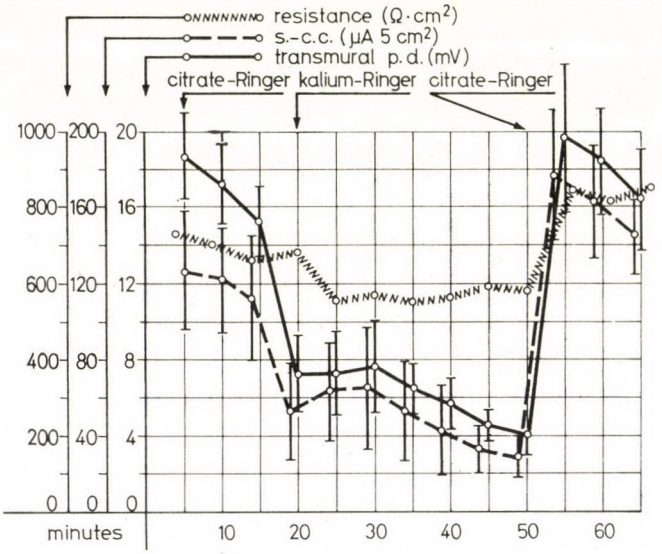


*Fig. 8.* Potential difference, short-circuit current and resistance vs. time. Potessium-Ringer and Na-citrate-Ringer as bath fluids were subsequently placed in the chambers

a preincubation in bicarbonate- or potassium-Ringer, the potential difference as well as the short-circuit current increased to a multiple of the initial value. Changes in these parameters had a fairly constant character (Figs 7 and 8).

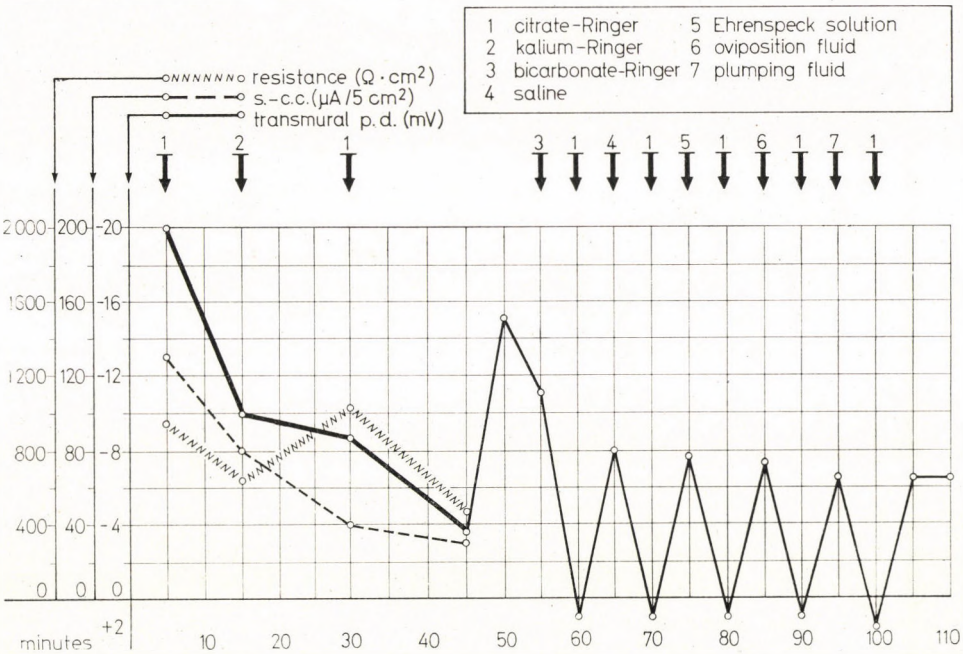


*Fig. 9.* Effect of different media on potential difference of short-circuited tissue. Left: potential difference, short-circuit current and resistance of one group in citrate- or potassium-Ringer. Right: an individual experiment; citrate-Ringer medium in all but one experiment. Transmural potential difference zero, or +1, +3 mV; polarity of mucosa obviously reversed. In all cases polarity was normalized by citrate-Ringer

Finally, as shown in Fig. 9, after several incubation periods in saline, Ehrenspeck medium, plumping or oviposition fluid the transmural potential difference was zero, or +1, +3 mV, that is the polarity had normalized on the addition of citrate-Ringer.

## Discussion

The nature of the relationship between metabolic activity and the movement of calcium through the shell-gland tissue were studied by EHRENSPECK et al. (1967). For our purpose, i.e. for electrophysiologic measurements, this method was not suitable. Therefore first an appropriate method had to be worked out and a simple and easily manipulated and cleaned mounting device was constructed. The device for potential difference as well as for short-circuit current measurements was easy to handle and proved to be precise.

Very often after the first rapid decrease — e.g., from 10 to 6 mV or from 20 to 12 mV — the potential had stabilized on this lower level for 1—2 hours and then gradually dropped to zero.

The classical bicarbonate and phosphate buffered Krebs—Ringer solutions were used as baths. Our attempts to achieve a high and relatively stable potential difference in $SO_4$-medium (FERREIRA et al. 1966) failed. EHRENSPECK et al. (1967) found that 16 mM of glucose and 10 mM of succinate are suitable substrate concentrations. If not otherwise specified, we too added these substrate concentrations to the media.

The next step was obviously to try other media, i.e. other components in the bath in order to achieve a long-term increase and/or stabilization of the transmural potential difference of these preparations. With citrate-Ringer as bath, potential difference and short-circuit current values increased in all experiments independently of the previously applied medium. Citrate-Ringer raised the potential difference from zero to 5—6 mM; moreover on the serosal side the values were —2, —3 mV, thus, contrary to the normal situation, the potential difference was reversed and increased markedly.

A slightly hypertonic medium described by EHRENSPECK et al. (1967), as well as compositions corresponding to the plumping and oviposition fluids of the shell-gland were also tried, considering the data of EL JACK and LAKE (1967). These media neither increased nor stabilized the transmural potential difference of the preparations. At present the classical bicarbonate buffered Krebs—Ringer solution supplemented with glucose and succinate seems to be the most suitable bathing solution for the examination of the uterus in vitro. Other media with different ionic composition were not more effective.

The surviving shell-gland is highly sensitive to anoxia (Fig. 4). In this respect its behaviour bears a likeness to the membrane preparation from the rat colon (EDMONDS and MARIOTT 1968). $CO_2$ has a definite effect upon the potential of the frog skin. If instead of air, oxygen is used to aerate the Ringer solution on both sides of the frog skin, the potential drops to about zero. Even lower $CO_2$ tensions (1—2%) suffice to depress considerably the potential difference.

The membrane of the shell-gland is not particularly sensitive to $CO_2$. It was found to consume $O_2$ fairly well from a mixture of 50% $O_2$ + 50% $CO_2$.

By changing the bath some information was obtained on the electrophysiological aspects of the effect of certain ion types on the shell-gland. Exposure of the tissue to Na-free potassium-Ringer for 15—20 min was often accompanied by a rapid drop in potential difference and short-circuit current.

The calcium content of the oviposition fluid is fairly high; the potential difference of the shell-gland decreases rapidly in this medium, too. But the shell-gland is the only part of the oviduct in which oxygen consumption is independent of the environmental calcium ion concentration (MISRA and KEMÉNY 1964).

In the experiment shown in Fig. 9, the polarity of the membrane reversed in all but one medium. The values for potential difference and short-circuit

current were measured alternately. When a similar experiment was performed earlier measuring only the potential difference (Fig. 3), no reversal of the polarity was observed. Apparently, the reversal of tissue membrane polarity was in the above cases only an artifact.

These observations formed the basis for the investigation of shell-gland electrogenesis. Ouabain produced a significant decrease in potential difference when added to either chamber (HURWITZ et al. 1970), therefore the existence of a sodium pump in the uterine wall was suggested. It is evident that further studies are required before a final conclusion can be drawn.

The present approach is inadequate to disclose the effects of substrate molecules on potential difference and short-circuit current. It is, however, certain that glucose, succinate and acetate at concentrations of 16 mM, 10 mM and 1 mM, respectively, cause no changes in either value.

As to the effect of citrate on potential difference and short-circuit current of the shell-gland there are two possible alternatives: it may be due to a change in the shunt path and/or to a metabolic process as an additional source of electric current. Our studies of the citrate effect have, however, failed to produce convincing evidence. In the case of frog skin very high potentials may be obtained by using the slowly penetrating sulphate ion instead of chloride or by treating the outside of the skin with traces of copper ions (a treatment leading to a very low chloride permeability). In this relation it is important to bear in mind Ussing's two membrane hypothesis (USSING and ZERAHN 1951).

Citrate concentration in the shell-gland and the isthmus is high (200—400 mg/100 g fresh weight), about a hundred times higher than in brain tissue and three to four hundred times higher than in liver tissue (HERTELENDY and TAYLOR 1964; KEMÉNY et al. 1970). The published data are not sufficient for drawing convincing conclusions as to the physiological significance of the exceptionally high citric acid level in the isthmus and the shell-gland. A rise in citrate has been accepted as a primary manifestation of the operation of the citric acid cycle and of the metabolic level of an individual organ (POTTER et al. 1951; FIRSCHEIN and BELL 1961).

In conclusion we believe that the present technique will allow to prepare a relatively stable isolated avian shell-gland which should be useful in the elucidation and interpretation of the mechanism of transport of different ions in this tissue.

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# PLASMA INSULIN-LIKE ACTIVITY AND INSULIN SENSITIVITY IN RATS AFTER HYPOTHALAMIC DEAFFERENTATION

By

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After medial hypothalamic deafferentation either complete (CD) or at frontal (FD), fronto-lateral (FL), dorsal (DD) levels, parallel with adrenocortical hormone determination, sensitivity to insulin and plasma insulin-like activity were followed for two weeks post-operatively. Insulin increased after FD and decreased after CD or DD. Adrenal hormone production increased after FL and CD and was unchanged after FD and DD. Mechanical interruption of nervous connections between ventromedial (VMH) and ventrolateral (VLH) nuclei did not lead to hyperphagia or changes in the fasting blood glucose level. The behaviour of insulin is attributed to the action of growth hormone and adrenocortical activity; it was not due to decreased hepatic glycogenesis. Without denying the direct role of the central nervous system, the results pointed to a direct connection between plasma ACTH concentration and insulin-like activity and showed that the increase in insulin-like activity was not due to hyperglycaemia.

In a previous paper (HEFCO and JITARIU 1971) it has been shown that certain types of hypothalamic deafferentation result in a change of hypothalamo-pituitary-adrenal activity, but this alteration is not accompanied by variations in the fasting blood glucose level. Based on some functional tests as well as on hepatic glycogen determination, the normal course of carbohydrate metabolism was shown to require the presence of nervous connections of the hypophysiotrophic area with both extrahypothalamic and hypothalamic structures outside the hypophysiotrophic area (HEFCO et al. 1971).

In the present work, carbohydrate metabolism has been studied after different types of hypothalamic deafferentation in the rat, parallel with the determination of pituitary-adrenal activity, and of insulin sensitivity and insulin-like activity. Food intake was also determined after separation of VMH and VLH nuclei connections.

## Methods

Male Wistar rats, maintained at constant temperature and Mac Collum's standard diet were used. Hypothalamic deafferentation was carried out according to the stereotaxic method of HALÁSZ and PUPP (1965), with the difference that the height of the sectioned area was 2 mm and the maximum width at the base was approximately 1.8 mm. FD began just behind the optic chiasma and spread up to the anterior part of the VMH nucleus. FL began at the same level and behind the chiasma it included part of the mammillary bodies. The size of the isolated area after CD was identical to FL, only that posterior connections were

also sectioned. The isolated area extended laterally up to the level of the fornix. In the case of DD, the shape of the section is shown in Fig. 2, a section extended antero-posteriorly to the same distance as in the case of CD. On this occasion thalamic connections were sectioned at the level of the paraventricular nucleus. Deafferentation was performed under intraperitoneal pentobarbital (45 mg/kg) anaesthesia, followed by streptomycin powdering of the skull. No post-operative infections were noticed. Sham-operated animals were subjected to the same surgical procedure, with the difference that the knife reached only the upper part of the hypothalamus. The insulin tolerance test was performed after 18-hour fasting, 12 days post-operatively, by the subcutaneous injection of crystalline zinc insulin (0.125 U/kg) dissolved in Krebs—Henseleit buffer solution with 200 mg per 100 ml of gelatin. Blood was sampled from the tail vein before, and 30, 60 and 120 minutes after insulin injection. Blood sugar was determined by the method of ASATOOR and KING (1956), insulin-like activity by bioassay with rat epididymal fatty tissue, according to BELLENS (1961), after 18-hours fasting, 14 days post-operatively.

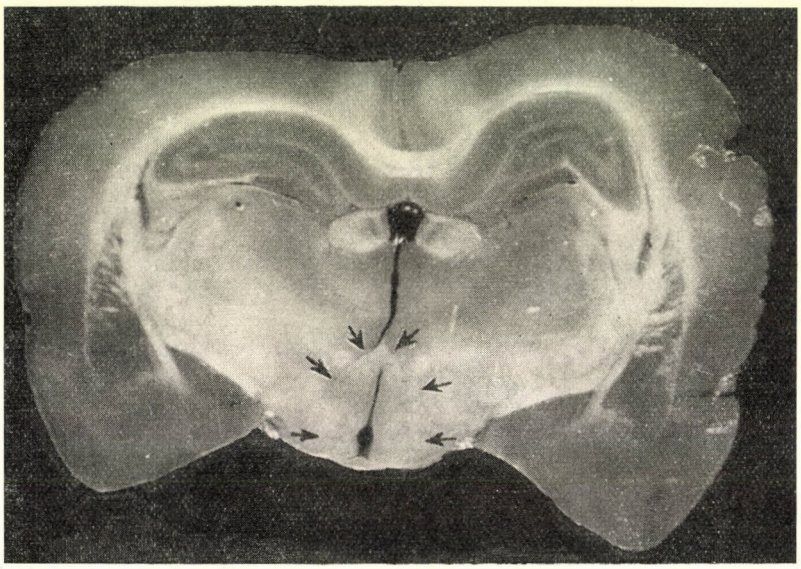

*Fig. 1.* Maximum size of deafferentated area in the case of FD, FL or CD. Arrows indicate the cut. Legend as in Table I

Adrenal corticosteroid production in vitro was determined by the method of VAN DER VIES et al. (1960). Total corticosteroid production in vitro expressed in $\mu$g/100 mg adrenal/hour served as the index of pituitary ACTH release (VAN GOCH et al. 1963). Histological control of the brain deafferentation was performed according to GUZMAN-FLORES et al. (1958). The stereotaxic atlas of SZENTÁGOTHAI et al. (1968) was used. Food intake was daily measured using RUFEGER (1968) type metabolic cages.

The results were analysed statistically, using Student's *t*-test.

## Results

The maximum size of the isolated area in the case of FD, FL and CD is seen in Fig. 1. Fig. 2 shows the level where the section was made in the case of DD.

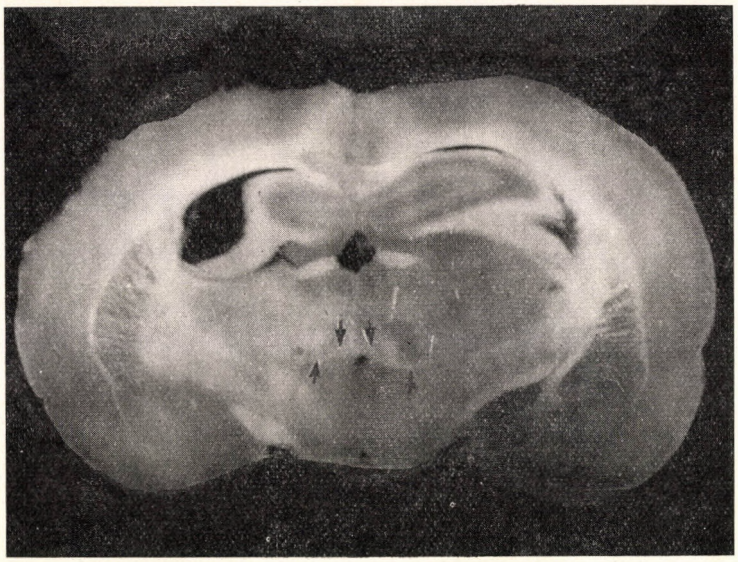

*Fig. 2.* Dorsal deafferentation of the medial hypothalamus. Arrows indicate the cut

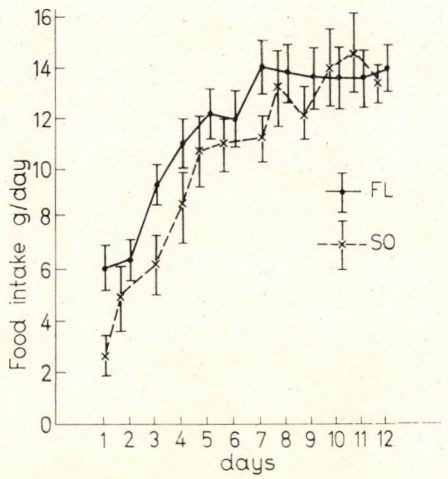


*Fig. 3.* Effect of fronto-lateral deafferentation on food intake in rats. Mean ±SE

*Effect of fronto-lateral hypothalamic deafferentation on the food intake*

In the first days after the interruption of nervous connections between VMH and VLH nuclei, the rats displayed a significantly increased food intake as compared to sham-operated animals (Fig. 3). The difference disappeared on the following days. Though food consumption increased in the first days after FL, the animals were not hyperphagic as their total food consump-

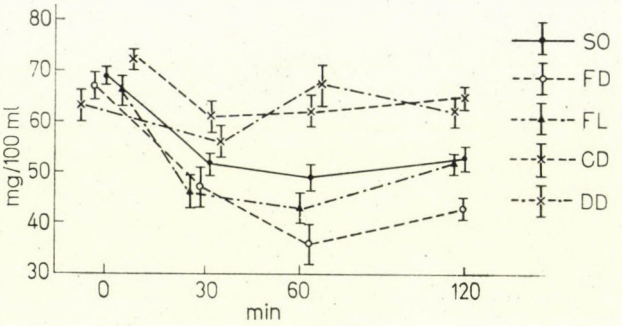


*Fig. 4.* Insulin sensitivity (0.125 U/kg) of rats with hypothalamic deafferentation. Legend as in Table I

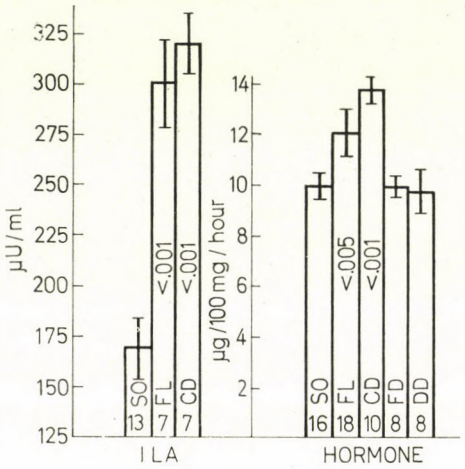


*Fig. 5.* Plasma insulin-like activity and adrenal corticosteroid production in vitro in rats after hypothalamic deafferentation. Numbers in columns represent number of rats and significance

tion was less than the normal one of about 14 g/day. Hypophagia ceased 4 days after surgery.

*Effect of the partial and complete deafferentation of the hypothalamus on the insulin sensitivity and plasma insulin-like activity*

Results are summarized in Figs 4 and 5. Insulin sensitivity of FL rats in the first 60 minutes after injection was somewhat higher than that of sham-operated animals; the difference was not significant statistically. Conversely, FD rats reacted to insulin by a high decrease in blood sugar level for 2 hours. In contrast, animals with CD and especially those with DD, presented a reduced sensitivity to exogenous insulin, as differences between

them and the sham-operated animals were significant throughout the experiment. The decrease observed 30 minutes after insulin in the group with DD was non-significant as compared to that at zero hour, after which the values oscillated around those estimated before insulin injection.

Before insulin loading, the blood sugar level in the studied groups showed no significant differences.

In CD and FL rats plasma insulin-like activity was significantly increased.

*Hypothalamo-pituitary-adrenal activity*

Experimental data are presented in Table I and Fig. 5. The weight of the neurohypophysis decreased in all cases of deafferentation, that of the adenohypophysis increased after FD. In DD, adrenal weight was not modified significantly, and after the other types of deafferentation it increased significantly. Adrenal corticosteroid production in vitro was not modified after FD and DD; after FL and CD, it increased.

In the animals, in which insulin tolerance test and insulin-like activity were determined, no significant variations were observed in body weight.

**Table I**

*Changes of some indices after hypothalamic deafferentation*
(Mean ± S. E. Number of rats is given in brackets)

| Groups | Body weight, g | | Pituitary wt. mg/100 g b.w. | | Adrenal wt. mg/100 g b.w. |
|---|---|---|---|---|---|
| | Initial | At autopsy | Adenohypophysis | Neurohypophysis | |
| SO | 239±4.5 | 235±5 | 2.57±0.08 | 0.52±0.026 | 14.64±0.38 |
| | (20) | (20) | (18) | (20) | (14) |
| FL | 232±4.2 | 222±6.5 | 2.74±0.08 | 0.44±0.022 | 16.90±0.70 |
| | (15) | (15) | (18) | (18) | (17) |
| | NS | NS | NS | $p < 0.05$ | $p < 0.01$ |
| | 245±6.1 | 225±6 | 2.40±0.09 | 0.332±0.023 | 18.30±1.05 |
| CD | (14) | (14) | (12) | (14) | (12) |
| | NS | NS | NS | $p < 0.001$ | $p < 0.01$ |
| | 246±5 | 244±6 | 2.82±0.09 | 0.381±0.029 | 15.96±0.46 |
| FD | (10) | (10) | (8) | (10) | (9) |
| | NS | NS | $p < 0.05$ | $p < 0.002$ | $p < 0.05$ |
| | 237±6.9 | 231±6.5 | 2.65±0.08 | 0.460±0.01 | 15.20±0.40 |
| DD | (8) | (8) | (8) | (8) | (8) |
| | NS | NS | NS | $p < 0.05$ | NS |

NS = non-significant
SO = sham-operated
FL = fronto-lateral deafferentation
CD = complete deafferentation
FD = frontal deafferentation
DD = dorsal deafferentation

## Discussion

The results showed that frontal isolation of the medial hypothalamus is accompanied by a marked increase of insulin sensitivity without a decrease of adrenocortical activity. After an insulin dose 10 times higher than that applied in the present experiments, FD rats die or develop hypoglycaemic coma, while in sham-operated, blood sugar level decreases markedly but no hypoglycaemic coma is observed.

