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Physiologia

TRACER KINETIC STUDIES ON *IN VIVO* FATTY ACID METABOLISM IN WHITE ADIPOSE TISSUE OF WELL-FED AND STARVING NEWBORN RABBITS DURING ACUTE OR PROLONGED EXPOSURE TO COLD

By

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(Received December 11, 1975)

Pool size (M), and specific radioactivities (SA) of lipids of white adipose tissue (WAT), as well as the flow rates (m_s) of free fatty acids (FFA) from plasma into WAT were studied by injecting ^{14}C-1-palmitate ($20 \cdot 10^6$ cpm/100 g body weight) into 7-day-old rabbits reared in a thermoneutral (Group I) or a cold environment (Group II) or subjected to starvation at an ambient temperature (T_a) of 35° C (Group III) or at T_a 20° C (Group IV). Experiments were carried out at T_a 20° C in all four groups of rabbits.

The pool size of esterified and non-esterified fatty acids of WAT was reduced in both the well-fed animals raised in the cold and in the starving ones. The SA of tissue FFA and phospholipid fatty acid (PL-FA) was highest in Group III and Group IV indicating an increased FFA metabolism of WAT in animals subjected to starvation prior to acute exposure to cold.

The flow rate (m_s) of FFA between plasma and WAT increased twofold in Group IV but remained about one fifth that of the m_s between plasma and brown adipose tissue (BAT) indicating that the contribution of WAT to cold-induced calorigenesis of the whole animal must be regarded as of secondary importance.

White adipose tissue (WAT) is considered to represent an energy store (*Shapiro* 1965; *Renold* and *Cahill* 1965; *Jeanrenaud* et al. 1970) supplying fatty acids on demand and serving as an insulator preventing or reducing heat loss in a cold environment (*Brück* 1961; *Heim* 1971; *Hey* and *Katz* 1970; *Kajtár* et al. 1976; *Ryser* and *Jecquier* 1972; *Sulyok* et al. 1976).

Beside these functions the question has repeatedly been raised whether WAT also served like an electric blanket (*Ball* and *Jungas* 1961) taking part besides brown adipose tissue (*Aherne* and *Hull* 1966; *Dawkins* and *Scopes* 1965; *Heim* et al. 1966, 1967a, b, 1968; *Silvermann* et al. 1964) in cold-induced thermogenesis in the newborn rat, rabbit and especially in the human infant (*Hahn* et al. 1966; *Novák* and *Monhus* 1972, 1973; *Novák* et al. 1965/66, 1969, 1971, 1972a, b).

The purpose of the present study was to investigate the metabolic activity of white adipose tissue *in vivo* during acute and prolonged exposure to cold.

Methods

Newborn rabbits (n = 134) were divided into four experimental groups.

Group I (n = 42): well-fed rabbits, reared in the doe's nest (32—35° C). Since newborn animals huddle together in the nest made from straw and fur and are often covered by the sitting doe, their environmental temperature was found to be thermoneutral under these circumstances (*Bernard* and *Hull* 1964). The body weight of animals in Group I with stomach and bladder content was 120 ± 3.8 g (M ± S.E.) on the 7th day of life.

Group II (n = 21): well-fed rabbits, taken away from the mothers on day 4, kept individually in thermostatically controlled cages at an ambient temperature of 20 °C until the 7th day of life. Since rabbits normally suckle once daily, animals of this group were returned to the nursing doe to feed every morning. Each of them was weighed before and after the feed to measure the amount they had received. In 15—20 minutes they usually had suckled an amount of milk corresponding to 20—30% of their body weight. The body weight of rabbits with stomach and bladder content in Group II on the 4th day was 101 ± 2.9 g and on the 7th day, 133 ± 4.5 g.

Group III (n = 39): rabbits removed from the nest on day 4, and kept unfed in individual cages at a controlled environment of 35° C until day 7. Body weight with stomach and bladder content of animals in Group III was on the 4th day 98 ± 2.9 g and on the 7th day, 80 ± 2.1 g.

Group IV (n = 32): rabbits taken out of the nest on the 5th day of life and starved at an ambient temperature of 20° C until the 7th day of life. Body weight with stomach and bladder content of animals in Group IV was on the 5th day 102 ± 3.0 g and on the 7th day, 87 ± 2.6 g.

On the morning of day 7, one hour before the beginning of the tracer kinetic study the 30 animals the experimental results of which are reported in Tables II—V of this paper, were exposed to an environment of 20° C, thus thermoregulatory heat production was triggered and maintained throughout the surgical procedure and intravenous injection of the radioactive tracer. ^{14}C-1-palmitic acid was prepared as described previously (*Heim* et al. 1974) and injected ($20 \cdot 10^6$ cpm/100 g body weight) under light ether anaesthesia into the external jugular vein in 4—5 seconds. In another series of experiments the turnover and pool size of circulating FFA in 38 animals of the four groups were determined using a mathematical approach (*Heim* et al. 1974) published earlier (*Schenk* et al. 1975a).

Samples of 100—300 mg from inguinal white adipose tissue were excised, from the 20th minute up to the 80th minute after the tracer injection, immediately washed in physiological saline, frozen in liquid nitrogen and weighed. After homogenization and extraction (*Folch* et al. 1957) the lipids were separated by column (*Borgström* 1952; *Winkler* et al. 1972) and thin layer chromatography and subjected to quantitative and radioactivity measurements (*Schenk* et al. 1974) for glycerides (*Kaplan* and *Lee* 1965), phospholipids (*Fiske* and *SubbaRow* 1925) and free fatty acids (*Novák* 1965). Radioactivity was measured by a Packard Tri-carb liquid scintillation counter, Model 2001.

According to the estimations of *Dawkins* and *Hull* (1964) no cholesterol and a negligible amount of cholesterol esters was found in white adipose tissue of newborn rabbits. Therefore, the neutral fat fraction of both adipose tissues could be regarded as a homogeneous pool of mono-, di- and triglycerides.

After the experimental procedure the animals were sacrificed and their inguinal, subcutaneous, perirenal, and axillar white adipose tissue was dissected and weighed in order to estimate the pool size of white fat.

Finally, all experimental values, i.e. tissue weight, lipid concentration, lipid pool, specific radioactivity were standardized to 100 g body weight and to $20 \cdot 10^6$ cpm/100 g body weight of injected radioactivity.

Since the specific radioactivity of plasma free fatty acids and that of the esterified and non-esterified fatty acids in all three main lipid classes were in a steady state from 20 minutes up to 80 minutes after the