In man and in some animals, a failure of glycogenesis appears to be the primary defect in adrenal insufficiency which is responsible for the severe insulin sensitivity (reviewed by FAJANS 1961). In our experiments in rats with different types of hypothalamic deafferentation no decrease in hepatic glycogen content has been observed (HEFCO et al. 1971). Recently, KOKKA and GEORGE (1969) have reported a hypersensibility to insulin in normal and adrenodemedullated rats, after lesions in the rostral hypothalamic area or after hypophysectomy. From the plasma corticosterone level these authors concluded that the increased insulin sensitivity of rats with such lesions does not seem to be due to a reduction of ACTH secretion. The same conclusion may be drawn from our results. The fact that adrenal corticosteroid production is not significantly modified after FD, indirectly supports the experiments performed by SPIRTOS and HALMI (1959), who suggested that a growth hormone (STH) deficiency represents the main cause of the increased insulin sensitivity. HALÁSZ's data (1968) support this conception as he observed an almost significant decrease in the plasma and pituitary STH content in FD rats and a significant decrease in plasma and pituitary STH after CD. In this case, however, sensitivity to insulin is reduced. This might be due to the high adrenocortical activity after CD. FAJANS (1961) also reported an increased insulin sensitivity in persons with glucocorticoid excess.

The increase in adrenocortical activity after FL is probably compensated by the reduction of STH, which results in an almost normal sensitivity to insulin. If we consider the action of these two hormones, the presented data show that degree of insulin sensitivity is a resultant of their action.

DD rats, though displaying a normal adrenal activity, show a high resistance to exogenous insulin, exceeding that of CD rats. This phenomenon may be explained, only on speculatory grounds. Since in DD rats, no significant decrease in blood sugar level appears after a 0.125 U/kg dose of insulin, the resistance to insulin might be due to an increase of STH release after the isolation. Our supposition is based on YOUNG's investigations (1963) which revealed a normal blood sugar in dogs and cats treated with STH for several days; their sensitivity to exogenous insulin was reduced. DEBODO et al. (1963) also reported that STH increases glucose production in the normal animal and prevents the facilitation of glucose consumption by injected insulin.

Glucagon and epinephrine are also preventing glucose consumption by insulin-dependent tissues. An increase in the amount of glucagon occurs also under the influence of STH (YOUNG 1963). We believe, however, that a marked increase of the epinephrine content after insulin injection is unlikely, since a parallel increase of coagulation time has been observed in the rats treated in this manner, and only in them. Epinephrine usually enhances blood coagulation. These speculations might indicate that fibres with an inhibitory role on STH secretion reach the hypophysiotrophic area from the dorsal side.

The question may arise whether the insulin sensitivity changes are a result of hormonal variations appearing after hypothalamic deafferentation, or can be attributed partly to the interruption of some connections between the insulin-sensitive nervous centres and the pituitary, or due to the interruption of nervous pathways through which insulin-sensitive central areas would act on the periphery. The existence of insulin-sensitive nervous centres has been demonstrated in a series of papers. Thus, CHOWERS et al. (1966) showed that the fall in systemic blood sugar level, consecutive to intracisternal insulin administration, was mediated by insulin action on some brain structure. They suggested parasympathetic brain centres as a possible site of action for a direct effect of insulin on the central nervous system. This may only be one of many. Indeed, GOODNER and FREINKEL (1961) have demonstrated insulin effects on the anterior pituitary of the rat and the calf, both after in vivo and in vitro administration of the hormone.

Considering that part of the mechanisms implied in glucose homeostasis is released after the action of insulin on the insulin-sensitive nervous areas, from the present results it follows that the connections between the centres activating these mechanisms reach the medial hypothalamus frontally, and those with an inhibitory role, posteriorly and/or dorsocaudally. Thus, sensitivity to insulin increased after FD of the hypophysiotrophic area, and decreased after posterior or dorso-caudal deafferentation. However, for the precise explanation of the observed phenomenon, further studies are needed. For the moment, taking into account the effect of STH and ACTH on insulin sensitivity, the presented data show that it is their resultant action on which the degree of insulin sensitivity depends.

FL and CD rats presented a higher plasma insulin-like activity than the sham-operated animals. An increased insulin content has been observed by HALES and KENNEDY (1964) in hyperphagic obese rats after VMH lesion. This increase of plasma insulin occurred in the animals with free access to food, and was explained by the chronic action of hyperglycaemia on the endocrine pancreas. Our data obtained after FL showed that an increase in the insulin-like activity may occur without the appearance of hyperphagia, followed by hyperglycaemia. The increased plasma insulin-like activity observed under the present experimental conditions could also occur by the agency of

nervous pathways, as suggested by recent investigations. The VMH nucleus is considered to exert an inhibitory action on the VLH nucleus. The connections between these two areas have exactly been described (AREES and MAYER 1967). Stimulation of the lateral hypothalamic area results in a decrease of the blood sugar level (SHIMAZU et al. 1966) and a picture characteristic of an enhanced insulin secretion was observed at the level of the pancreas (KUROTSU et al. 1957). The pathways for this reaction involve the participation of the vagus, which is connected to the VLH nucleus (MAYER 1966). After vagal stimulation, FROHMAN et al. (1967) observed an increase of insulin secretion. Our results have fully confirmed this possibility, since the interruption of connections between the medial and lateral hypothalamic areas after CD or FL disinhibits the function of the VLH nucleus and this may result in a neurogenic rise of the plasma insulin level. The same conclusion has been arrived at by FROHMAN et al. (1969) in investigations into the effect of lesions of the VMH nucleus.

This is a possibility for a change in pancreatic B cell activity, the physiological significance of which remains to be solved. Beside this nervous mechanism, one must not overlook the possibility of modifying pancreatic activity by an endocrine pathway, especially by ACTH. This action has been confirmed by a series of investigation carried out recently. Thus, ACTH administration in the intact dog, or ACTH perfusion of rat isolated pancreas results in increased insulin release (reviewed by MAYHEW et al. 1969). This might account for the normal blood sugar level observed by us in rats with hypothalamic deafferentation, which enhances adrenal activity although the connections between the VMH and VLH nuclei are not sectioned, as it was observed after preoptic deafferentation (HEFCO et al. 1971). We have also shown that a lesion of the VMH nucleus which does not affect the median eminence, is accompanied by a significant increase in both adrenal weight and in its hormone production (unpublished data). This change could determine the alteration in endocrine pancreatic activity observed in experiments with VMH lesion.

Thus, without denying the direct role of the central nervous system in the control of insulin secretion, our results point to a direct connection between the plasma ACTH level and insulin-like activity. Thus, under normal conditions, the endocrine pancreatic secretion may be under the control of the central nervous system, the endocrine system and of many other pathways (RENOLD 1970; MAYHEW et al. 1969).

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# CORTICAL AND HIPPOCAMPAL BIOELECTRIC ACTIVITY DURING ASPECIFIC VASODEPRESSOR REACTION

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The aspecific vasodepressor reaction in superficially anaesthetized rabbits is associated with a tonic bioelectric arousal. At the same time, spontaneous tonic arousals are also accompanied by a fall of the blood pressure. The phasic EEG changes are not associated with a vasomotor response.

It was found that the obligatory indicator of the vasomotor response was not the cortical desynchronization but the appearance of the slow hippocampal rhythm.

The characteristic EEG pattern elicited by the stimulation of reticular structures is associated, at low voltage stimuli, with a depressor response, and with a pressor response at high voltage ones.

It is assumed that the aspecific vasodepressor response forms a vegetative component of the subcortical orienting reaction if the pattern of the descendent vegetative processes is predominated by inhibition.

It has been shown (SUCH 1959) that in rabbits superficially anaesthetized with urethan or ether, various auditory stimuli evoked decreases of 15—30 mm Hg in blood pressure lasting about 30 seconds. Since other nociceptive and non-nociceptive stimuli brought about the same effect, the reaction was not considered a specific vasomotor response and the possibility has been pointed out that the vegetative reaction evoked by essentially arousing stimuli represented an integrant component of a behavioural response, presumably an orientation reaction, of higher organization.

The experiments to be reported were designed to study the bioelectric activity of the cortical and rhinencephalic structures in the course of the aspecific vasodepressor response, and the functional connection of the ascendent and descendent function of the reticular formation during the organization on that reaction. The vasomotor centre is known to constitute a semispecialized part of the reticular formation, isolated neither structurally nor functionally from the other parts of the system.

## Methods

Experiments were carried out in 25 rabbits weighing 2.5—3.5 kg, anaesthetized intravenously with 1 g/kg of urethan. The head of the animals was fixed in a holder specially designed for the purpose (SUCH and MADARÁSZ 1962) which could be mounted upon the common stereotactic device and thus permitted the use of the rabbit brain atlas of SAWYER et al. (1954). Interventions were undertaken in local anaesthesia. Electrical activity was recorded from the frontal and occipital cortical areas. The electrodes were enamelled phonograph needles nailed in the cranium. Hippocampal recording was made monopolarly, by glass-insulated

silver electrodes. Arterial blood pressure was monitored in the femoral artery by means of a Statham transducer.

Noise producing devices and a square wave generator, connected to a loudspeaker were used for acoustic stimulation. Tactile, pain and interoceptive stimuli were applied as well. The reticular structures (mesencephalic reticular formation and the hypothalamic reticular nucleus) and the cortical areas were stimulated with square wave impulses by means of bipolar silver electrodes (pole distance, 1.0—1.5 mm).

## Results

### a) *Simultaneous analysis of the aspecific vasodepressor response and the ascendent (cortical and hippocampal) reticular effects*

Acoustic and other wakening stimuli, applied in superficially sleeping or superficially anaesthetized animals, bring about a generalized electric arousal in the cortex, displaying a phasic or a tonic form depending on the conditions (SHARPLESS and JASPER 1956). Simultaneously with the desynchronization, slow rhythmic waves of 5 c/s appear in the hippocampus (JUNG and KORNMÜLLER 1939; GREEN and ARDUINI 1954).

It has been found that the above bioelectric changes associated with the aspecific vasodepressor response commonly exhibit a tonic form (Fig. 1).

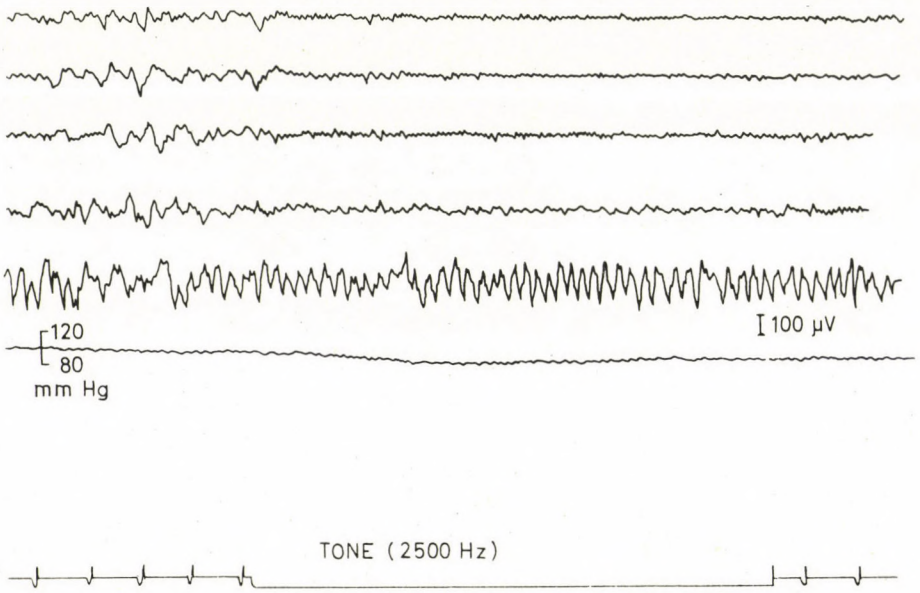


*Fig. 1.* Vasodepressor reaction and the simultaneous changes in cortical and hippocampal activity in a rabbit (3.1 kg) anaesthetized intravenously with 1 g/kg of urethan. Due to the delay of the manually controlled signal, the hippocampal theta activity begins about 1 sec earlier. The registrates from above: left fronto-occipital, right fronto-occipital, bifrontal, bioccipital, right hippocampal activity, and blood pressure

A correlation was observed between the biological intensity of the applied stimulus, the degree of blood pressure decrease and the duration of the arousal. In the case of short phasic arousals the blood pressure does not change (Fig. 2). All "spontaneous" arousals in the course of the present experiments were accompanied by a decrease in blood pressure (Fig. 3).

Non-nociceptive (auditory or tactile) stimuli repeated in one-minute intervals evoke habituation phenomena after the fourth of fifth application, manifesting in a reduction of the duration of arousal and of the extent of blood pressure depression.

The aspecificity of the vasodepressor response could be extended to interoceptive stimuli (distension of the urinary bladder or of the rectum) if they resulted in tonic arousal. However, on its repetition the effectivity of the interoceptive stimulus rapidly decreased and was finally abolished, and the EEG changes and the vegetative response disappeared simultaneously.

Neocortical desynchronization was often slight or failed to appear after moderate stimulation or in cases where a decreased excitability of the nervous system could be assumed. Regular hippocampal activity was, however, maintained. This paleocortical activation (GREEN and ARDUINI 1954) or inhibition (GRASTYÁN et al. 1959) was always associated with a fall in blood pressure (Fig. 4). The aspecific vasodepressor response was regularly accompanied by hippocampal theta rhythm. This sign sensitively indicated the vegetative reactions. Consequently, it was not the cortical arousal but the regularization

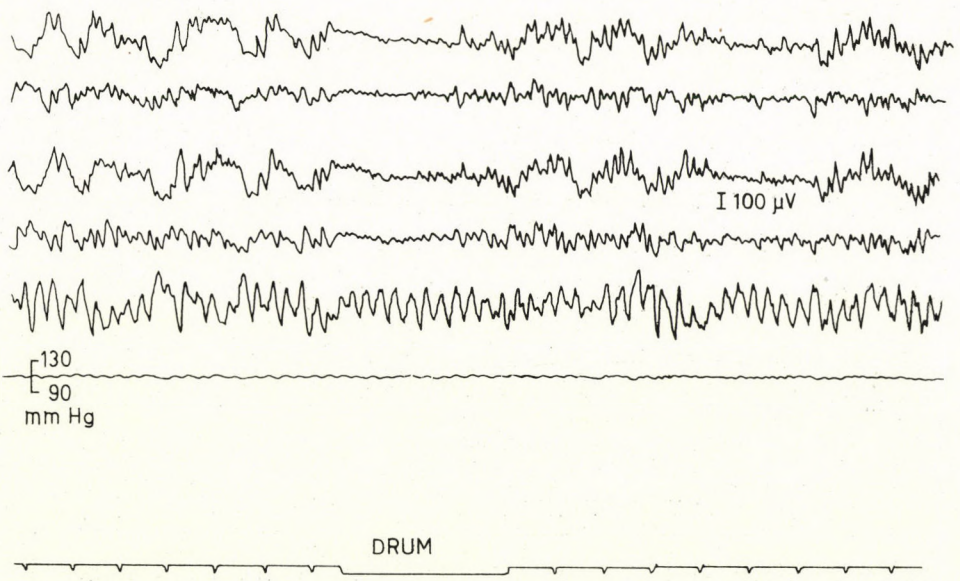


*Fig. 2.* Same animal as in Fig. 1. Phasic arousal is not associated with a vasomotor response

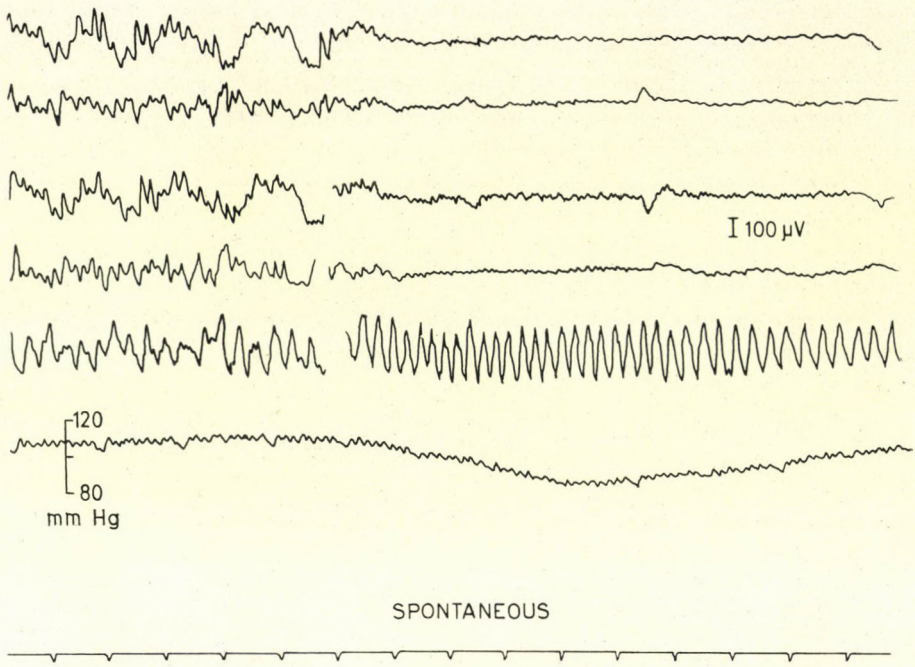


*Fig. 3.* Same animal as in Fig. 1. "Spontaneous" arousal is associated with hypotension

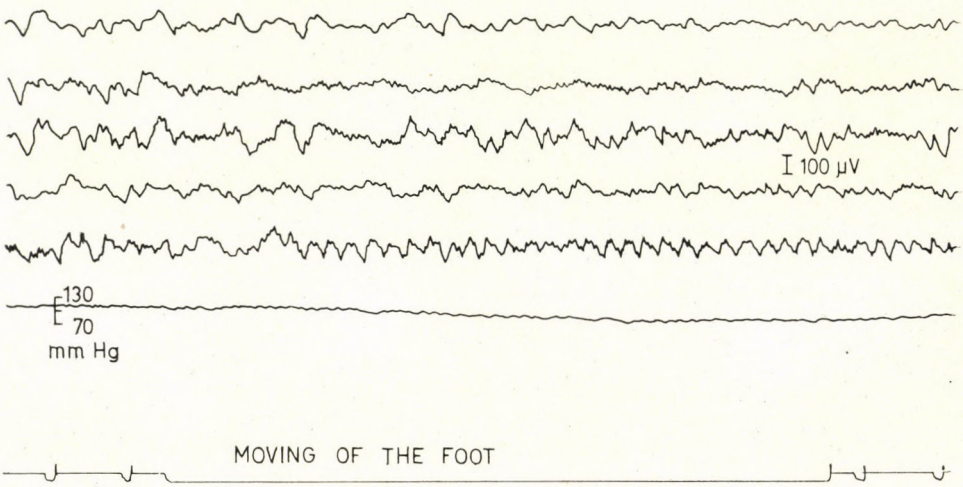


*Fig. 4.* Rabbit, 3.4 kg; 1 g/kg urethan intravenously. The aspecific vasodepressor response is reflected only by the alteration in hippocampal activity. Neocortical activity is unchanged

of the hippocampal rhythm that proved to be characteristic of the aspecific vasomotor reaction.

b) *Effect of chlorpromazine on ascendent reticular activity and the aspecific vasodepressor reflex*

With regard to the essential role of the reticular formation in the awakening and vasomotor reactions, the effect of chlorpromazine on the aspecific vasodepressor reaction was examined. According to several data, the site of central depressive action of chlorpromazine is the brain stem reticular formation (LONGO et al. 1954; BRADLEY and HANCE 1955, 1957). Chlorpromazine prevention of the arousal after afferent stimulation was described by LONGO et al. (1954), HIEBEL et al. (1954), BRADLEY and HANCE (1957) and BRADLEY and KEY (1958). According to MADARÁSZ (1962) the slow hippocampal rhythm following exteroceptive stimulation cannot be evoked in cats pretreated with chlorpromazine.

The effectiveness of regularly repeated stimuli on the aspecific vasodepressor reaction exhibited a gradual decrease after the intravenous administration of 5 mg/kg of chlorpromazine. Neither hypotension, nor hippocampal theta activity nor cortical desynchronization could be evoked 20 minutes later. After the extinction of the pharmacological effect the responses returned (Fig. 5).

In this connection we refer to our previous experiment (SUCH 1959) where the vasodepressor reactions blocked by anaesthetics were reactivated by pentamethazole, a drug with a reticular site of action.

c) *Effect of the stimulation of reticular structures on vasomotor functions, and cortical and hippocampal activity*

Stimulation of diverse reticular structures (mesencephalic reticular formation, nucleus reticularis hypothalami) resulted in the bioelectric response illustrated in Fig. 1. However, alterations in the parameters of stimulation significantly influenced the character and direction of the vegetative responses. The usual stimulus of 200 c/s, 0.5 msec and 0.5 V brought about a decrease in blood pressure and the appearance of the typical EEG pattern. By elevating the voltage, the unaltered bioelectric response was associated with a biphasic, then with a pressor response in the blood pressure curve (Fig. 6).

Stimulation of the convexity of the brain (motor, limbic and somaesthetic areas) at different parameters evoked exclusively hypotension. No electric activity was registered in these experiments but on the basis of the foregoing it may be assumed that again the same cortical and hippocampal effects prevailed.

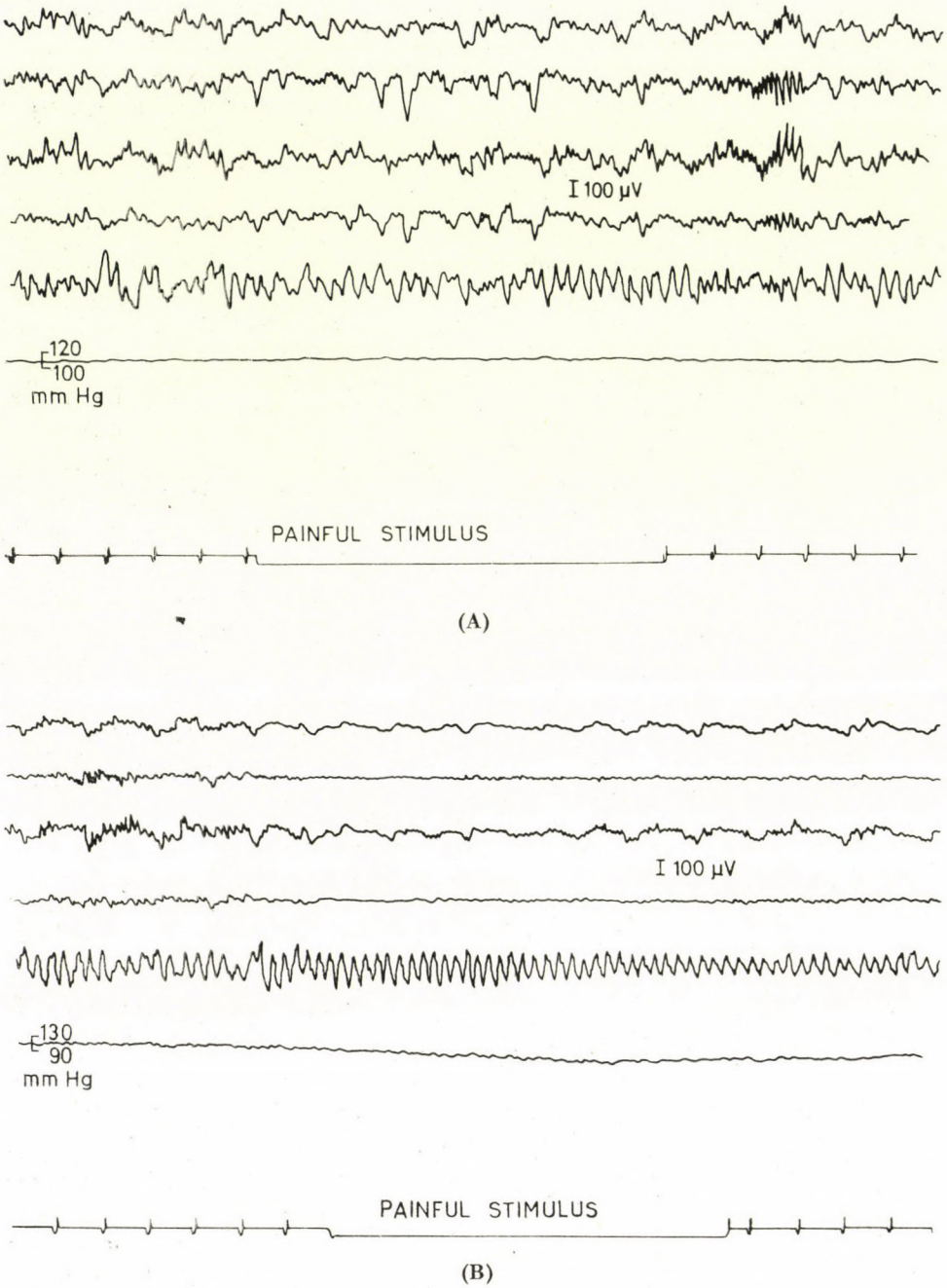


*Fig. 5.* Rabbit, 3.2 kg; 900 mg/kg urethan intravenously. Effect of 5 mg/kg of chlorpromazine on EEG and the vasomotor response 20 minutes (A) and 45 minutes (B) after intravenous administration

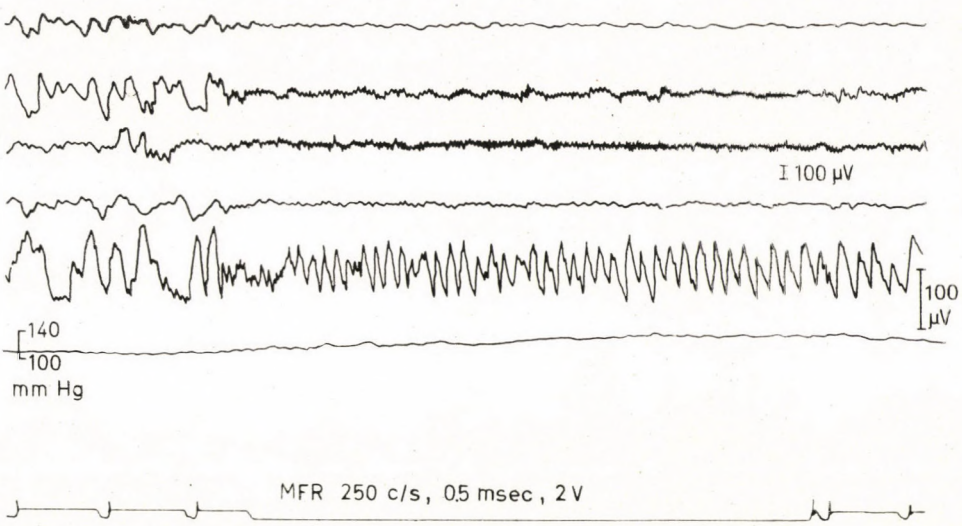


*Fig. 6.* Rabbit, 2.9 kg; 1 g/kg urethan intravenously. Stimulation of mesencephalic reticular formation with 2 V results in an increase in blood pressure and arousal response

## Discussion

Although the ascendent and descendent effects of the reticular formation were investigated mostly separately especially in the relation of somatic reflex activity several authors were studying the two-way effects. MORUZZI and MAGOUN (1949) and MOLLICA et al. (1953) found a simultaneous appearance of ascendent activation and descendent inhibition. Other authors, however, observed a parallelism of cortical arousal and the facilitating effects directed towards the spinal regions (MAGOUN 1950; LINN 1953; HUGELIN 1955a, b; GRASTYÁN et al. 1955; HIMWICH and RINALDI 1955/56). According to NARIKASHVILI and BUTKHUZI (1958) the reticular formation is not activated diffusely; reticular stimulation may have both an unidirectional and a bidirectional effect of the same or of opposite signs. The descendent inhibitory effect manifests itself with simultaneous cortical desynchronization only under chloralose anaesthesia (15—20 mg/kg). Deepening of the anaesthesia results in the disappearance of the ascendent effect.

As regards the vegetative sphere, BONVALLET et al. (1954) found in immobilized conscious cats that the changes in cortical activity always ran parallel with changes in the sympathetic tone. Similar observations were reported by ROTHBALLER (1956), BAUST et al. (1968) and KAKOLEWSKI and TAKEO (1967). According to the last-named authors, acoustic stimuli failed to affect blood pressure, and the vasomotor effect evoked by tactile stimuli was of a pressor character.

The interaction of the ascendent and descendent effects as observed in our experiments was usually an inverse one in that cortical activation was accompanied by a decrease in blood pressure. A similar inverse reaction was reported by POMPEIANO and SWETT (1962) in connection with the stimulation of cutaneous nerves with low frequency impulses, although these experiments were carried out 36 hours after pentobarbital anaesthesia. According to our observations and to literary data, the dissociation of the ascendent and descendent effects should be ascribed to the anaesthesia. YOKOTA and FUJIMURI (1963) showed that a stimulation of the medullary depressor area in conscious animals failed to evoke hypotension except after the administration of a low dose of pentobarbital.

As mentioned above, neocortical activation was not an obligatory sign of the aspecific vasodepressor response. The vegetative response appeared always with the regularization of the hippocampal activity even if there was no change on the neocortex. This observation strongly suggests an intimate correlation between the aspecific vasodepressor reaction and the function of the hippocampus.

The hippocampal theta rhythm is regarded by GREEN and ARDUINI (1954) as a palaeocortical activation. According to GRASTYÁN et al. (1959), it is a bioelectric manifestation of the orientation reflex; a sign of the structure's inhibition. This means the release from the hippocampal inhibition of the reticular formation and of the posterior hypothalamus. Whatever be the case, it is obvious that in the conscious state the increased sympathetic tone is associated with the appearance of hippocampal theta waves. Of course this does not involve that every vasomotor response must be a consequence of hippocampal activation or inhibition. According to TOKIZANE et al. (1960), the hippocampus reflects the activity of the posterior hypothalamus, the stimulation of which results in a simultaneous activation of the hippocampus and of the sympathetic tonus. The anterior hypothalamus exerts an opposite effect. According to TORII and KAWAMURA (1960), stimulation of the sinus nerve is followed by hypotension and fast hippocampal activity. Slow hippocampal activity is regarded as an indication of the increased sympathetic tone by TORII (1961) and YOKOTA and FUJIMURI (1964).

As already mentioned, the aspecific vasodepressor response in the superficially anaesthetized rabbits is a consequence of the anaesthetic effect. However, it has to be explained why the pressor response observed in the conscious state, is reversed during anaesthesia and why seemingly ineffective stimuli (e.g. sound) bring about hypotension, i.e. an inhibition of the sympathetic system. The complex multineuronal structure and the sensibility to anaesthetics of the pressor mechanism would account only for the disappearance of the pressor response. Thus we assume that under physiological conditions any stimulus evokes a double process in the central nervous system. The char-

acter of the descendent activity is controlled by the balance and quantitative relations of opposite neural processes. It is the central mechanism of the sympathetic excitatory processes which shows the greatest sensibility to anaesthetics resulting thus in the predominance of the inhibitory mechanism. The character of the ascendent reticular effects is abolished but not reversed by the anaesthetics. The difference in the pharmacological sensibility of the ascendent and descendent effects does not exclude the possibility that inhibitory components are involved in ascendent reticular activity. The homeostatic role of the inhibition in ascendent reticular activity was shown by DELL (1963).

Summing up, the aspecific vasodepressor reaction observed in superficially anaesthetized rabbits is regarded as a vegetative manifestation of a behavioural reaction (orientation reflex), organized at a higher structural level. The reaction is characterized by the predominance of the inhibitory component of the descending neural activity, due to the elimination of excitatory connections by the narcotics. The comparatively great reactivity of the sympathico-inhibitory system in the superficially anaesthetized rabbit cannot be observed in other animal species. The phenomenon is probably accounted for by the inefficiency of the vegetative compensatory processes in the domesticated rabbit.

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# INTRALOBAR REDISTRIBUTION OF TISSUE CIRCULATION IN THE CANINE LIVER, II

## EFFECT OF THE STIMULATION OF CERTAIN PERIPHERAL NERVES

By

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Changes in tissue blood flow in the central (perihilar) and peripheral regions of the hepatic lobe have been estimated in mongrel dogs by means of flexible Hensel thermocouples after electrical stimulation of the hepatic plexus, splanchnic and left vagal nerve (on the neck and at the cardia), and the right and left phrenic nerves (on the neck). Stimulation of the right phrenic nerve was performed also subsequent to the circumcision of the diaphragm around the inferior vena cava. It was found that

1) stimulation of the splanchnic, cervical vagus and right phrenic nerves resulted in a significant difference in tissue blood flow between the central and peripheral lobar regions;

2) the efferent pathway of the reflex eliciting the restrictive circulation was found in the hepatic plexus system;

3) stimulation of the vagus leads to a restriction of flow through a fall in blood pressure; stimulation of the vagus at the cardia fails to evoke any change in intrahepatic circulation;

4) the right phrenic nerve exerts no vasoconstrictive effect on the system of the hepatic vein; with the diaphragm uninjured, stimulation of the right phrenic nerve results in a restriction of intrahepatic flow through a mechanical effect.

DANIEL and PRICHARD (1951a, b, c) drew attention to the fact that the blood flow of the perihilar tissues (henceforth central region) and of the lobar peripheral parenchyma (henceforth peripheral region) changes in a different way after intravenous adrenaline administration and stimulation of the hepatic nerves in that the normal "diffuse" circulation is replaced by a "restrictive" circulation. In the rat, during restriction the radioopaque medium appeared only in the perihilar vessels and reached the hepatic and inferior caval veins more rapidly than normal, without the sinusoids being outlined.

BRAUER (1963) saw the essence of the restrictive mechanism in an active vasoconstriction in the portal system. In a previous paper (UNGVÁRY and VARGA 1971) it has been shown that restrictive circulation in the liver, although not produced by noradrenaline and adrenaline which cause a vasoconstriction in the portal bed, was consistently evoked by some drugs inducing a fall in systemic blood pressure, for instance acetylcholine, isoproterenol and histamine and, in addition, by haemorrhagic hypotension. The restriction is presumably due to the reflex described by GINSBURG and GRAYSON (1954).

consisting in a portal vasoconstriction during systemic hypotension. In the rabbit liver, an elevation of intraabdominal pressure elicited a restrictive circulation (OLERUD 1953). The efferent fibres of the relevant reflexes are localized in the splanchnic (preganglionic fibres) or the vagus or the right phrenic nerves, and the hepatic plexus.

The present study was aimed at (1) the exploration of the efferent fibres; (2) answers were sought in addition to the questions whether the restriction is elicited by the (normovolaemic) hypotension due to cervical vagal stimulation, and (3) whether the right phrenic nerve had a motor effect on the system of the hepatic vein.

## Methods

Observations were made on 20 mongrel dogs of both sexes weighing 10 to 16 kg. In the dog under chloralose-urethan anaesthesia (0.075 g chloralose plus 0.75 g urethan per kg body weight intravenously) blood flow in the central and peripheral parenchyma of the right medial

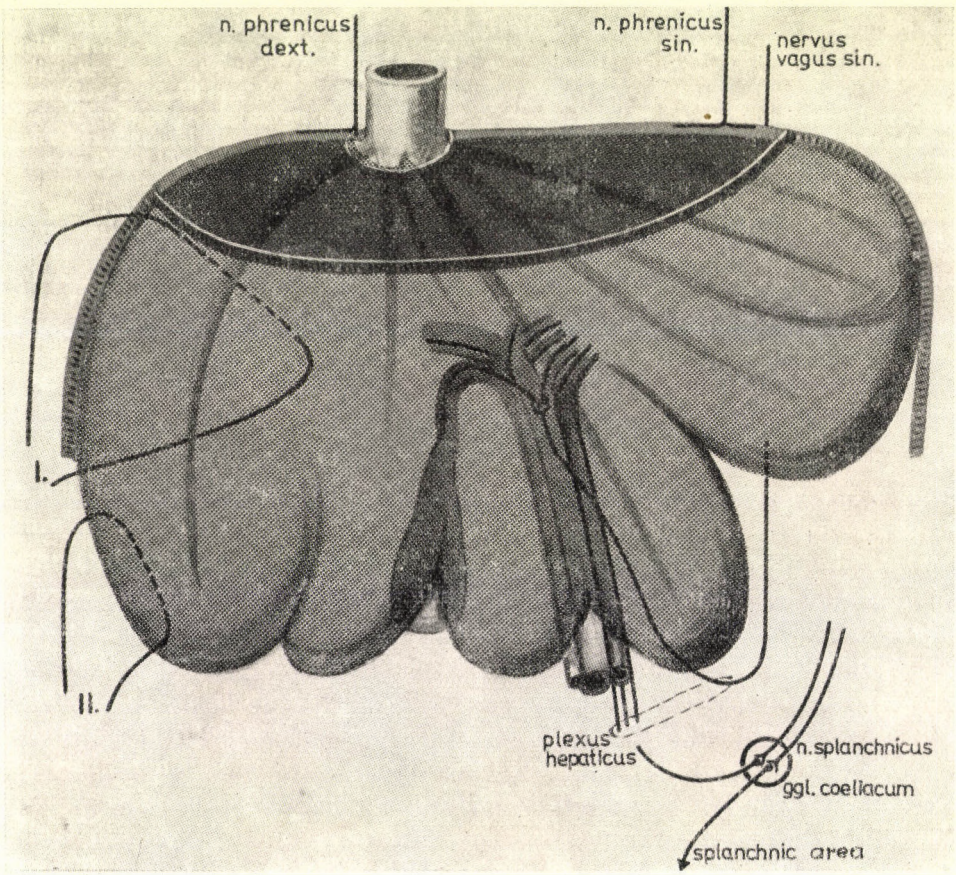


*Fig. 1.* Schematic drawing of canine liver with the electrically stimulated nerves. I.: flexible thermocouple inserted into central area of the lobe. II.: flexible thermocouple inserted into peripheral area of the lobe; the diaphragm was circumcised along the inferior vena cava

hepatic lobe were recorded on a two-channel Fluvograph apparatus using flexible Hensel thermocouples, the method being based on tissue heat conduction (HENSEL et al. 1954). Localization of the thermocouples was subsequently checked in histological or corrosive preparations. Blood pressure in the femoral artery and portal vein, pulse and respiratory rates were measured with a Statham pressure transducer, a Hellige integrator and a thermistor, respectively. All these parameters were recorded on a Hellige multiscriptor.

Electrical stimulation was applied to the hepatic plexus, the splanchnic, the left vagus (in the neck and at the cardia), the right phrenic (in the neck and mediastinum) and the left phrenic nerves (in the neck). Electrical stimulation was performed for 2 minutes on the peripheral stump of the divided nerves using a Multistim (DISA) apparatus at 20 cycles (imp/sec), 1 msec (impulse length) and 5 V (amplitude) as technical parameters. In 6 animals thoracotomy was performed and with a circular dissection of the diaphragm enclosing the hepatic veins the inferior vena cava was exposed, avoiding damage of the right phrenic nerve fibres running to the large veins. After the circumcision the right phrenic nerve was stimulated again in the animals respirated mechanically (Fig. 1).

Statistical analysis was performed according to Student's $t$ -test.

## Results

Electrical stimulation of the hepatic plexus resulted in an elevation of portal pressure ($p < 0.05$) and temporary apnoea. During, and even some time after, the stimulation, blood flow in the central and peripheral parenchyma underwent a change, control values, with a reduction in peripheral tissue flow exceeding the decrease of central tissue flow ($p < 0.05$; Fig. 2).

Stimulation of the splanchnic nerve brought about an increase in arterial blood pressure ($p < 0.001$). Portal pressure was at first enhanced ($p < 0.01$) then decreased ($p < 0.05$). On discontinuing the stimulation, a moderate elevation in pressure occurred ($p < 0.05$), the pulse rate diminished ($p < 0.01$) and transitory apnoea was observed. Following a temporary increase there was a reduction in tissue blood flow in both the central and the peripheral regions ($p < 0.05$ and $p < 0.02$, respectively). In the peripheral area the decrease was more marked and protracted. A significant difference in blood flows was found between the two regions ($p < 0.05$; Fig. 3).

Stimulation of the cervical section of the left vagus nerve elicited a decrease in femoral blood pressure ($p < 0.01$) and an elevation in portal pressure ($p < 0.01$). There was a transitory drop to almost zero in the pulse rate ($p < 0.001$), and an apnoea of nearly 30 sec duration was recorded. Hilar and peripheral tissue blood flows were both reduced, with a deeper and more prolonged drop in the peripheral flow. The difference between the two regional blood flows proved significant ($p < 0.05$; Fig. 3).

Stimulation of the vagus nerve at the cardia failed to produce measurable changes in the parameters observed.

Apart from the transitory apnoea no detectable alterations were brought about by the cervical stimulation of the left phrenic nerve (Fig. 5).

In animals with uninjured diaphragm and thoracic cavity, an elevation in portal pressure ($p < 0.001$) and a temporary apnoea were elicited by the cervical stimulation of the right phrenic nerve. Tissue blood flow in the central

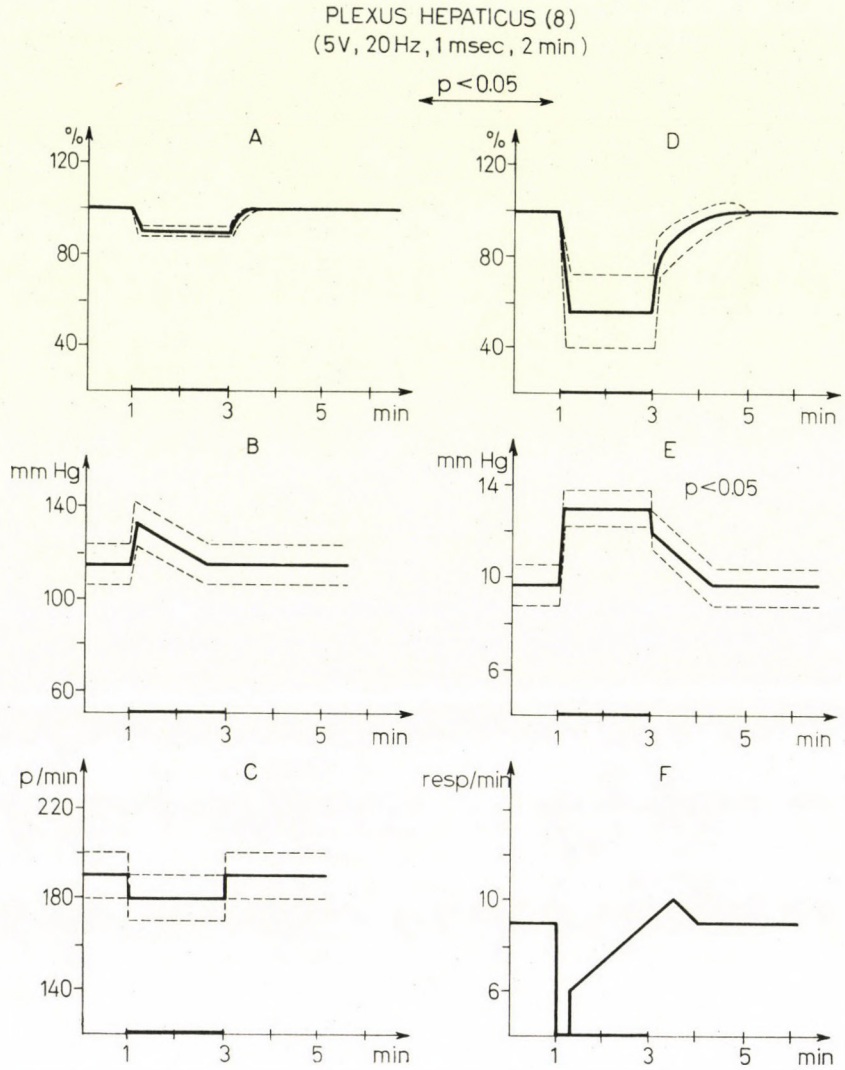


*Fig. 2.* Effect of electrical stimulation of the hepatic plexus on central (A) and peripheral (D) lobar blood flow, arterial (B) and portal (E) pressure, pulse (C) and respiratory (F) rate. On the abscissa, each spacing equals 1 minute. (8): number of animals tested. ——— mean ± S.E. The heavy line abscissa segments mark the duration of electrical stimulation

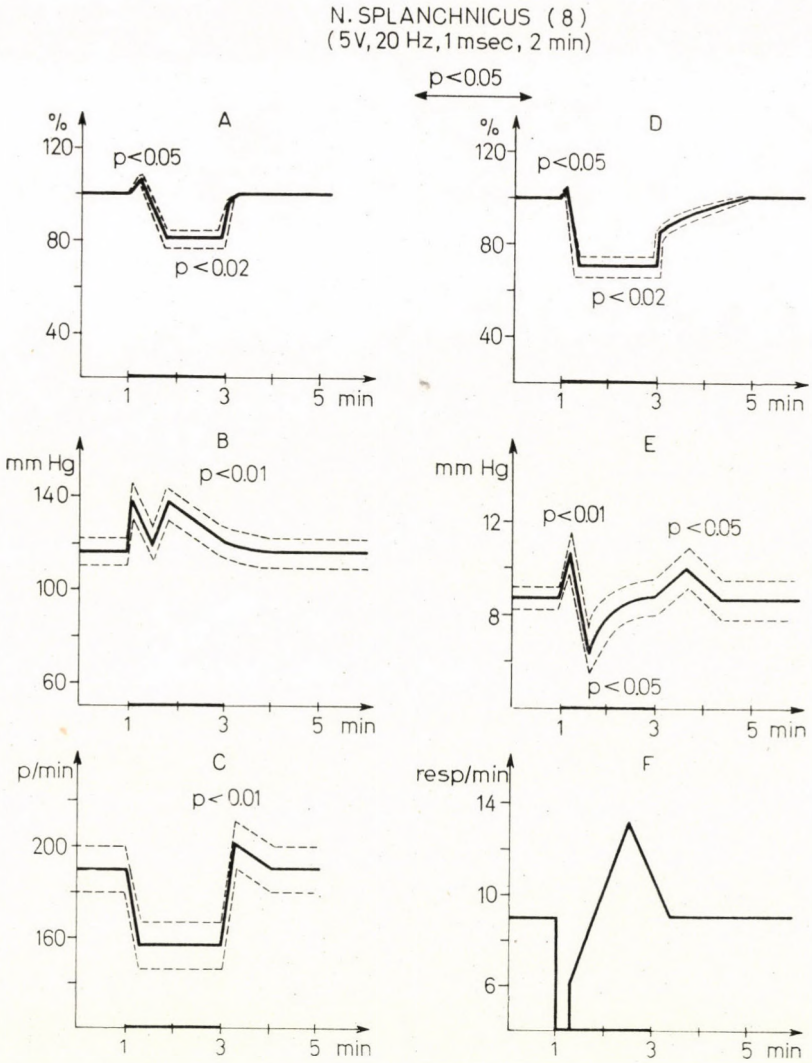


*Fig. 3.* Effect of electrical stimulation of splanchnic nerve on central (A) and peripheral (D) lobar blood flow, arterial (B) and portal (E) pressure, pulse (C) and respiratory (F) rate. On the abscissa each spacing equals 1 minute. (8): number of animals tested. ═══ mean ±S.E. The heavy line abscissa segments mark the duration of electrical stimulation

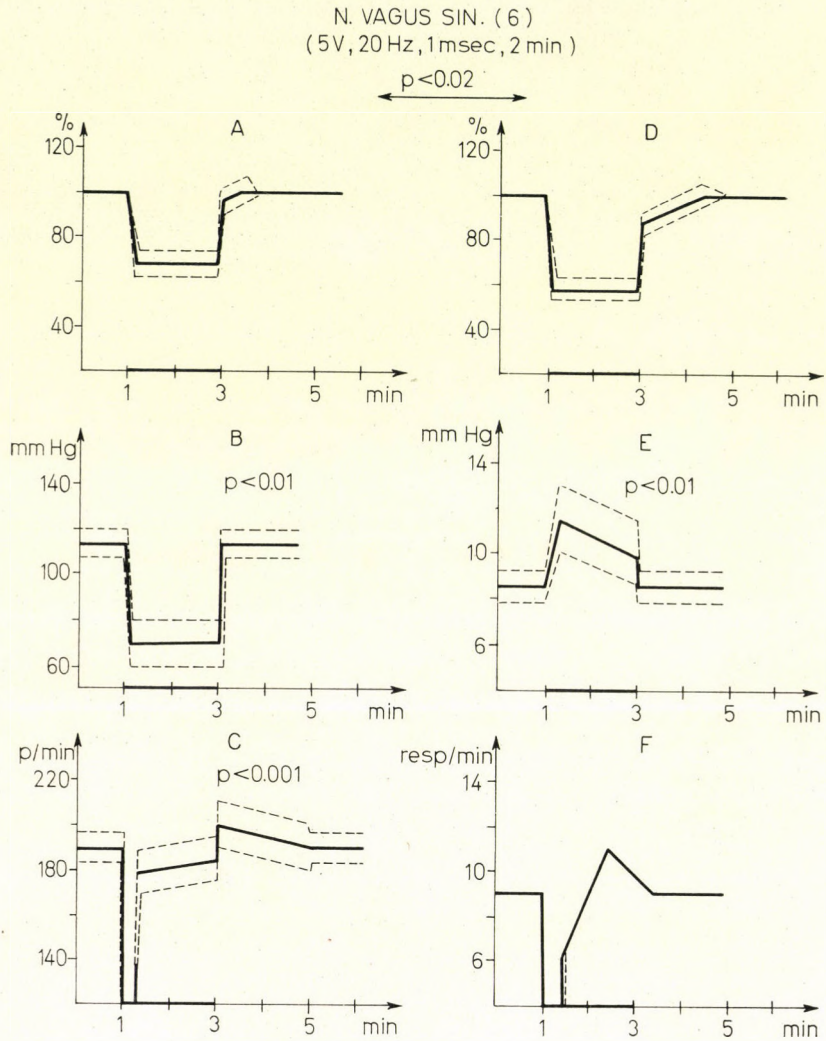


*Fig. 4.* Effect of electrical stimulation of left cervical vagus nerve on central (A) and peripheral (D) lobar blood flow, femoral (B) and portal (E) pressure, pulse (C) and respiratory (F) rate. On the abscissa, each spacing equals 1 minute. (6): number of animals tested. ------ mean ± S E. The heavy line abscissa segments mark the duration of electrical stimulation

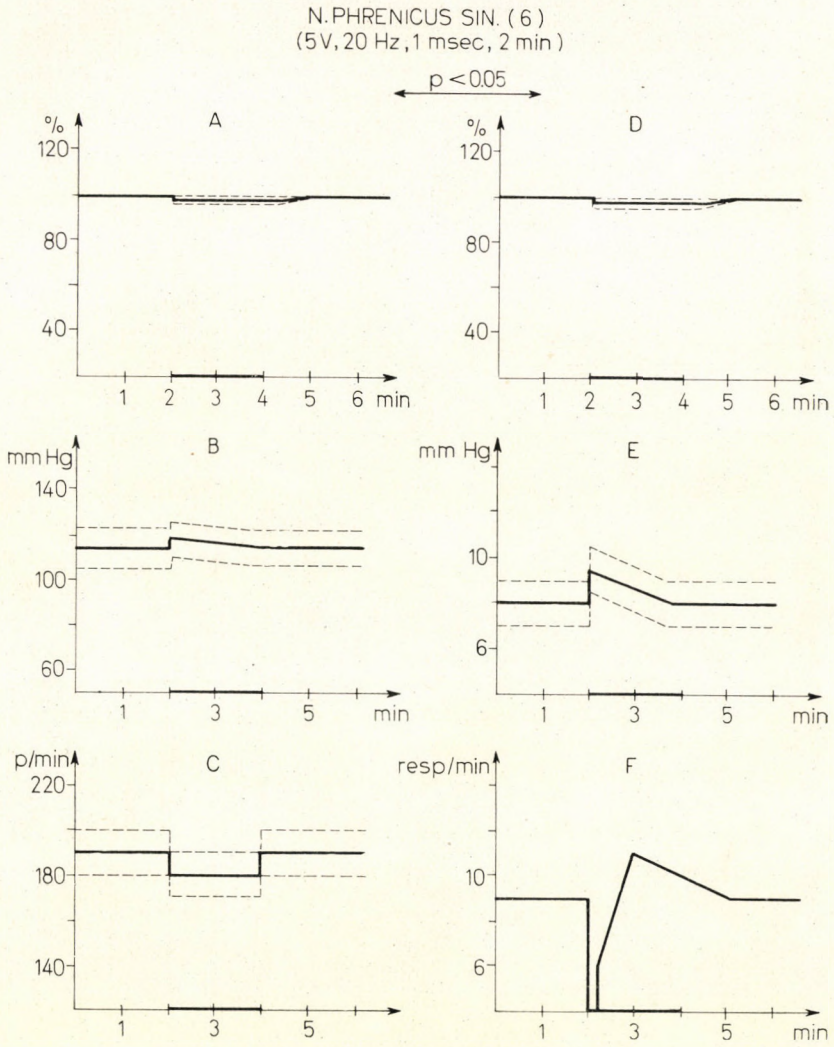


*Fig. 5.* Effect of electrical stimulation of left phrenic nerve on central (A) and peripheral (D) lobar blood flow, femoral (B) and portal (E) pressure, pulse (C) and respiratory (F) rate. On the abscissa, each spacing equals 1 minute. (6): number of animals tested. ——— mean ±S.E. The heavy line abscissa segments mark the duration of electrical stimulation

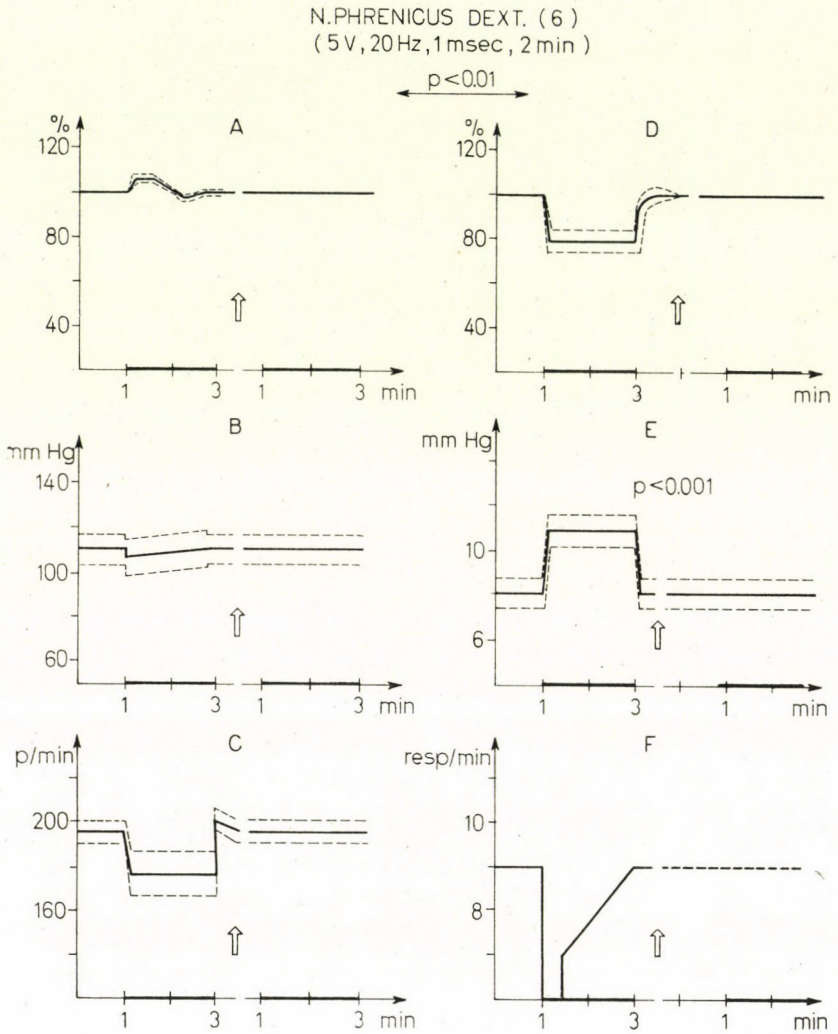


*Fig. 6.* Effect of electrical stimulation of right phrenic nerve on central (A) and peripheral (D) blood flow, arterial (B) and portal (E) pressure, pulse (C) and respiratory (F) rate. On the abscissa, each spacing equals 1 minute. (6): number of animals tested. ——— mean ±S.E. ↑: moment of circumcision. The heavy line abscissa segments mark the duration of electrical stimulation

region showed a moderate ($p < 0.05$) increase, to decrease to the original value at the end of the stimulation period. A pronounced decrement in peripheral tissue flow was observed ($p < 0.02$); the difference in tissue blood flow between the two regions was significant statistically ($p < 0.01$; Fig. 6).

No appreciable change was caused by mediastinal stimulation of the right phrenic nerve subsequent to circumcision of the diaphragm (Fig. 6).

## Discussion

The bundles of the hepatic plexus are entering the liver mainly along the hepatic artery. These bundles contain sympathetic postganglionic fibres from the coeliac ganglion (mostly from the left sympathetic trunk), parasympathetic preganglionic (chiefly right) vagal fibres and further fibres from the right phrenic nerve. It is beyond doubt that the restrictive circulation is elicited by stimulation of the hepatic plexus if it is to be elicited through the nervous system at all. In the above experiments there was a significant difference in tissue flow between the central and the peripheral regions of the liver during stimulation of the hepatic plexus. A shifting of intrahepatic circulation ensued in every case in contrast to the study of DANIEL and PRICHARD (1951b) who failed to observe the restriction in every case of hepatic nerve stimulation. It should, however, be noted that by means of a current of less than 5 V we also failed to stimulate the complete hepatic plexus and to induce restriction in every case. Thus the efferent fibres of the hypothetical reflex leading to restriction are obviously comprised by the hepatic plexus. As to the intrahepatic distribution of the hepatic plexus, histological evidence has been presented (UNGVÁRY and DONÁTH 1969) that the plexus sends essentially monoaminergic terminal branches along the branches of the hepatic artery, the portal and hepatic veins, and an especially sturdy bundle is formed in the wall of the hepatic vein just before its joining the inferior caval vein. Although the hepatic blood vessels, at least in most animal species, have been shown to be supplied with monoaminergic fibres only, noradrenaline and adrenaline still failed to cause a consistent restriction (UNGVÁRY and VARGA 1971). Obvious explanations of the phenomenon are offered either by the way of administration of the catecholamines or by the difference between the exogenous and endogenous catecholamine effect. The overwhelming majority of the monoaminergic fibres originates in the coeliac ganglion (UNGVÁRY and LÉRÁNTH 1970), consequently a similarity of the effects of splanchnic nerve and hepatic plexus stimulation was to be expected. In fact, a close resemblence was found between the results of the two stimulations. The only conspicuous difference was that in the case of splanchnic stimulation the major and prolonged reduction in tissue flow was preceded by its moderate and brief increase. This

was ascribed to the fact that the response to splanchnic stimulation was a vasoconstriction in the whole splanchnic area and not only in the intrahepatic field (increase, decrease then another elevation in portal pressure). The vagal fibres cannot be regarded as an efferent leg of the reflex arc, as no change in hepatic circulation was observed on stimulation of the vagus at the cardia. This is an agreement with the data of GINSBURG and GRAYSON (1954) who also failed to record any change in hepatic circulation after stimulation of the peripheral vagal stump.

Since after circumcision a stimulation of the right phrenic nerve failed to influence the diaphragm, then it had no vasomotor effect and consequently cannot be considered the efferent leg of the reflex arc producing restriction.

Stimulation of the cervical vagus by a current of more than 5 V resulted in a marked depression of blood pressure. The elevation in portal pressure might have been a consequence of the sudden congestion in the venous system. There was a significant discrepancy in blood flow between the central and peripheral regions, as the reduction was much less in the central than in the peripheral flow. It has been shown previously that a pronounced fall in systemic blood pressure is always followed by a restrictive circulatory distribution in the liver. Hypotension (vagal effect) may again be considered as the primary cause: it launches the reflex action described by GINSBURG and GRAYSON (1954) and leads to restriction by means of portal vasoconstriction.

BÁNFAI et al. (1953) reported on a pressure drop in the inferior vena cava with a simultaneous enlargement of the liver after stimulation of the right phrenic nerve. The phenomenon was attributed to the fact that motor innervation of the so-called hepatic sphincters described by AREY and SIMMONDS (1920) are supplied by the right phrenic nerve. An electron microscopic evidence of phrenic nerve fibres running to the hepatic veins was presented by UNGVÁRY and LÉRÁNTH (1970) using the degeneration method. The number of these fibres, however, cannot be large, as only a few degenerating axons were found in the adventitia. It is undecided whether a vasomotor role may fall to the right phrenic nerve in the innervation of the sphincters. Cervical stimulation of the left phrenic nerve, as expected, failed to produce any change in hepatic circulation. Cervical stimulation of the right phrenic nerve, however, effected a significant elevation in the central and a decrease in the peripheral hepatic tissue flow; i.e. it resulted in a significant difference between the lobar regions. After circumcision of the diaphragm along the inferior caval vein and the hepatic veins, by preserving the nerve branches of the veins, repeated stimulation of the nerve failed to evoke the phenomenon. Consequently, the right phrenic nerve has no constrictive effect on the hepatic veins. Various explanations may be offered concerning the opposite changes occurring in the central and peripheral blood flows with an intact diaphragm. Contraction of the diaphragm might lead to a reflectory restrictive circulation through an

increase in intraabdominal pressure. In the rabbit OLERUD (1953) raised the intraabdominal pressure by intraperitoneal air insufflation and observed a restriction of flow on the serial portogram. An elevation of intraperitoneal pressure may, however, be produced also by a stimulation of the left phrenic nerve, which was not followed by any change in intrahepatic flow distribution. The solution of the problem must be hidden elsewhere. First, a direct mechanical effect on the liver is exerted by the movements of the diaphragm, by which primarily the thinner peripheral lobar regions are influenced and blood is diverted to the central area as a result of the local increase in sinusoid resistance. On the other hand, the areas of the diaphragm around the inferior vena cava may be considered a functional suprahepatic sphincter of hepatic circulation. To settle the problem detailed investigations are warranted.

**Acknowledgement**

We are indebted to E. STARK, Director of the Institute of Experimental Medicine of the Hungarian Academy of Sciences, for advices in the course of this study.

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# PHASIC STRETCH REFLEX IN THE EXTRAOCULAR MUSCLES OF ENCÉPHALE ISOLÉ CATS

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The phasic stretch reflex of the extraocular muscles in encéphale isolé cats has been studied by means of an electromyographic method. The response is followed by a complete or incomplete silent period in the electric activity. The factors influencing the silent period, as well as the role of the phasic stretch reflex in the regulation of the eye movements are discussed.

The introduction of the electromyographic examination of the eye muscles in the last two decades has enhanced research related to the oculomotor system, and BREININ and PERRYMAN (1964), HYDE (1964) and others have solved the kinesiologic and physiologic problems of the eye muscles.

Since in animal experiments stretch reflexes could not be elicited in the eye muscles (MCCOUCH and ADLER 1932; WHITTERIDGE 1960, 1962), we have attempted to examine by means of electromyographic registration whether a response could be elicited by phasic stretch in the extraocular muscles of encéphale isolé cats. In the skeletal muscles, electromyography proved to be suitable for the examination of reflexes (MERTON 1951; JANSEN and RUDJORD 1964), but on the cat's extraocular muscles no such experiments have been performed. This has made it necessary to develop a method for the recording of electrical activity, which would allow to spare the walls of the orbit.

## Methods

The examinations were performed on 10 adult cats, weighing 1.8—3.0 kg. In superficial ether anaesthesia into the femoral vein a cannula was introduced, and through this 80 mg/kg body weight of thialbarbital was given for maintaining the anaesthesia. Transection of the spinal cord was performed at the level of $C_2$. In the course of the experiment the $CO_2$ content of the expired air was controlled by means of a Godard capnograph. Body temperature was kept between 37—38° C. The head was fixed in a stereotaxic device; having prepared down the skin of the glabella and the periosteum, a steel-needle was fixed into the bone. The nictitating membrane was removed.

After awaking of the animal, 1% tetracaine solution was instilled into the conjunctival sac, then DISA coaxial electrodes were introduced transconjunctivally into the extraocular and the orbicularis oculi muscles. Electrical activity was transmitted by a DISA amplifier into an Orion EMG-1552 type oscilloscope and photographs were taken with a MOM camera. The activity of one or two muscles was examined simultaneously. Synchronous recording of the activity of several muscles is difficult in cats since the electrodes may move from their place.

Changes in the electromyogram related to spontaneous eye movements indicated that the electrode was located in the target muscle. In order to elicit the reflex, the metal needle placed into the glabella was struck with a metal hammer every two seconds, to induce a short vibration in the extraocular muscles. On the basis of the time-course of the reaction it could be established that the phenomenon was no artefact due to vibration of the skull. The sound due to the percussion was transmitted by a microphone and an amplifier to one ray of the oscilloscope and in this way the establish time of the stimulus could be registered. The response to the latter was examined in the relaxed and in a slightly contracted state of the muscles. Recording was done on stationary films at 5 msec/mm sweep speed, and on mobile films — by rays deviated only in vertical direction — at 40 and 160 mm/sec film speed.

## Results

A reaction to the phasic stretch stimulus could be revealed in every eye muscle preparation of encéphale isolé cats. The reflex proved to be identical in the four rectus muscles and in the two oblique ones. The latent period was

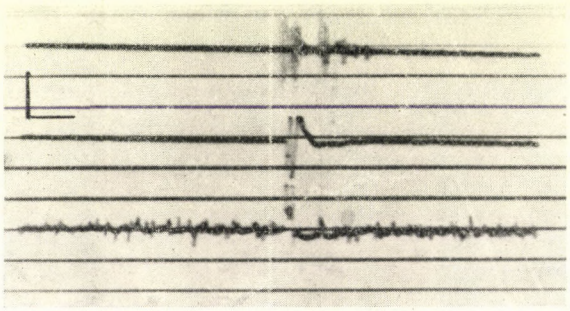

*Fig. 1.* Electromyogram of phasic stretch reflex in cat's eye muscle. Above: stimulus involved in eliciting the reflex. At centre: rectus externus muscle. Below: electric activity of rectus internus muscle. Calibration: 100 $\mu$V, 50 msec

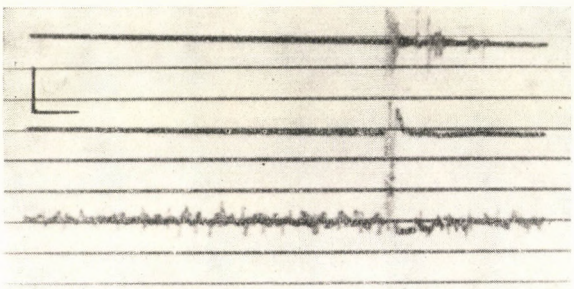

*Fig. 2.* Above: sign of stimulus. Centre: rectus externus muscle. Below: rectus superior muscle. Calibration: 100 $\mu$V, 50 msec

found to last 2—3 msec, corresponding to the short latent period of the monosynaptic myotatic reflex. The latent period was constant in one and the same preparation.

The reflex manifested itself in the form of strongly synchronized action potentials originating from several motor units. These action potentials could well be differentiated from spontaneous activity and the eye bulb displayed no movements. If prior to the stimulus the eye muscle had been slightly

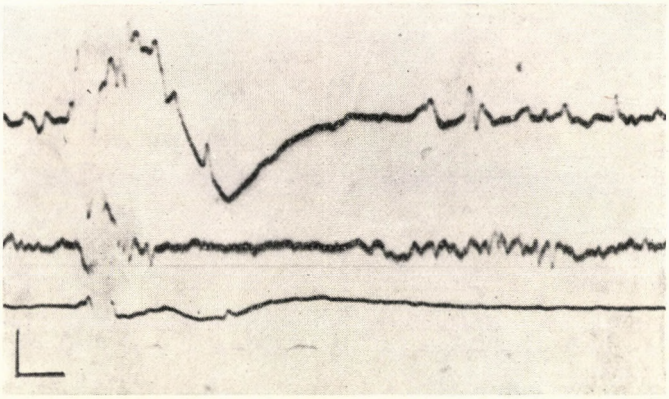

*Fig. 3.* Incomplete silent period. Above: rectus superior muscle. Centre: rectus inferior muscle. Below: orbicularis oculi muscle. Calibration: 100 $\mu$V, 15 msec

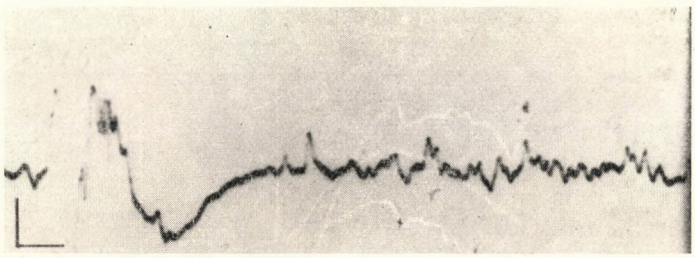

*Fig. 4.* Incomplete silent period. Inferior oblique muscle. Calibration: 100 $\mu$V, 15 msec

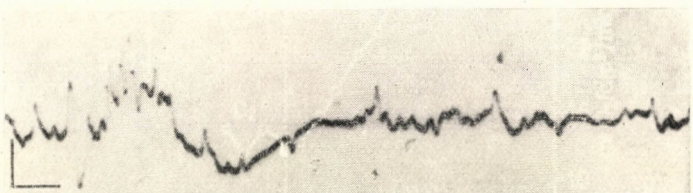

*Fig. 5.* Incomplete silent period. Inferior rectus muscle. Calibration: 100 $\mu$V, 15 msec

contracted, electric activity after the reflex response was interrupted for about 35—110 msec (silent period, Figs 1, 2). The duration of this period was constant within one preparation. Upon the application of a slight stimulus, the silent period proved to be incomplete but its duration did not change. In such cases a decrease or cessation of spontaneous activity was mostly observable in the final part of the period (Figs 3, 4, 5). MERTON (1951) observed

similar incomplete silent periods on the electromyogram of the human adductor pollicis muscle. Owing to a shifting of the electrodes, in some of our registrates the basic line was slowly decreasing, to increase following the reaction to the stimulus. By the end of the silent period muscle activity generally returned to the pre-stimulus level. In a few cases, after a period of electric silence, synchronized action-potentials ("overshooting") and a silent period manifested themselves again (Fig. 6).

As a special feature of the myotatic reflexes, the reflex did not show habituation. The F wave described by MAGLADERY and MCDOUGAL (1950) could not be identified on the electromyogram of the extraocular muscles.

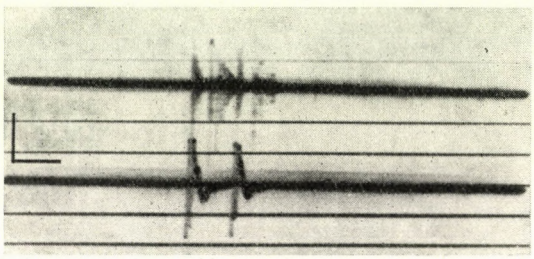

*Fig. 6.* "Overshooting". Above: sign of stimulus. Below: inferior rectus muscle. Calibration: 100 $\mu$V, 100 msec

## Discussion

The stretch reflexes are of a decisive importance in the organization of every kind of movement (for a review, see GRANIT 1970). Few myotatic reflexes were described from the innervation area of the cranial nerves. One of the most important reflexes is the masseter reflex involved in the process of mastication (SZENTÁGOTHAI 1948). On the other hand, little is known on the role of the stretch reflex in ocular movements. In animal experiments the common methods have failed to register constant and well-evaluable phasic stretch reflexes (MCCOUCH and ADLER 1932; WHITTERIDGE 1960, 1962). Though SHERRINGTON in 1893 described a stretch reflex in the ocular muscles of the cat, he considered it inconstant. Owing to the contradictory data in the literature, we have attempted to elicit the reflex in encéphale isolé cats. Electromyography seemed to be the most suitable procedure for the registration of the reaction, since changes manifest themselves in electric activity even in the case of weak reflexes (DODT and GOHL 1952).

By percussion of the glabellar area, a phasic stretch reflex could be elicited in every case. This appeared on the electromyogram, in the form of synchronized action potentials and a silent period following them. No movement of the eye bulb could be observed since the slight contraction occurred si-

multaneously in the antagonistic muscles. The phasic stretch reflex elicited in the cat's ocular muscles differs from similar reflexes in the skeletal muscles in that the afferent pathway does not convey inhibitory impulses to the motoneurons of the antagonistic muscle; the response occurs simultaneously in all extrinsic eye muscles, spontaneous electric activity did not decrease in any of the muscles.

The question arose, what kind of nerve terminals had been stimulated under the given experimental conditions. Proprioception of eye muscles has long been a controversial subject in physiology and ophthalmology. It is well-known that no muscle-spindle is found in the ocular muscles of the cat. However, several kinds of sensorial nerve terminal have been described in them, from which the impulses reach the brain stem (FILLENZ 1955) and inhibit, in the reticular formation, the vestibular responses (GERNAND 1968). BACH-Y-RITA and ITO (1966) found among the proprioceptors of the extraocular muscles some connected "in line" with the muscle fibres and others connected "parallel" with them. The latter might be identical with the spiral-like terminals described by COOPER and DANIEL. The parallel-connected receptors have adapted themselves if the stretch was maintained for a longer time; in some of their properties they differed from the muscle-spindle, in others they resembled it.

On the electromyogram the silent period which follows the reaction is characteristic of the proprioceptive reflex. This silent period is elicited by the muscle-jerk related to the phasic stretch reflex, but may be induced by whatever jerk superposed to the voluntary contraction. The silent period in the skeletal muscles of human and of encéphale isolé cats was described by HOFFMANN (1920) and later (1934) he explained it with the refractory period of the motoneuron. SHERRINGTON (1928) showed that the silent period occurring after a stimulation of the anterior root was essentially shorter if the muscle had been de-afferented. Thus the afferent impulses are accounting for the greater part of the period. MATTHEWS (1963) showed that the regular discharges arriving from the muscle spindle are suspended during the time of the muscle-jerk. JANSEN and RUDJORD (1964) also ascribe a decisive importance to the arrest of the afferent impulses of the muscle spindle. The possible role of other mechanisms was also envisaged in the literature. In the skeletal muscles, in the early phase of the silent period a recurrent inhibition might be effective by way of the collaterals and Renshaw's cells (ECCLES et al. 1954). This possibility might be excluded as regards the ocular muscles, since the ocular motoneurons have no recurrent collaterals (RAMON Y CAJAL 1911). Other authors assigned a role to the autogenous inhibition induced by the afferent impulses of the tendon receptors (HUFSCHMIDT 1960). JANSEN and RUDJORD, on the other hand, called attention to the fact that the tendon receptors in the soleus muscle have no determined role in bringing about the

silent period. According to the view of GRANIT (1970) the equilibrium of the two systems, of the muscle spindles and the tendon receptors, the actual feature of the motoneuron activation is determining which afferentation will be decisive in a given case. The tendon receptors might also be involved in the silent period described by us; their role, however, seems to be of minor importance. In HOSOKAWA's (1961) opinion, the tendon receptors described in the ocular muscles are not typical Golgi organs. BACH-Y-RITA and ITO (1966) in the cat's inferior oblique muscle found rather few "in line" receptors (2 out of 49).

According to our measurements, the silent period in the cat's ocular muscles lasted 35—110 msec while in the human skeletal muscles it lasts 100—120 msec (STEINBRECHER 1965), and in the cat's soleus muscle it was found by JANSEN and RUDJORD (1964) to last 80—200 msec. The relatively short duration recorded in our own material was due presumably to the extremely rapid function.

FULTON and PI-SUNER regarded the silent period as an evidence of the existence of a mechanism regulating muscle contraction. The physiologic phenomena connected with the stretch reflex have actually to be considered a function of the loop regulating the length of the muscle, where the negative feed-back is based on informations arriving from the muscle proprioceptors, inhibiting an extreme shortening of the muscle (MERTON 1951). The fact that small jerks are followed by incomplete silent periods, as was observed by MERTON in the human adductor pollicis muscle and by us in the extraocular muscles of encéphale isolé cats, shows that the mechanism is involved in fine movements.

The phenomenon of "overshooting" occasionally manifesting itself after the silent period and the consecutive electric silence which we have observed in the ocular muscles of the cat, was registered by JANSEN and RUDJORD (1964) in the cat's soleus muscle and ascribed to an instability of the regulating system. Regarding the second augmentation of the sound signal, the electric response may also be interpreted as a sound resonance effect.

The movements performed by means of the skeletal muscles are at present considered intricate physiologic processes assisted by the gamma-loop ("servo-assisted", GRANIT 1970). The cat's ocular muscles have no muscle spindle, nor does a gamma-loop regulate the complex movements related to their gaze. The length of the muscle, however, can be measured also with a device more simple than the muscle spindle. The phasic stretch reflex described by us in the ocular muscles of encéphale isolé cats, "assists" at the level of a simple reflex mechanism the regulating function of the oculomotor system.

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# *Pathophysiologia*

## CHANGES IN BROMSULPHOPHTHALEIN* (BSP) AND INDOCYANIN-GREEN** (ICG) METABOLISM IN MAN AFTER BARBITAL INDUCTION

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Enzyme induction has been examined in man after administration of phenobarbital in a dose of 8 mg/kg daily for three days and in patients who for suicidal purposes had taken single doses of 3.0—9.0 g. The results were as follows.

1. Distribution and excretion half-time of BSP were moderately shortened in the plasma.

2. The $T_m$ of BSP increased from 8.8 $\pm$ 0.8 mg/min to 19.1 $\pm$ 1.1 mg/min ($p < 0.001$).

3. The half-time of plasma ICG was shortened from 3 min 20 sec $\pm$ 12 sec to 1 min 12 sec $\pm$ 7 sec ($p < 0.001$).

When introduced into the organism, various drugs may produce as a nonspecific effect in the main organ of detoxication, the liver, changes which are easy to characterize morphologically and biochemically and by which detoxication can be accelerated (enzyme induction; REMMER and MERKER 1965; CONNEY 1967; KUNTZMAN 1969).

Examinations so far have shown that nearly a hundred substances have an inductive effect. Among these the commonest is barbital (HARGRAVES 1968; Drugs and Enzymes 1970).

Enzyme induction by phenobarbital was first used in clinical practice by YAFFE et al. (1966); these authors succeeded in decreasing the serum bilirubin level with phenobarbital in newborns suffering from nonhaemolytic jaundice.

In rodents treated with phenobarbital the excretion of exogenous bilirubin and the production of bile increased (ROBERTS and PLAA 1967).

In addition to accelerating the processes of detoxication, phenobarbital pretreatment increased the conjugation and excretion of bilirubin and also the secretion of other anions (KLAASSEN and PLAA 1968).

Evoked enzyme induction in mice accelerated the excretion of BSP and increased bile production (FUJIMOTO et al. 1965).

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Phenobarbital, when given to rats in a dose of 75 mg/kg/day for three days, increased the excretion of glutathione conjugated with BSP and of nonbiotransformable DBSP (phenol-3,6-dibromophthalein-disulphonate) and also that of indocyanin-green.

In humans we obtained similar data in that phenobarbital given in a dose of 8 mg/kg/day considerably shortened the plasma half-time of ICG and BSP in adults (GÓGL and JÁVOR 1971).

In the present series of experiments we examined in more detail the changes in cholephilic anion excretion in humans after induction.

## Methods

The material consisted of 10 males and 9 females aged between 16 and 39 years. In 8 patients induction was produced by a single dose of phenobarbital and/or amobarbital (3.0—9.0 g) taken for suicidal purposes. Enzyme induction was elicited in 11 healthy individuals (8 males and 3 females) by giving them 8 mg/kg/day of phenobarbital on three consecutive days. In this last group, the examinations were carried out also before treatment.

BSP loading was carried out according to the method of CASTENFORS and HULTMAN (1962): 20 mg/kg dye was given intravenously and the changes in its plasma level were followed for two hours.

Determination of $T_m$ was based on WHEELER's method (WHEELER et al. 1960); the administration of a single 100 mg dose of the dye was followed by its infusion at a rate of 10 mg/min and 5 mg/min. The plasma level was repeatedly estimated and maximum excretion was computed.

In the same patients we aspirated the bile continuously with a Bartelheimer type triple lumen gastrointestinal tube, measured the concentration and quantity of BSP in the bile and analysed the metabolites by paper-chromatography (COMBES and STAKELUM 1960).

Excretion half-time of nonbiotransformable ICG was determined according to CAESAR et al. (1961): after giving 0.4 mg/kg dye intravenously, its concentration in the serum was determined at 2, 4, 6, 8, 15 and 25 minutes.

## Results

The excretion of cholephils (exogenous anions) from the organism can be divided into three stages: uptake by the liver cells, metabolic transformation and excretion with a carrier substance.

Fig. 1 shows plasma BSP concentration in nine induced patients. Dye excretion in a normal adult gives two exponential curves, one for saturation and one for excretion. The half-time of the saturation phase in a normal adult is $11 \pm 1$ min, in an induced subject 8 min $\pm$ 40 sec. The excretion phase in a normal adult is $27 \pm 3$ min and in a pretreated adult $22 \pm 2.5$ min. These results showed a 20% shortening of excretion time, but the difference was not significant.

Fig. 2 shows the results of BSP $T_m$ determinations. The value of $8.8 \pm 0.8$ mg/min obtained before induction was practically the same as the 8.6 mg/min given in the literature (HARGRAVES 1968). After induction, the rise in $T_m$ was significant, $19.1 \pm 1.1$ mg/min. Only on one occasion was there no

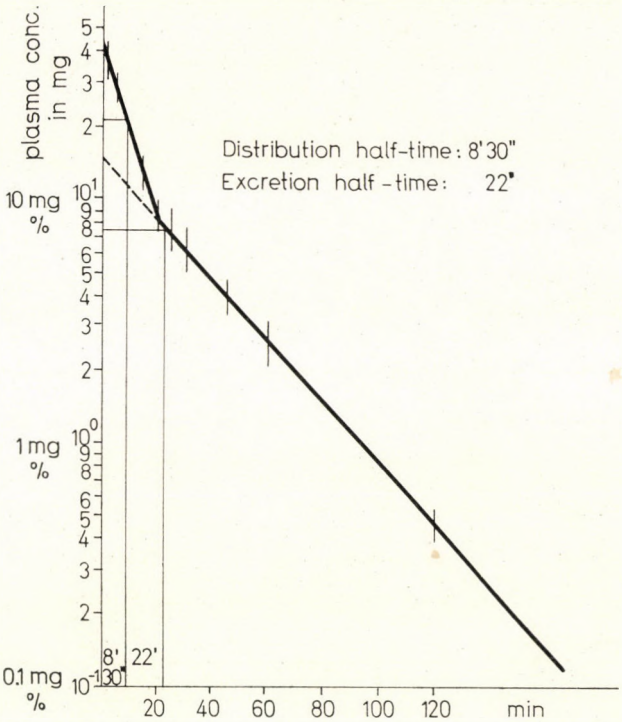


*Fig. 1.* Plasma BSP concentration after induction by 20 mg/kg of phenobarbital. Number of cases: 9

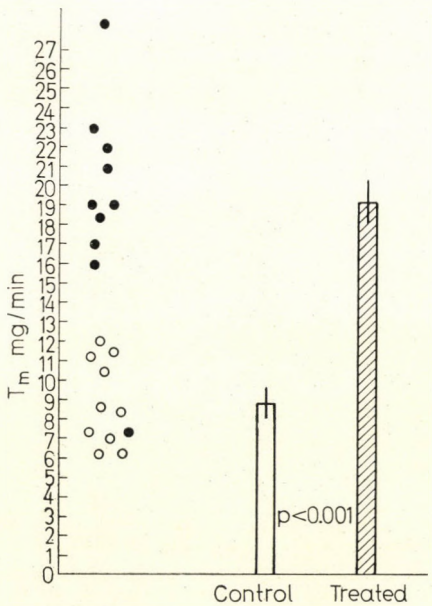


*Fig. 2.* $T_m$ values for BSP in controls (n = 10) and in patients treated with phenobarbital (n = 10) in a dose of 8 mg/kg daily for 3 days

change after the above-described treatment. In the available literature we could not find data concerning this phenomenon.

Paper chromatography demonstrated in the bile one ninhydrin-positive metabolite at the beginning of excretion, then three or in some cases four at the time of maximum excretion (at 60—120 min). Only one metabolite was demonstrated in the bile obtained by the traditional BSP test; in this case the quantity of the dye administered was 5 mg/kg. These data suggest a change in BSP metabolism dependent on loading.

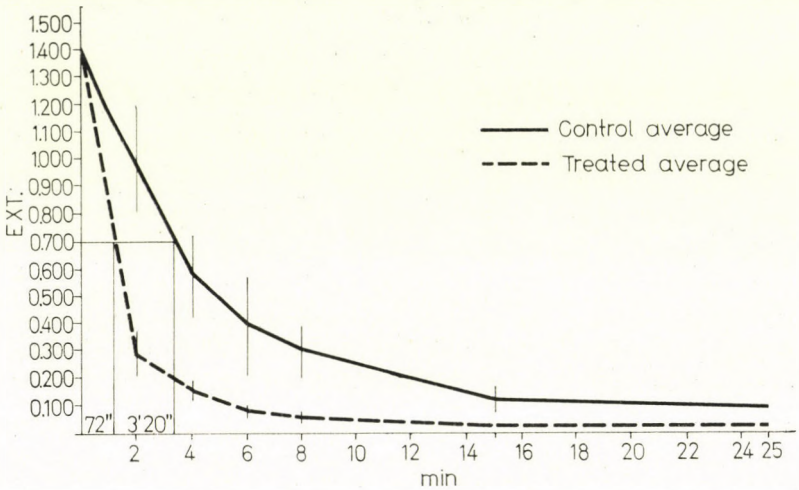


*Fig. 3.* Plasma ICG concentration in controls (n = 10) and in patients after treatment (n = 9)

The changes in plasma ICG concentration before and after induction are shown in Fig. 3. The mean half-time in 10 normal subjects was 3 min 20 sec ± 12 sec. The same value for the induced group was 1 min 12 sec ± 7 sec ($p < 0.001$).

## Discussion

Phenobarbital enhances enzymatic metabolism of the most varied substances in animals (REMMER 1962). Acceleration of the conjugation of bilirubin with glucuronic acid has also been demonstrated in the microsomal fraction of rat liver in vitro (SCHELLHAS et al. 1965).

In vivo, an increase of conjugation and bile production can be achieved also in man and of this effect use is made in the treatment of neonatal jaundice and cholestatic conditions (YAFFE et al. 1966; GÓGL and JÁVOR 1970; Drugs and Enzymes 1970). One of the factors in increased excretion may be the ac-

celerated activity of conjugation, although the biliary excretion of nonbiotransformable ICG is also enhanced by phenobarbital. According to KLAASSEN's experiments (KLAASSEN and PLAA 1968) the excretion of nonbiotransformable DBSP and that of ICG is equal to or in some cases even higher than, that of BSP in the rat. In view of our results, these statements may be extended to humans and it may be concluded that with inductive pretreatment an increase in the excretion of anions and an acceleration of general metabolism is obtained in man, too. The $T_m$ of BSP increases two-fold, while the half-time of plasma ICG is considerably shortened.

Considering that in the bile not only cholephils but also several other substances (bilirubin, oestrogen, steroid, thyroxin, serotonin, adrenaline, adipiodone) are excreted through the above mechanism, the phenomenon and its analysis deserve special attention.

## Acknowledgement

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# *Pharmacologia*

## PHARMACOLOGY OF 1-(2-OXO-1-PYRROLIDINO)-4-(2-CHLOROETHYL-METHYLAMINO)BUT-2-YNE·HCl (DSO-16), I

### ITS CENTRAL CHOLINOMIMETIC AND CHOLINOLYTIC EFFECTS ON MICE

By

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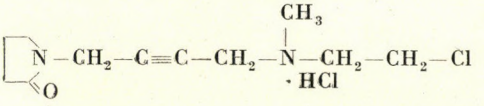
A new central cholinomimetic and cholinolytic agent, 1-(2-oxo-1-pyrrolidino)-4-(2-chloroethyl-methylamino)but-2-yne·HCl (DSO-16) was synthetized and found to induce in mice tremor, analgesia, and myasthenia for 1 to 2 ½ hours, and to potentiate pentobarbital and hexobarbital anaesthesia. The central cholinomimetic activity of the compound attained 10—35% of that of oxotremorine. The first symptoms were followed by a phase in which anticholinergic activities manifested themselves: DSO-16 showed anti-oxotremorine, anti-physostigmine, anti-arecoline, and, to a lesser degree, anti-nicotine properties. In the second phase a single dose was observed to be effective for 24, 48 and even 72 hours.

Some analogous derivatives of tremorine and oxotremorine are known to possess cholinomimetic and cholinolytic effects (CHO and JENDEN 1964; DAHLBOM et al. 1966; LÉVY and MICHEL-BER 1966, 1967a, b; NEUMEYER et al. 1967).

The halogen-hydrogen acid salts of tertiary amines containing a haloethyl, notably chloroethyl, group are mostly compounds of lasting effects. No oxotremorine analogue has hitherto been described in which the pyrrolidine ring was substituted by chloroethylamine and the rest of the molecule retained. Assuming that a compound of this type might possess lasting cholinergic actions we built a methyl-chloroethyl-amino group in the oxotremorine molecule and developed 1-(2-oxo-1-pyrrolidino)-4-(2-chloroethyl-methylamino)but-2-yne·HCL (DSO-16).



DSO-16

In view of the presence of the oxo-pyrrolidine group it was expected that DSO-16 might have a cholinomimetic activity, and since most chloroethyl amine derivatives have lytic properties, possible anticholinergic activities had to be anticipated. The present work describes only the central activities of DSO-16 observed in mice.*

## Methods

Swiss mice of either sex, weighing 17—21 g, were randomized into groups. Tremor was assessed visually by an observer who did not know what treatment the animals had been given. Myasthenia was assayed using an iron rod, 13 mm in diameter, operated at 7 rotations/min. Activity of the compound was regarded as positive for previously trained mice unable to cling to the rotarod for the first 180 sec (OELSSNER 1961). The hot-plate method, with the plate at 52°C, was used to evaluate the analgesic activity (WOOLFE and MCDONALD 1944; PÓRSZÁSZ and HERR 1950), and spontaneous motility was quantitatively estimated by means of the motimeter of KNOLL and VAJNOVSZKY (1961). The $LD_{100}$ for intravenous nicotine and for intraperitoneal physostigmine was determined daily, and this dose was administered after physiological saline and after pretreatment with DSO-16. Mydriatic activity was assessed in a stereomicroscope at forty-fold magnification.

Compounds: 1-(2-oxo-1-pyrrolidino)-4-(2-chloroethyl-methylamino)but-2-yne · HCl, DSO-16, M.w. 266 (STERK and DEÁK, to be published later), arecoline HBr, atropine sulphate, nicotine hydrogen tartrate, pentobarbital sodium (Nembutal®-Abbott), physostigmine salicylate, oxotremorine oxalate.

Doses were calculated as the salt and given intraperitoneally, except where noted in the text. Relative activities were counted from the quantities of the bases.

## Results

*DSO-16 possesses central cholinergic activity.* In mice an intraperitoneal dose of 1 or 2 mg/kg gave rise to symptoms similar to those seen from oxotremorine: tremor, ataxia, myasthenia, and salivation. DSO-16 prevented the animals from clinging to the rotarod, where its mean effective dose came to fall between 1 and 2 mg/kg. The 2 mg/kg and the 5 mg/kg dose each produced the above constellation of symptoms which then persisted for about 60 min

**Table I**

*Analgesic activity of DSO-16 in mice assessed on the hot plate*
(Activity of oxotremorine, see Table V)

| Dose of DSO-16 | n | Onset of reaction (second ± S.E.) | Increments in reaction time (seconds ± S.E.) | |
|---|---|---|---|---|
| | | | 30 min | 60 min |
| 0.2 mg/kg | 9 | 20.2±0.8 | 13.7±5.7 | 15.8±5.1 |
| 0.5 mg/kg | 20 | 21.2±1.3 | 20.4±3.1 | 12.1±2.2 |
| 1.0 mg/kg | 10 | 22.3±0.7 | 31.5±3.0 | 22.7±4.8 |

* The parasympathomimetic activity of DSO-16 on the cat's blood pressure and on the rat's isolated ileum was 50—100% that of oxotremorine. These results will be published later

**Table II**

*Influence of DSO-16 on spontaneous motility*
Continuous 30-min count ± S.E.

| Compound | n | Absolute values | Percentage of controls |
|---|---|---|---|
| NaCl | 14 | 277±31 | 100 |
| 0.1 mg/kg DSO-16 | 14 | 135±22 | 49 |
| 0.5 mg/kg DSO-16 | 14 | 65±18 | 23 |
| 1.0 mg/kg DSO-16 | 14 | 74±18 | 27 |
| NaCl | 24 | 187±22 | 100 |
| 0.02 mg/kg oxotremorine | 24 | 101±12 | 54 |
| 0.05 mg/kg oxotremorine | 24 | 43±8 | 23 |

and 150 min, respectively. In the rotarod experiments the mean effective dose of oxotremorine was 0.2—0.25 mg/kg, and that of DSO-16 was 10% to 14% thereof.

The hot-plate test showed DSO-16 to have analgesic activity (Table I). This was of lower intensity at 60 min than at 30 min after treatment, i.e. about 35% of that of oxotremorine (cf. Table V).

DSO-16 restricted spontaneous motility (Table II). This effect of the compound was roughly 15% of that of oxotremorine.

Table III shows the potentiating effect of DSO-16 on pentobarbital and hexobarbital-induced narcosis at 30 min and 60 min after administration. Given 24 hours before pentobarbital, neither the 1, 2 or 5 mg/kg dose

**Table III**

*Narcosis-potentiating activity of DSO-16*

| Treatment | Min before narcotic | Narcotic | n | Sleeping time (min ± S.E.) | Sleeping time in per cent of control |
|---|---|---|---|---|---|
| NaCl | 10 | 40 mg/kg pentobarbital | 10 | 42.3± 9.1 | 100 |
| 0.1 mg/kg oxotremorine | 10 | intraperitoneally | 10 | 79.5± 8.6 | 188 |
| NaCl | 30 | 40 mg/kg pentobarbital | 10 | 48.2± 6.7 | 100 |
| 0.5 mg/kg DSO-16 | 30 | intraperitoneally | 10 | 77.9±10.4 | 162 |
| 1.0 mg/kg DSO-16 | 30 | | 10 | 92.7±10.8 | 192 |
| NaCl | 60 | 50 mg/kg hexobarbital | 10 | 8.8± 2.8 | 100 |
| 2.0 mg/kg DSO-16 | 60 | intravenously | 9 | 31.2± 6.8 | 367 |
| 5.0 mg/kg DSO-16 | 60 | | 10 | 37.6± 7.4 | 442 |

of the compound exerted any influence on the narcotic effect (these data have not been included in the table).

A subcutaneous dose of 2 mg/kg b.w. of atropine sulphate inhibited the analgesic action of DSO-16, and its ability to cause myasthenia as measured by the rotarod test.

*DSO-16 possesses central anticholinergic activity.* The rotarod activity of 0.5 mg/kg b.w. of oxotremorine was 100%; DSO-16 inhibited this and also the oxotremorine-induced tremor. The $ED_{50}$ of the compound estimated at different times was calculated from the effects of two doses by graphic interpolation (Table IV). Up to the 48th hour the rotarod test showed practically no decrease in the anti-oxotremorine activity of DSO-16.

DSO-16 inhibited arecoline tremor for a shorter time than it inhibited the oxotremorine-induced tremor. A 20 mg/kg dose of arecoline produced a tremor lasting for 5 min in all the control animals. The inhibitory $ED_{50}$ of DSO-16 was 2.9 (1.7—5.1) mg/kg (95% confidence limit) when administered 4 hours before arecoline, and about 10 mg/kg when given 24 hours before it.

DSO-16 inhibited oxotremorine analgesia for considerable periods of time (Table V).

DSO-16 inhibited physostigmine lethality. Twenty hours after pretreatment it still afforded a marked protection (Table VI). Its activity against nicotine (3.0—3.5 mg/kg) was of shorter duration and weaker. Two hours after pretreatment, a 4 mg/kg dose of DSO-16 (approx. $ED_{50}$) conferred protection against the effect of an intravenously administered lethal dose of nicotine. Twenty-four hours after pretreatment, no anti-nicotine activity was observed from either a 2, 5, or 10 mg/kg dose of DSO-16.

In the rotarod experiments three consecutive 5 mg/kg doses of DSO-16 given at 0, 185, and 290 min had 10/12, 6/12, and 2/12 activity, respectively.

*Toxicity and pupillary activity of DSO-16.* The intraperitoneal $LD_{50}$ of the compound was 47 mg/kg (37.3—59.2) at 48 hours, and the intravenous $LD_{50}$ was 3.8 mg/kg (3.1—4.7) at 3 hours.

**Table IV**

*Anti-oxotremorine activity of DSO-16 assessed on the rotarod*

| DSO-16 preceding oxotremorine by | n | DSO-16 $ED_{50}$ (approx.), mg/kg |
|---|---|---|
| 4 hours | 36 | 1.5 |
| 24 hours | 33 | 3.5 |
| 48 hours | 44 | 4 |
| 72 hours | 40 | 10 |

$ED_{50}$ = the dose preventing the activity of 0.5 mg/kg oxotremorine in half of the animals

**Table V**

*Influence of DSO-16 on the analgesic activity of oxotremorine*

| Treatment | Preceding oxotremorine by | n | Onset of reaction (second ± S.E) | Increment in reaction time after 0.1 mg/kg oxotremorine (second ± S.E) |
|---|---|---|---|---|
| NaCl | 24 hours | 12 | 19.6±0.6 | 14.5±2.2 |
| 2 mg/kg DSO-16 | 24 hours | 12 | 22.0±1.6 | 5.8±3.8 |
| 5 mg/kg DSO-16 | 24 hours | 12 | 19.4±1.1 | 3.5±1.6 |
| NaCl | 48 hours | 12 | 17.9±0.9 | 19.1±4.7 |
| 2 mg/kg DSO-16 | 48 hours | 12 | 17.8±0.9 | 3.2±3.5 |
| 5 mg/kg DSO-16 | 48 hours | 12 | 19.0±1.2 | −0.7±0.5 |

**Table VI**

*Anti-physostigmine activity of DSO-16*

| DSO-16 preceding physostigmine by | n | Physostigmine dose mg/kg | DSO-16 $ED_{50}$ mg/kg |
|---|---|---|---|
| 5 hours | 64 | 1.25 | 2.7 (1.7—4.4) |
| 20 hours | 36 | 1.20 | approx. 2 |
| 20 hours | 48 | 1.50 | approx. 5 |
| 72 hours | 72 | 1.5 | 10 and 20 inactive |

$ED_{50}$ = the dose preventing the lethality of the intraperitoneal $LD_{100}$ of physostigmine in half of the animals

**Table VII**

*Cumulative toxicity of DSO-16*

| Daily treatment on the 1st, 2nd and 3rd day | n | Total number of deaths after the 24 hours | | | Survivors | DSO-16 given on 4th day, mg/kg | Number of further deaths within 24 hours |
|---|---|---|---|---|---|---|---|
| | | 1st | 2nd | 3rd | | | |
| NaCl | 40 | 0 | 0 | 0 | 20 | 50 | 7 (35%) |
| | | | | | 20 | 100 | 14 (70%) |
| 5 mg/kg DSO-16 | 40 | 4 | 6 | 6 | 17 | 50 | 1 (6%) |
| | | | | | 17 | 100 | 5 (29%) |
| 10 mg/kg DSO-16 | 40 | 12 | 12 | 12 | 14 | 50 | 1 (7%) |
| | | | | | 14 | 100 | 3 (21%) |
| 20 mg/kg DSO-16 | 40 | 20 | 20 | 20 | 10 | 50 | 1 (10%) |
| | | | | | 10 | 100 | 4 (40%) |

Three consecutive doses of 5, 10 or 20 mg/kg of DSO-16 daily reduced the lethality of a fourth dose of 50 or 100 mg/kg of DSO-16 (Table VII).

A 2 mg/kg dose of DSO-16 produced unimpressive mydriasis, and one of 5 mg/kg gave rise to myosis followed by mydriasis. Control value: $3.6 \pm 0.5$ U. Values at 30 min and 360 min after administration of the compound were $2.3 \pm 0.6$ and $16 \pm 1.2$ U, respectively. (Twelve mice, arithmetical mean and S.E.) At 20 hours no mydriasis could be observed.

## Discussion

The tremorigenic, myasthenic, and analgesic activities of DSO-16, as well as its ability to potentiate anaesthesia and to reduce motility, are similar to those of oxotremorine but lower in intensity (10 to 35%) and never of longer duration. The compound behaves in the manner of a typical partial agonist: after its mimetic activity has worn off it assumes a central anticholinergic activity.

The question arises whether in antagonising the action of oxotremorine and other cholinomimetics and in reducing its own activity, the effect of DSO-16 may not be a phenomenon analogous to acute oxotremorine-tolerance (GYÖRGY et al. 1970; GYÖRGY et al., in press). The two phenomena have many common as well as widely differing properties. One injection of oxotremorine inhibits the effect of the next one for a few hours only whereas a single dose of tremorine has a more lasting anti-oxotremorine activity (presumably because of the slowed-down metabolism) which however, still falls far behind the duration of the DSO-16 effect (24, 48 and even 72 hours).

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# *Communicationes breves*

## BIOCHEMICAL CHANGES ASSOCIATED WITH CONDITIONED REFLEXES ESTABLISHED UPON HYPOXIA

By

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The conditioned increase in oxygen consumption, established upon a 3—6% hypoxia in the rat, is associated with increased blood glucose and lactic acid levels and increased cerebral GABA and glutamic acid contents. The conditioned decrease of metabolism built upon moderate hypoxia with an oxygen content above 10%, results in opposite biochemical changes. The direction of the conditioned reflex established upon hypoxia depends on the intensity of the unconditional stimulus and the reactivity of the two parts of the vegetative nervous system; it corresponds to that vegetative response which had been reinforced by the corresponding nervous structures of the highest function in the course of the conditioning.

In previous experiments it has been observed that conditioned metabolic responses could be established in various experimental animals by reducing the oxygen content of the inspired air (OBÁL et al. 1953, 1955, 1962; OBÁL and VICSAY 1963; OBÁL 1966). It was also observed that, especially in the first stages of ontogenesis, the effect of the conditioned reflex manifested itself with a reduction of metabolism (OBÁL 1966; VICSAY et al. 1966a; OBÁL et al. 1969). The conditioned reflex character of these vegetative responses was confirmed by EEG studies (MADARÁSZ et al. 1965; OBÁL 1966). For a further analysis of these phenomena the experiments were completed by biochemical studies (VICSAY et al. 1966b), the results of which will now be summarized.

### Methods

Wistar rats kept on a standard diet were used. The weight of the animals varied between 260 and 290 g, and in some groups between 200 and 230 g. The animals were grouped and 3—3 animals were sacrificed in the various stages of conditioning. As an unconditional stimulus, a mixture of air of 3, 6 and 10% oxygen content was applied. Hypoxia lasting for 15 min was associated with an auditory stimulus (1000/min). Oxygen consumption was registered by means of a Noyons-Kipp diaferometer. In the diverse stages of conditioning the animals were decapitated, their blood was sampled for glucose and lactic acid and the GABA and glutamic acid content of the brain was estimated. Blood glucose was analyzed by HULTMANN's (1959) method, lactic acid was estimated by the method of BARKER and SUMMERSON (1941). GABA and glutamic acid were determined by the chromatographic method of ROBERTS and FRANKEL (1950) as modified by FÖLDI et al. (1966).

## Results

During conditioning with hypoxia, the oxygen consumption of rats of 260—280 g bw. decreased (Fig. 1). This decrease became slighter in the course of repetitions. On eliciting the conditioned reflex there was a significant increase in metabolism. As demonstrated in the bottom of Fig. 1, the decreased oxygen consumption during hypoxia was accompanied by an elevation of the

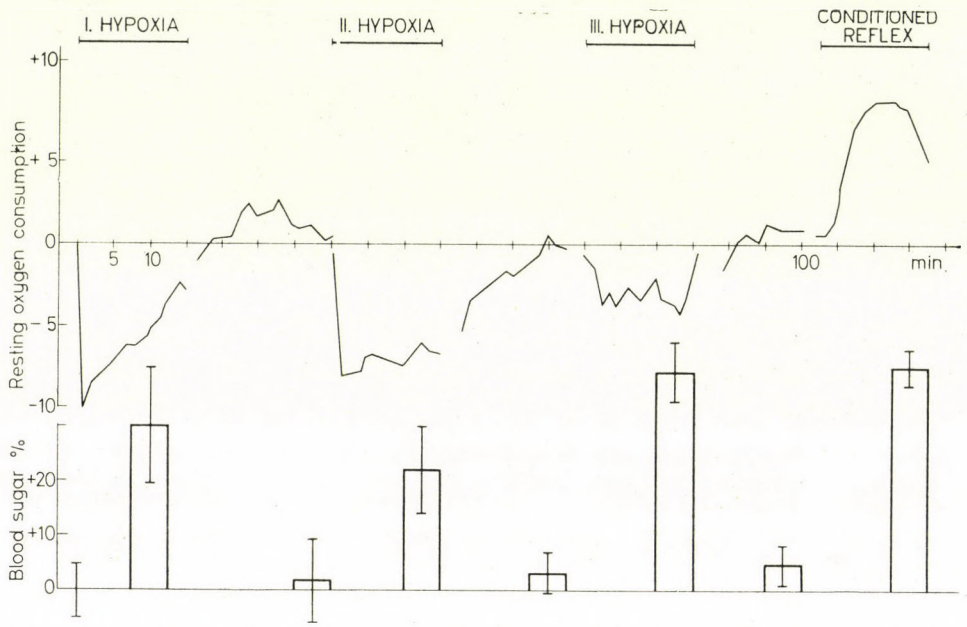


*Fig. 1.* Establishment of conditioned reflex in the course of repeated conditionings. 15-min periods of inspiration of an air mixture with 6% oxygen content are alternated by 20-min periods of room air breathing. Above, the changes in oxygen consumption are shown, related to the resting metabolism. The columns demonstrate the changes in blood sugar level at the end of the single periods, related to the preexperimental value (n = 6)

blood glucose level. Between conditionings, during the 20-min interval with a normal oxygen content, the blood glucose level returned approximately to the starting value. Application of the conditional stimulus alone after conditioning resulted in hyperglycaemia. However, this could not be attributed to a direct effect of the auditory stimulus since the same sound effects, repeatedly applied without hypoxia in other experiments, were ineffective.

The blood lactic acid level and the cerebral GABA and glutamic acid contents exhibited cyclic changes resembling to those of the blood sugar level. These changes are summarized in Fig. 2, as compared to the initial values.

The first column demonstrates the relative changes during hypoxia, the second one shows the changes measured in the intervals between two hypoxic periods, the third group illustrates the mean alterations found during the conditioned reflex. It may be seen that the unconditional stimuli resulted in an increased level of the substances observed, these levels more or less returned to normal in the intervals between conditionings and the conditional stimuli evoked the metabolic changes characteristic of hypoxia.

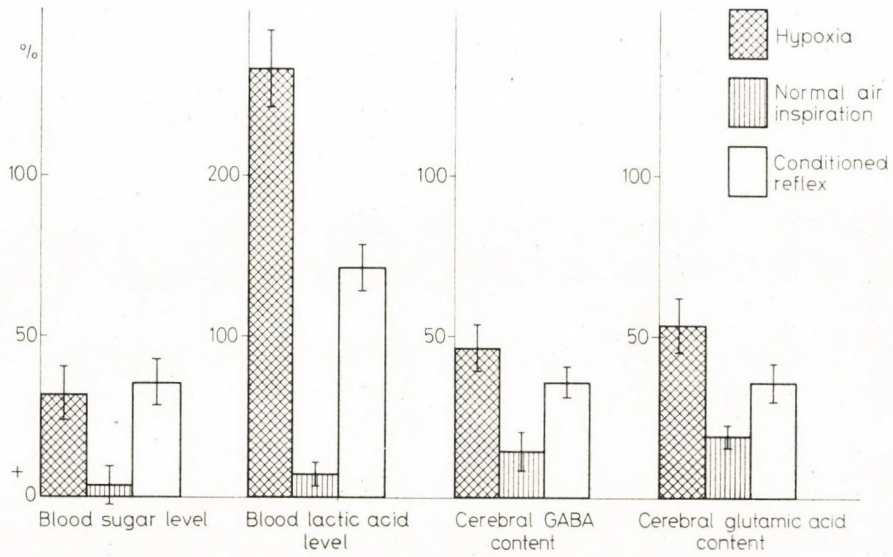


*Fig. 2.* Blood sugar and lactic acid levels and cerebral GABA and glutamic acid contents in the periods of hypoxia, normal air inspiration and conditioned reflex. Changes are illustrated in per cents of control values, on the basis of data obtained from 18 animals. Inspiration of an air mixture with 6% oxygen content was the unconditional stimulus

As already mentioned, the conditioned reflex established in the early stage of ontogenetic development, manifested itself with a decrease in metabolism. In the present experimental series the conditioned reflex, established in rats of 200—230 g b.w., manifested itself with a reduction of metabolism, especially when moderate hypoxia ($O_2$ content above 10%) was applied as the unconditional stimulus. Fig. 3 demonstrates such a conditioned response. Oxygen consumption is shown in the top, blood glucose and lactic acid levels before and after the conditioned reflex in the bottom of the figure. The conditioned reflex, manifesting itself in a reduction of metabolism, is accompanied here by a moderate increase in the blood glucose and lactic acid levels.

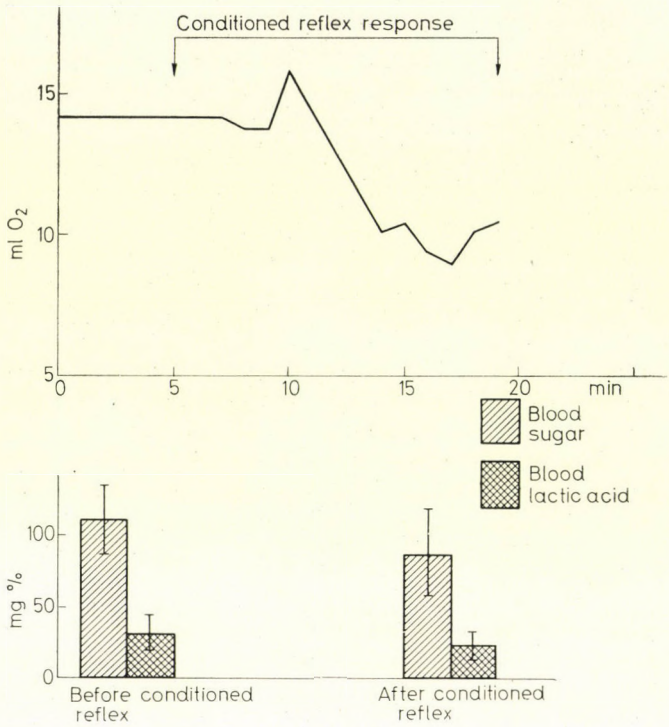


*Fig. 3.* Above: oxygen consumption; below: changes in blood glucose and lactic acid levels before and at the end of the conditioned reflex. The conditioned reflex, manifesting itself with a reduction of metabolism is accompanied by a moderate decrease of the blood glucose and lactic acid levels. Unconditional stimulus: air mixture with 10% oxygen. Summarized data obtained in 12 experiments

## Discussion

It has been known since CANNON's (1929) studies that sympathetic excitation results in an elevation of the blood glucose level. The increase in blood lactic acid may also be regarded as a sign of sympathico-adrenal activity. Hence, the hypoxic conditioned responses may be due to the increased tone of the sympathetic system, in spite of the decrease in metabolism. Even in those cases where the conditioned reflexes are associated with an increase in oxygen consumption, blood glucose and lactic acid level, the basis of the temporary connection is the activation of the sympathetic nervous system. The cerebral GABA and glutamic acid content runs parallel with the changes of the blood sugar. According to our observations, the changes in the amino acid levels may be in connection with the changes in the intensity of carbohydrate metabolism.

What accounts for the metabolism reducing conditioned reflex? Hypoxia activates both parts of the vegetative nervous system, with a predominance

of either the sympathetic or the parasympathetic system (VAN-LIERE and CRISLER 1933; BACQ et al. 1939; GELLHORN 1954 etc.). Thus, the metabolism reducing conditioned reflex may logically be attributed to parasympathetic effects. The dual role of the vegatative nervous system in these phenomena is also suggested by our studies because the establishment of a temporary connection with a stronger hypoxic unconditional stimulus in the animal displaying conditioned parasympathetic effect reverses the reflex into one of a sympathetic character. In younger animals or in conditions where the compensatory processes are less developed, the parasympathetic effects are dominating. Therefore, the direction of a conditioned reflex, elicited by a stimulus of given intensity, depends on the reactivity of the two components of the vegetative nervous system. They also control the level and stability of the initial level which will be reflected in the reflex response according to the law of initial value (WILDER 1931, 1967). These factors, together with the intensity of the stimulus and the number of its repetitions will result in such vegetative responses which had been reinforced by the corresponding highest nervous structures in the course of the conditionings (OBÁL 1966).

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# MICROPHYSIOLOGICAL ANALYSIS OF PYRAMIDAL AND A KIND OF INHIBITORY INTERNEURONAL (PROBABLY BASKET CELL) ACTIVITY IN THE DORSAL HIPPOCAMPUS OF THE CAT*

By

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(Received June 16, 1970)

Laminar analysis of the dorsal hippocampus of the cat revealed the selective extracellular recordability of the pyramidal and a kind of inhibitory interneuronal activity. It is highly probable that the bursts of smaller amplitude spikes, recorded at 200—250 $\mu$ below the alveolar surface of the hippocampus show the activity of basket cells.

a) The reactivity of the pyramidal cells is suppressed when the stimulus (light flash) is administered shortly after the spontaneous burst of small amplitude spike;

b) under the effect of chloralose the activity of suspected basket cells is selectively suppressed, indicating a similar effect of the drug in the hippocampus and on spinal Renshaw activity (cf. HAASE and VAN DEN MEULEN 1961; SHIMAMURA et al. 1968);

c) simultaneously, the inhibitory effect of lateral septal stimulation on pyramidal-cell activity (recurrent inhibition) is abolished.

Recurrent inhibition has been recognized in the spinal cord (RENSHAW 1941) and subsequently in the hippocampus (KANDEL et al. 1961), thalamus (ANDERSEN and ECCLES 1962), cerebellum (ANDERSEN et al. 1963b) and neocortex (STEFANIS and JASPER 1964). Attempts to identify responsible interneurones were successful in the cerebellum (SZENTÁGOTHAI 1965; ECCLES et al. 1966) and the thalamus (MARCO and BROWN 1966). On the basis of an extensive study of hippocampal neural elements, ANDERSEN et al. (1963a) suggested that the basket cells were responsible for the recurrent inhibition of hippocampal pyramidal cells.

Whereas — until very recently — selective recording of hippocampal basket cell activity has not been reported, the data obtained from the three series of experiments described below may reflect this kind of cellular activity. Preliminary note has been reported earlier (ARUTYUNOV et al. 1969).

The first series of experiments involved the extracellular laminar analysis of the dorsal hippocampus ($CA_3$) with the help of micropipettes of 1—1.5 $\mu$ tip diameter, and filled with 1.5 M sodium citrate. These recordings were taken from anaesthetized (pentobarbital, 40—45 mg/kg, intraperitoneally) as well

* This work has been supported in part by a research grant (1968—69) from the Hungarian Academy of Sciences, to the first author. His permanent address: Institute of Physiology, University Medical School, Pécs, Szigeti út. 12., Hungary

as unanaesthetized gallaminated and artificially ventilated cats. In the latter animals, the operations were made under ether anaesthesia, and pressure points and wound edges were infiltrated with procain solution before the cessation of the anaesthesia, repeatedly during the experiment. Special care was taken to avoid any discomfort to the animal.

Typical patterns of spontaneous pyramidal bursts were recorded; in addition, 250—300 $\mu$ below the alveolar surface, we repeatedly recorded "little spike bursts" of cellular but not of pyramidal origin (Fig. 1; and cf. ARUTYUNOV et al. 1969). Since the basket cells are located at this depth (RAMON Y CAJAL 1911; LORENTE DE NÓ 1934), the basket cell origin of the "little spike bursts" seems very probable.

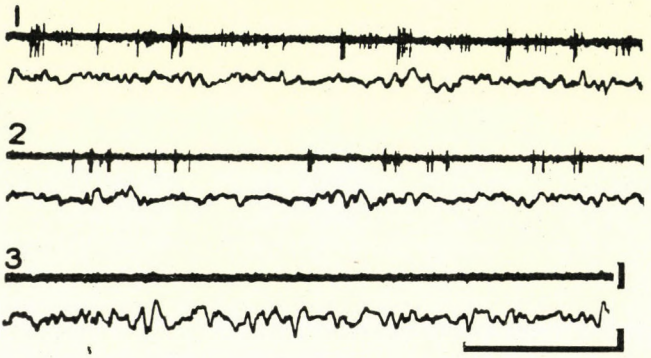


*Fig. 1.* Spontaneous activity of the different layers of the dorsal hippocampus of the cat. A: 200 $\mu$; B: 400 $\mu$; C: 650 $\mu$, below the alveolar surface. Pentobarbital anaesthesia (45 mg/kg, intraperitoneally). Upper tracings: unit activity of the dorsal hippocampus; lower tracings: records with the same microelectrode, through low pass filters. Calibration: 1 mV and 0.5 sec

Histological studies (LORENTE DE NÓ, 1934) have shown that each basket cell supplies a number (up to 200—500) of pyramidal cells with synapses, and that each pyramidal cell receives synapses from a number of basket cells, so it was expected that a reciprocal relationship between the pyramidal and the suspected basket cell activity will be rare. The analysis of photically evoked activity of a pyramidal cell revealed such a relation (Fig. 2). While the latent period between the stimulation artifact and the elicited discharge varied widely, the time interval between the last spike of the "little spike bursts" and the first pyramidal spike was remarkably stable at 120 msec. This time span would reflect the decay of the hyperpolarizing effect of the basket cell burst. Fig. 2C is of particular interest; the enhanced pyramidal burst demonstrates that the effect of the visual stimulus reached the pyramidal cell during its postanodal hyperexcitability.

The recently reported finding that chloralose was a selective inhibitor of the Renshaw cells in the spinal cord (HAASE and VAN DEN MEULEN 1961;

SHIMAMURA et al. 1968), provided a pharmacological possibility for the identification of the "little spike bursts" as basket cell activity. The spontaneous hippocampal activity at 250—300 $\mu$ below the alveolar surface showed a typical mixture of hippocampal firing and the "little spike bursts", both in the anaesthetized and in the unanaesthetized paralyzed animals (Fig. 3A). Chloralose (20 mg/kg) administered intraperitoneally selectively blocked the "little spike bursts" in 15—20 minutes (Fig. 3B, C, D), while the pyramidal spikes remained unchanged in both amplitude and shape. With the deepening

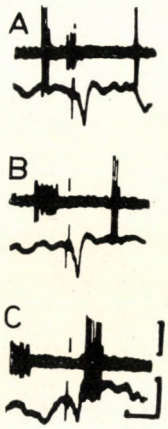


*Fig. 2.* Effect of "little spike bursts" on the reactivity of pyramidal neurones to light flash administered: A) during the "little spike burst" or inhibitory phase; B) shortly after the cessation of "little spike burst" or after recovery from the inhibitory phase; C) following cessation of "little spike burst" during postanodal hyperexcitability (120 msec). Cat under pentobarbital anaesthesia (45 mg/kg, intraperitoneally). Upper tracing: neural activity of dorsal hippocampus; lower tracing: gross records from visual cortex. Calibration: 1 mV and 80 msec

of the chloralose effect, the number of spikes per individual pyramidal burst decreased and the average inter-burst interval increased (Fig. 3D).

In order to verify our electrophysiological and pharmacological data, we performed a third series of experiments involving an adapted version of the classical Renshaw experiment. Six unanaesthetized, gallaminized cats were used. Retrograde activation of pyramidal axon collaterals and basket cells was induced by electrical shocks administered to the lateral septum. The pyramidal background activity in the ipsilateral hippocampus was thus inhibited (Fig. 4A). This inhibition was particularly pronounced when the septal shock has been administered during an artificially enhanced pyramidal background activity produced by preliminary peripheral or midbrain reticular formation stimulation.

Within 15 minutes after chloralose administration, the inhibitory effect vanished, simultaneously with the gradual disappearance of the "little spike

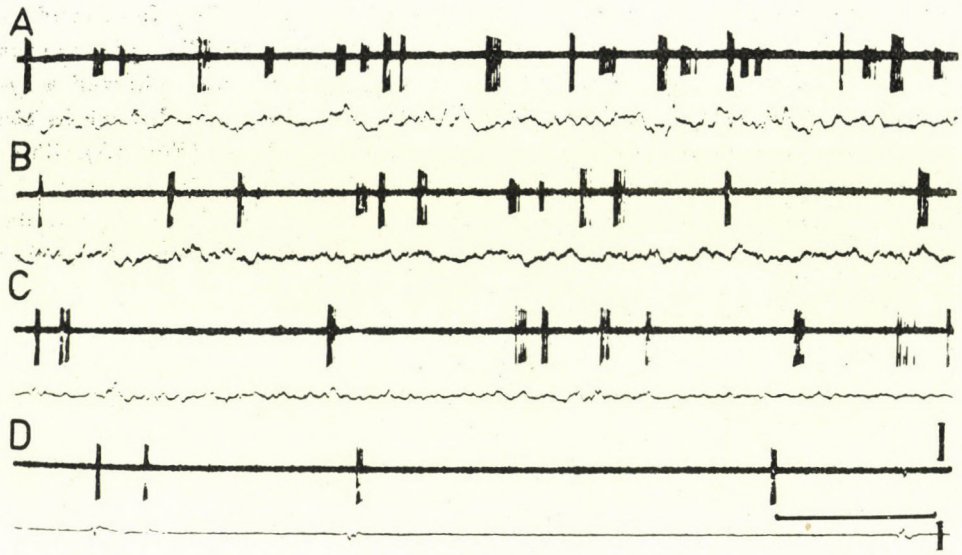


*Fig. 3.* Effect of 20 mg/kg chloralose intraperitoneally on the spontaneous neural activity of the dorsal hippocampus in the gallaminized cat. Upper tracing: unit activity of dorsal hippocampus; lower tracing: record with the same microelectrode, through low pass filters. A: 2 min; B: 5 min; C: 15 min; D: 20 min after chloralose administration. Calibration: 1 mV and 1 sec

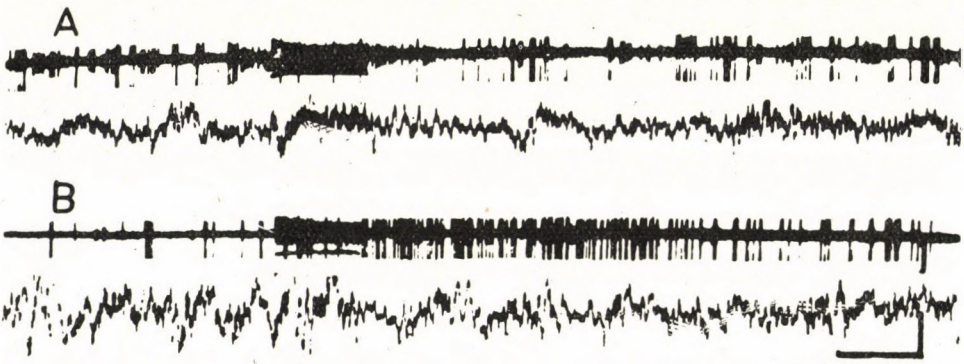


*Fig. 4.* Effect of septal stimulation (5 V, 100 cps, 0.2 msec) on the activity of hippocampal pyramidal cells. A: before chloralose; B: after (70 mg/kg) chloralose. Unanaesthetized gallaminized cat. Calibration: 1 mV and 1 sec

bursts". This was followed by an increase in the activation of pyramidal cells, showing the hitherto masked facilitating effect of septal stimulation (Fig. 4B) which probably takes the transcortical-entorhinal-perforant path route. The simultaneous disappearance of the little spike bursts and the cessation of the recurrent collateral inhibition provide a functional verification of the basket cell origin of the former type of activity.

The above results are in accordance with the recent observations of DICHTER and SPENCER (1969) and of ITO and OLDS (1971), whose data obtained

in epileptic and normal hippocampus are suggesting the extracellular recordability of the inhibitory interneurones.

The relative importance of pyramidal and basket cells in the genesis of the electrohippocampogram may, perhaps, be recognized in future studies on the basis of the above findings. Further research is also required to show whether or not the basket cells are supplied with afferentation other than the pyramidal axon collaterals. The answer to this question may be of assistance in the identification of the sources and the evaluation of the role of the extrahippocampal influences in the genesis of hippocampal electrical patterns.

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# EFFECT OF ADRENERGIC RECEPTOR BLOCKING AGENTS ON THE DEVELOPMENT OF EXPERIMENTAL ULCER IN THE RAT

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Phenoxybenzamine significantly inhibits the development of Shay ulcer and reserpine ulcer but fails to influence that of the immobilization ulcer. Propranolol has no effect on these kinds of ulcer. Thus, alpha receptors seem to have an important role in the formation of Shay and reserpine ulcer.

Previously it was shown that the inhibiting effect of epinephrine on gastric secretion in the rat was mediated through adrenergic beta receptors (GÁTI et al. 1967, 1968). The development of Shay ulcer was prevented by the continuous blockade of alpha receptors while the blockade of beta receptors was ineffective in this regard (PUCSOK et al. 1970).

In the present experiments we have studied the effect of the alpha blocking agent phenoxybenzamine (Dibenzyline, Smith Cline and French Lab. Ltd., Welwyn Garden City, Herts, England) and of the beta blocker propranolol (Inderal, I. C. I. Cheshire, England) on the development of ulcer evoked by pyloric ligation (SHAY et al. 1945), by restrain (ROSSI et al. 1956) or by the intramuscular administration of reserpine (LA BARRE and DESMAREZ 1957).

The control and experimental animals were sacrificed 18 hours after provocation. The ulcerous changes of the gastric mucosa were statistically evaluated for ulcer index (Mann-Whitney method) and ulcer frequency ($\chi^2$ procedure).

Fig. 1 summarizes the results obtained with phenoxybenzamine given intramuscularly 30 min prior to the ulcer provoking procedure. The drug significantly inhibited the development of Shay ulcer and reserpine ulcer while failed to affect the restrain ulcer.

The effect of propranolol is shown in Fig. 2. The drug was administered intraperitoneally, 30 min before and 5 hours after the provocation; it failed to influence ulcer development.

These results are in agreement with the data in literature. Ulceration of the gastric mucosa, irrespective of its aetiology, is a consequence of the disequilibrium between the physiological factors lesioning and protecting the

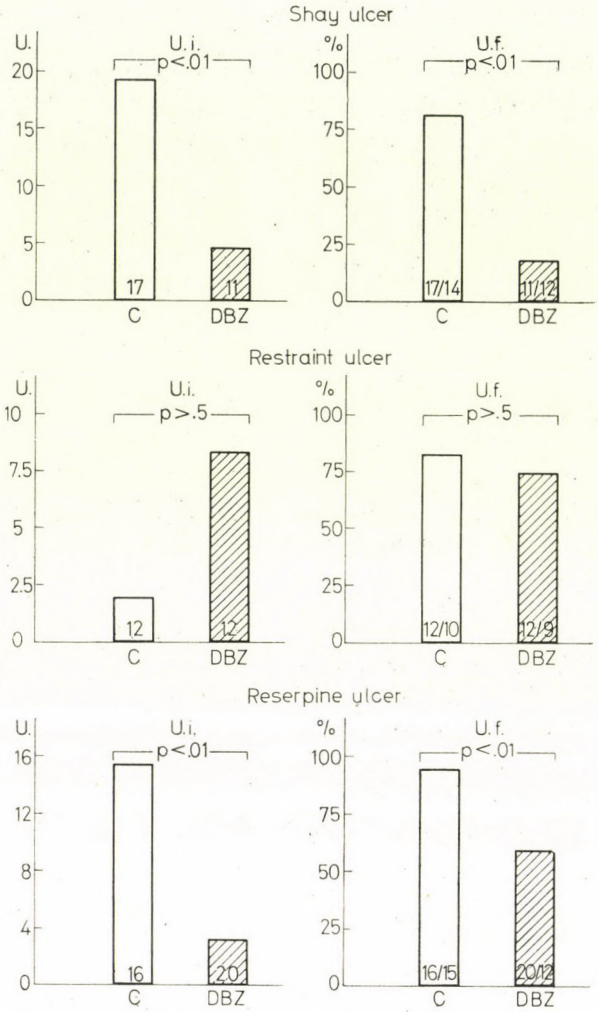


*Fig. 1.* Effect of phenoxybenzamine (5 mg/100 g, intramuscularly) on experimental ulcer development in the rat. Ui: ulcer index; U: unit; Uf: ulcer frequency; in bars: number of animals; C: control; DBZ: phenoxybenzamine

gastric mucosa (BOCKUS 1964). Alpha blocking increases the blood catecholamine level and has an antisecretory effect (JOW et al. 1960; BASS and PATTERSON 1967) while beta blockade has no significant effect on gastric secretion (MISHER et al. 1969). The conclusion may be drawn that the alpha receptors play an important role in the development of the Shay and reserpine ulcer. By blocking these receptors, i.e. by moderating the attacking effect, a protective effect may be obtained.

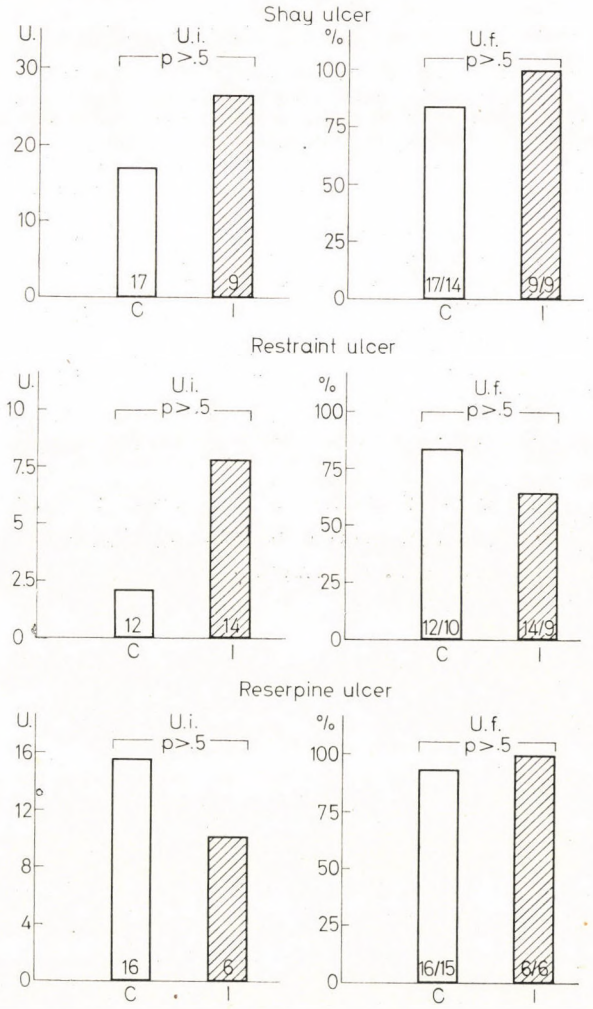


*Fig. 2.* Effect of propranolol (twice 0.5 mg/100 g, intraperitoneally) on experimental ulcer development in the rat. Ui: ulcer index; U: unit; Uf: ulcer frequency; in bars: number of animals; C: control; I: propranolol

## Acknowledgement

We are indebted to Mrs. K. BŐLE, Mrs. J. IMRE and Mrs. E. CSURGAY for skilful technical assistance.

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# *Recensiones*

CL. PFISTER (Ed.)

## Physiologie von Erregung und Kontraktion, Physiologie des Zentralnervensystems

Ergebnisse der experimentellen Medizin. Vol. 3. Verlag Volk und Gesundheit, Berlin 1970. 560 pages, with 200 figures. Price: M 45.80

This volume contains the abstracts of papers presented in the different Sections of the Meeting of the Deutsche Gesellschaft für experimentelle Medizin in Leipzig. The 54 papers from different institutes of the GDR and some from abroad offers a view of the recent results of research achieved in the GDR.

K. LISSÁK

F. BRONNER and A. KLEINZELLER (Eds)

## Current Topics in Membranes and Transport

Vol. I. Academic Press, New York and London 1970. XII + 243 pages, with 88 figures. Price: $ 12.50

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K. LISSÁK

E. GLÁZ and P. VECSEI

## Aldosterone

Akadémiai Kiadó, Budapest 1971. 626 pages

The monograph consists of two parts. The first part is written by P. VECSEI and deals with the methodology applied in aldosterone research, biosynthesis, transport, metabolism, biological activity and the control of aldosterone secretion.

The second part written by E. GLÁZ, discusses the clinical problems connected with aldosterone, the clinical feature of the different forms of hyper- and hypoaldosteronism and the therapeutical approaches to these conditions.

The long years' experience of the authors in aldosterone research makes the monograph one of the most comprehensive surveys on the subject in the last few years. The methodological, theoretical and clinical implications of the problem will offer useful help for physiologists, biochemists, endocrinologists and clinicians interested in the up-to-date problems of aldosterone.

G. TELEGDY

W. L. HERRMANN (Ed.)

## Gynecologic Investigation

International Journal of the Science of Reproduction. Vol. 1. No. 1 (pp. 1—56), 1970. S. Karger, Basel, München, New York

This new periodical "Gynecologic Investigation" is published with the purpose of establishing an international source of information on the science of reproduction. The question is propounded by the Editor, W. L. HERRMANN, and the Co-Editor, W. LEROY HEINRICHS, in the introduction of the first number: who requires today a new periodical when an estimated number of 3323 papers concerning medical sciences are published each day. It turns out from their answer that till now there has been no obstetric-gynaecologic journal printing exclusively the experimental results of this discipline. The new periodical is expected to meet such demands. Moreover, its issueing does not augment the large number of scientific journals, because it replaces the "Monatschrift für Geburtshilfe und Gynäkologie" established in 1885 (renamed Gynaecologia in 1949).

The papers in the first number achieve the purpose of the editors.

G. MIKHAIL investigated the secretion of ovarian hormones, by means of estimation of the hormone content in ovarian venous and peripheral blood. The main ovarian products isolated by the author were progesterone, oestradiol, 17α-hydroxy-progesterone and androstenedione. The corpus luteum synthetized 20α-hydroxy-pregn-4-ene-3-one as well. Small amounts of oestrone and testosterone were also produced. Only androstenedione if anything at all was secreted by the postmenopausal ovary. Most of the steroids were produced by the very gland in which the follicle was growing or in which the luteal body had been developed. The contralateral ovary actively secreted androgens, mostly androstenedione.

KLEBANOFF and SMITH reported that the uterine fluid of oestrogen-pretreated rats exerted an antimicrobiotic effect against some bacteria, fungi and viruses if conjugated with iodide ions and $H_2O_2$. The uterine fluid exhibited peroxydase activity and its antimicrobiotic effect in the presence of iodide and hydrogen peroxyde might be attributed probably to this enzyme.

In a study of the placental and foetal metabolism of RNA in 14-day pregnant rats, HAYASHI and KAZMEROWSKI demonstrated that $^{14}C$-d-glucose was the most efficient precursor of both kinds of RNA. It was also found that glucose was incorporated both in the ribosomes and in the base of the nucleic acid.

CHEZ observed active sodium and chloride transport and histamine responsive $H^+$-secretion in the gastric wall of new-born subhuman primates.

NAKAMURA et al. elaborated a micro and a normal complement fixation test for the estimation of human placental lactogen. The sensitivity of the method is 6 μg/ml, the calibration curve linear between 6 and 40 μg/ml. This specific method is suitable for the analysis of HPL in 0.1 ml of pregnant serum.

The make-up of the journal amounts to the well-known level of Karger's publications. All who are interested in the theoretical problems of obstetrics and gynaecology beside its practical relations, will be offered indispensable material by this new periodical.

L. LAJOS

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# EIGHTH INTERNATIONAL CONGRESS OF ELECTROENCEPHALOGRAPHY AND CLINICAL NEUROPHYSIOLOGY

MARSEILLE, FRANCE. – SEPT. 1–7, 1973

## PROGRAM

The emphasis of the Congress program will be on Free Communications. There will also be two EEG Round Tables and two EMG Round Tables and one Common Session of interest to all Congress participants.

Four full days will be devoted to the presentation of Free Communications. Ten minutes will be allowed to each speaker with 5 minutes for discussion. Time will also be provided for general discussion in each session. There will be facilities for 8—10 simultaneous sessions. Each session will consist of 8—10 related papers. Papers will be selected on the basis of originality and quality only.

The topics of the EEG Round Tables will be "Possibilities and Limitations of New Methods of Data Collection and Analysis" and "Basic Mechanisms of Epilepsy". The topics of the EMG Round Tables will be "Computer Analysis of the EMG" and "Histopathology of Nerve and Electrophysiological Correlations".

The Common Session will be on the "Clinical Neurophysiology of Speech" (including mechanisms of reading and writing).

There will also be a concurrent program of didactic lectures and demonstrations of interest to a wide variety of senior technicians, technologists, and clinicians.

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*Acta Physiologica Academiae Scientiarum Hungaricae 40, 1971*

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# РЕЗЮМЕ

## ВЛИЯНИЕ ДЕНЕРВАЦИИ НА СОСТАВ ФОСФОЛИПИДОВ В ТОНИЧЕСКИХ И ТЕТАНИЧЕСКИХ МЫШЦАХ

Л. ХЕЙНЕР, Й. ДОМОНКОШ и М. ВАРГА

Установлено, что

1. Количество фосфатидилхолина повышается в денервированной тетанической мышце, а в денервированной тонической мышце значительно не изменяется. В контралатеральных, иннервированных мышцах уменьшается количество фосфатидилхолина.

2. Количество сфингомиелина повышается как в денервированных, так и контралатеральных тонических и тетанических мышцах. Повышение более выраженное в тетанических и денервированных мышцах.

3. Количество фосфатидилетаноламина, фосфатидилинозита и фосфатидилсерина увеличивается в денервированной тонической и тетанической мышце. Повышение более выраженное в тетанической мышце. В контралатеральных, иннервированных мышцах количество фосфатидилетаноламина уменьшается.

## ТЕПЛОПРОДУКЦИЯ ПЕРИАОРТАЛЬНОЙ БУРОЙ ЖИРОВОЙ ТКАНИ ПОД ВЛИЯНИЕМ ГИПОКСИИ, ГИПЕРКАПНИИ, $\beta$-АДРЕНЕРГИЧЕСКОЙ БЛОКАДЫ И НАРКОЗА

М. СЕКЕЙ, М. КЕЛЛЕРМАЙЕР и С. ДОНХОФФЕР

Под влиянием холода, параллельно с повышением терморегуляторной теплопродукции, температура крови в грудной аорте сравнивая с температурой крови в дуге аорты повышалась у крыс, акклиматизированных к холоду. С уменьшением или прекращением терморегуляторной теплопродукции при применении гипоксии, гиперкапнии, пропранолола или гексобарбитала, одновременно уменьшалась или исчезала разница между температурами, измеренными на вышеупомянутых местах аорты, в тесном параллелизме с уменьшениями теплопродукции интерскапулярного бурого жира. Заключение авторов: на крысах периаортальный буровый жир в ходе терморегуляторной теплопродукции передает тепло крови аорты.

## ТЕРМОРЕГУЛЯТОРНАЯ РОЛЬ ПЕРИАОРТАЛЬНОЙ БУРОЙ ЖИРОВОЙ ТКАНИ НА КРЫСАХ, АККЛИМАТИЗИРОВАННЫХ РАЗЛИЧНЫМ ТЕМПЕРАТУРАМ СРЕДЫ

М. СЕКЕЙ, М. КЕЛЛЕРМАЙЕР и С. ДОНХОФФЕР

У взрослых крыс, содержащихся при комнатной температуре или акклиматизированных к холоду, измеряя температуру крови дуге аорты и грудной аорте над диафрагмой, наблюдалось относительное повышение температуры дистального места под влиянием

холодной среды. Тепло, передаваемое крови через стенку аорты исходит из периаортальной бурой жировой ткани. Эта передача тепла почти или совсем не повышается в холодной среде на крысах, акклиматизированных к теплу, вследствие атрофии периаортального бурого жира. У морской свинки и молодого кролика, у которых отсутствовала периаортальная бурая жировая ткань, действие холодной среды не повышала разницы температуры между двумя пунктами аорты.

## МЫШЕЧНАЯ АКТИВНОСТЬ ЧАСТИЧНО ИННЕРВИРОВАННЫХ КОНЕЧНОСТЕЙ ПРИ ХОДЬБЕ У Ambystoma

Г. СЕКЕЙ и Г. ЦЕХ

Потенциалы действия 8-и мышц передней конечности регистрировались одновременно у двигающейся свободно ambystoma (1-ая группа). У дальнейших 6-и групп животных большинство нервов, исходящих из сегментов спинного мозга, приводящих в движение конечности, было перерезано перед регистрацией, и конечности были иннервированы у отдельных экспериментальных групп только из следующих мест: из III-его сегмента спинного мозга (2-ая группа), из каудальной части III-его сегмента (3-ая группа), из IV-ого сегмента (4-ая группа), из краниальной или каудальной части IV-ого сегмента (5-ая и 6-ая группы) и из V-ого сегмента (7-ая группа).

У интактных животных первой группы наблюдалось длительное взаимодействие антагонистических мышц при ходьбе. В 2-ой и 3-ей группах из мышц конечностей животных регистрировалась большей частью протракционная активность, но некоторая из мышц была активной и в течение ретракции. У животных 4-ой группы была обнаружена активность, близкая к нормальной. В 5-ой и 6-ой группах в образце регистрированной мышечной деятельности можно было найти отдельные части мышечной деятельности, наблюдаемой у животных в 4-ой группе. В 7-ой группе преобладала ретракционная активность мышц, но у нескольких мышц замечено и протракционная активность. Вообще, в каждой экспериментальной группе активный период мышц был короче при иннервированных частью, чем при интактных конечностях.

Результаты показали, что 5 из 8-и исследованных мышц получили иннервацию из всех трех сегментов спинного мозга, а 3 мышцы из двух сегментов. В течение цикла шага полный период нормальной деятельности мышцы складывался из более или менее коротких периодов деятельности, исходящей из различных мест трех сегментов спинного мозга. Из распределения по времени коротких активных периодов мышц авторы пришли к заключению, что каждый сегмент или части их посылают циклические пачки импульсов к мышцам конечности.

## МЫШЕЧНАЯ АКТИВНОСТЬ, РЕГИСТРИРОВАННАЯ ОДНОВРЕМЕННО ИЗ НОРМАЛЬНЫХ И ПЕРЕСАЖЕННЫХ КОНЕЧНОСТЕЙ У Ambystoma

Г. ЦЕХ и Г. СЕКЕЙ

Добавочные конечности пересаживались в плечевую область личинок axolotl таким образом, что иннервация получалась ими из 3-его или 4-ого или 5-ого сегмента спинного мозга. При медленной ходьбе животных пересаженная конечность двигалась координированно и синхронно с нормальной передней конечностью, лежащей на той же стороне, при насильных же движениях она клонически коченеет, или остается неподвижной при медленном движении животного в воде. Пересаженные конечности совершили полное подобное шагу движение, если иннервировались из 4-ого сегмента; если иннервация исходила из 3-его или 5-ого сегментов, их ретракционные и протракционные движения были ограниченными. При иннервации трансплантатов из 3-его сегмента регистрации в двух опытах показали нерегулярную мышечную активность, гомологные мышцы работали вместе только в небольшой мере. В случае иннервации, получаемой из 4-ого сегмента, мышечная активность стояла близко к нормальной, гомологные мышцы большей частью сокращались симультанно, но имела место также и нерегулярная деятельность. Если конечность иннервировалась из 5-ого сегмента, на миографических записях из мышцы плеча обычно можно видеть нерегулярную активность, в то время, как мышцы предплечья показывали актив-

ность, характеризующую деятельность 5-ого сегмента. В нерегулярной деятельности часто было можно выявить образец деятельности другой мышцы.

Из полученных результатов было сделано заключение, что мышцы конечности не могут модулировать деятельность иннервирующих их центров спинного мозга. На основании вышеуказанных и ранних экспериментальных результатов движение пересаженной конечности, координированное и синхронное с нормальной конечностью, обяснялось тем, что любая ветвь plexus brachialis передает ритмические импульси, на действие которых пересаженная конечность отвечает шаго-подобным движением.

## ДЕЯТЕЛЬНОСТЬ СЕГМЕНТОВ СПИННОГО МОЗГА И КОНЕЧНОСТЕЙ, ДЕПЛАНТИРОВАННЫХ В СПИННОЙ ПЛАВНИК ЛИЧИНА urodela

Г. СЕКЕЙ и Г. ЦЕХ

Сегменты спинного мозга и конечности пересаживались в канал, приготовленный в спинном плавнике личинок urodela. В некоторых случаях к фрагменту спинного мозга клался и чувствительный узел. Всегда, когда между деплантированным спинным мозгом и конечностью возникала нервная связь, наблюдались спонтанные и рефлекторные движения конечности. Если фрагмент спинного мозга исходил из грудных сегментов, спонтанные движения состояли из нерегулярных сокращений конечности. Координированное, подобное ходьбе движение деплантированной конечности возникало, если фрагмент спинного мозга был взят из области плечевых сегментов. Мышечная активность, регистрированная из мышц плеча и предплечья, приближалась к нормальной. По гистологическим исследованиям к образованию координированных движений конечности была необходимой целостность структуры деплантированных сегментов.

Авторами было сделано заключение, что подвижной образец конечности программируется в структуре плечевых сегментов спинного мозга. Эта структура может координированно двигать конечность без их ритмических афферентных импульсов и направления супраспинальных центров.

## ИЗОЛИРОВАННАЯ МАТКА КУРИЦЫ, КАК ЭКСПЕРИМЕНТАЛЬНЫЙ ОБЪЕКТ. РАЗНОСТЬ ТРАНСМУРАЛЬНЫХ ПОТЕНЦИАЛОВ И ТОК В ВЫЖИВУЩЕЙ МАТКИ КУРИЦЫ

А. КЕМЕНЬ, Й. Б. ЛАКЛИА и Г. ЛЕНЧЕШ

Авторы выработали in vitro технику для измерения значений разности потенциалов и тока замыкания в матке курицы и для изучения некоторых факторов, оказывающих влияние на электрические свойства мембраны. Когда в матке находилось яйцо с мягкой или твердой скорлупой, разность трансмурального потенциала выживущей ткани колебалась между 5—23 $\mu V$; в большинстве случаев показывала 5—6 $\mu V$. Слизистая сторона была отрицательной сравнивая с серозной. Величина тока замыкания изменялась между 16—26 $\mu A/см$.

## ИНСУЛИНОПОДОБНАЯ АКТИВНОСТЬ В СЫВОРОТКЕ КРОВИ И ИНСУЛИН-ЧУВСТВИТЕЛЬНОСТЬ ПОСЛЕ ДЕАФФЕРЕНТАЦИИ ГИПОТАЛАМУСА У КРЫС

В. ХЕФКО, П. ЙИТАРИУ и П. РОТЕМБЕРГ

У крыс параллельно были исследованы гормоны коры надпочечников, чувствительность к инсулину и инсулиноподобная активность в сыворотке в течение двух недель после фронтальной, фронто-латеральной, дорзальной или полной деафферентации медиального гипоталамуса. Чувствительность к инсулину повышалась после фронтальной, и снижа-

лась после дорзальной или полной деафферентации. Продукция кортикостероидов увеличивалась после фронто-латеральной и полной деафферентации, но не изменялась после фронтальной или дорзальной. Механическая перерезка нервных связей между вентромедиальным и вентро-латеральным ядрами не приводила к гиперфагии или изменению уровня глюкозы в крови натощак. Степень чувствительности к инсулину приписывается действию гормона роста и активности коры надпочечника, и не является следствием пониженного синтеза гликогена в печени. Не отрицая непосредственной роли центральной нервной системы, результаты показали прямую связь между концентрацией АКТГ в сыворотке и инсулиноподобной активностью, и показали, что повышенная инсулиноподобная активность не являлась следствием гипергликемии.

## ИЗУЧЕНИЕ БИОЭЛЕКТРИЧЕСКОЙ АКТИВНОСТИ КОРЫ И ГИППОКАМПА ПРИ НЕСПЕЦИФИЧЕСКОЙ ВАЗОДЕПРЕССОРНОЙ РЕАКЦИИ

Г. ШУХ и И. МАДАРАС

Показано, что неспецифическая вазодепрессорная реакция сопровождается тоническим биоэлектрическим пробуждением у кроликов при поверхностном наркозе. В то же время при спонтанных тонических пробуждениях в каждом случае отмечается снижение кровяного давления. Физические изменения ЭЭГ не связаны с вазомоторным ответом.

Установлено, что обязательным признаком вазомоторного ответа является не кортикальная десинхронизация, а появление медленного ритма в гиппокампе.

Характерная картина ЭЭГ при электрическом раздражении ретикулярных структур низким напряжением сопровождается депрессорной реакцией, а при раздражении более высоким напряжением — повышением кровяного давления.

На основании опытов авторы предполагают, что неспецифический вазодепрессорный ответ является одним из вегетативных явлений подкорковой ориентировочной реакции при таких условиях, когда равновесие нисходящих вегетативных процессов сдвинуто в сторону торможения.

## ВНУТРИЛОБАРНОЕ РАСПРЕДЕЛЕНИЕ ТКАНЕВОГО КРОВООБРАЩЕНИЯ В ПЕЧЕНИ СОБАК, II

## ВЛИЯНИЕ РАЗДРАЖЕНИЯ НЕКОТОРЫХ ПЕРИФЕРИЧЕСКИХ НЕРВОВ

Г. УНГВАРИ и Б. ВАРГА

Изменение тканевого кровотока измерялось с помощью гибких термоэлементов Hensel в тех частях долей печени собак, которые лежат близко к гилюсу (центральные) и на расстоянии от него (периферические) при электрическом раздражении печеночного сплетения (plexus hepaticus), внутренностного нерва (n. splanchnicus), левого блуждающего нерва (n. vagus sinister; на шее и кардии), правого и левого диафрагмального нерва (n. phrenicus dexter, sinister; на шее). Раздражение правого диафрагмального нерва производилось и после обрезки той части диафрагмы, которая лежит около нижней полой вены. Установлено, что:

1. раздражение печеночного сплетения, внутренностного, шейного блуждающего и правого диафрагмального нервов вызывает значительную дифференцию между тканевым кровотоком в центральной и периферической частях печеночной доли;

2. эффективные волокна рефлекса, вызывающего ограниченное кровообращение, находятся в системе внутренностный нерв — печеночное сплетение;

3. раздражение блуждающего нерва путем гипотонии приводит к ограничению; раздражение блуждающего нерва на кардии не вызывает изменения внутрипеченочного кровообращения;

4. правый диафрагмальный нерв не оказывает сосудосуживающего действия на систему печеночной вены; при невредимой диафрагме правый диафрагмальный нерв посредством механического действия диафрагмы вызывает ограничение внутрипеченочного кровообращения.

## ФАЗОВЫЙ РАЗГИБАТЕЛЬНЫЙ РЕФЛЕКС НАРУЖНЫХ ГЛАЗОДВИГАТЕЛЬНЫХ МЫШЦ У КОШЕК „ENCÉPHALE ISOLÉ"

П. МАРЕК и Э. МАРКЕЛ

Авторам удалось вызывать фазовый разгибательный рефлекс наружных глазодвигательных мышц у кошек с изолированным мозгом. Рефлексы регистрировались электромиографическим методом. После рефлекторного ответа в фоновой электрической активности наблюдалась полная или частичная тишина. В работе дискутируются факторы, влияющие на возникновение электрической тишины, а также роль фазового разгибательного рефлекса в регуляции движения глаз.

## ИЗМЕНЕНИЯ МЕТАБОЛИЗМА БРОМСУЛЬФОТАЛЕИНА (BSP) И ИНДОЦИАНИН-ГРИНА (ICG) ПОСЛЕ ИНДУКЦИИ БАРБИТАЛОМ У ЧЕЛОВЕКА

А. ГОГЛ

Индукция энзимов исследовалась на людях, которым вводилось 8 мг/кг фенобарбитала ежедневно в течение 3 дней, или на людях, которые с целью самоубийства принимали фенобарбитал в дозе 3,0—9,0 г.

Установлено, что:

1. период дистрибуции и экскреции BSP постепенно укорачивался в сыворотке крови;
2. $T_m$ бромсульфоталеина повышалось с $8{,}8 \pm 0{,}8$ мг/мин до $19{,}1 \pm 1{,}1$ мг/мин ($p < 0{,}001$);
3. период полураспада ICG в сыворотке значительно снижался (с $200 \pm 12$ сек на $72 \pm 7$ сек).

## ФАРМАКОЛОГИЯ 1-(2-ОКСО-1-ПИРРОЛИДИНО)-4-(2-ХЛОР-ЭТИЛ-МЕТИЛ-АМИНО)2-БУТИН-ГИДРОХЛОРИДА (DSO-16), I

## ХОЛИНОМИМЕТИЧЕСКИЕ И ХОЛИНОЛИТИЧЕСКИЕ ДЕЙСТВИЯ DSO-16 В ЦЕНТРАЛЬНОЙ НЕРВНОЙ СИСТЕМЕ МЫШЕЙ

Л. ДЬЁРДЬ, Б. ГЕЛЛЕН, М. ДОДА, А. К. ПФЕЙФЕР и Л. ШТЕРК

DSO-16 был синтезирован и исследован авторами. На мышах DSO-16 вызывает тремор, аналгезию, мышечную слабость, снижение общей подвижности и усиливает наркоз, проводимый пентобарбиталом или гексобарбиталом. Эта центральная холиномиметическая активность вещества по отношению к активности оксотреморина составляет 0,1—0,35. Вышеупомянутые симптомы исчезают за 1—2,5 часа и потом возникает вторичная фаза действия DSO-16, в которой его анти-холинергическая активность доминирует. В этой фазе DSO-16 имеет свойства анти-оксотреморина, анти-физостигмина, анти-арехолина и в меньшей мере анти-никотина и тормозит и свою токсичность. Эта антагонистическая активность медикамента наблюдаема в течение 24—48—(72) часов после его введения.

## ИССЛЕДОВАНИЕ БИОХИМИЧЕСКИХ ИЗМЕНЕНИЙ, СОПРОВОЖДАЮЩИХ ВЫРАБОТАННЫЕ НА ГИПОКСИЮ УСЛОВНЫЕ РЕФЛЕКСЫ

Г. ШУХ, М. С. ВИЧАИ, Е. П. САБО, Г. БЕНЕДЕК и Ф. ОБАЛ

У крыс повышение потребления кислорода в течение условного рефлекса, выработанного на 3—6%-ную гипоксию, сопровождается повышением уровня сахара и молочной кислоты крови, увеличением содержания ГАМК и глютаминовой кислоты в мозге.

Условнорефлекторное метаболическое снижение, выработанное на более слабую гипоксию (кислород выше 10%-ов) вызывает биохимические изменения противоположного направления. По мнению авторов направление выработанного на гипоксию условного рефлекса зависит от интенсивности безусловного раздражителя и от реактивности двух частей вегетативной нервной системы, и соответствует тому вегетативному изменению, которое подкреплялось в течение сочетаний нервными структурами высшей деятельности (Обал 1966).

## МИКРОФИЗИОЛОГИЧЕСКИЙ АНАЛИЗ ПИРАМИДАЛЬНОЙ И ТОРМОЗНОЙ ИНТЕРНЕВРОНАЛЬНОЙ [КОРЗИНЧАТОЧНОЙ КЛЕТКИ (?)] АКТИВНОСТИ В ДОРЗАЛЬНОМ ГИППОКАМПЕ КОШКИ

П. МОЛЬНАР, В. С. АРУТЮНОВ и С. П. НАРИКАШВИЛИ

Послойный анализ дорзального гиппокампа кошки сделал возможным селективное внеклеточное отведение пирамидальной активности тормозного характера. Авторами предполагается, что пачка спаек с меньшей амплитудой, отведенная на глубине 200—250 $\mu$ от альвеолярной поверхности гиппокампа, показывает активность корзинчаточных клеток.

1. Реактивность пирамидальных клеток будет подавлена, если применять стимул (вспышка лампы) сразу после пачки спонтанных спаек с низкой амплитудой.

2. Хлоралоз селективно подавляет активность предполагаемых клеток, показывая с этим, что этот медикамент одинаково влияет и на гиппокамп, и на спинную активность Renshow.

3. Одновременно с этим исчезает влияние раздражения латеральной перегородки на активность пирамидальных клеток (возвратное торможение).

## ДЕЙСТВИЕ МЕДИКАМЕНТОВ, БЛОКИРУЮЩИХ АДРЕНЕРГОРЕЦЕПТОРЫ НА РАЗВИТИЕ ЭКСПЕРИМЕНТАЛЬНОЙ ЯЗВЫ У КРЫС

Т. ГАТИ, П. КЕСЛЕР, Т. ЗЕЛЛЕШ и Й. ПУЧОК

Дибензилин, блокирующий алфа-рецепторы, значительно тормозит развитие резерпиновой и Shay язв, но не влияет на развитие язвы, вызванной иммобилизацией. Индерал, блокирующий бета-рецепторы, не оказывает влияние ни на одну из упомянутых экспериментальных язв. На основании результатов установлено, что алфа-адренергорецепторы играют важную роль в развитии резерпиновой и Shay язв.

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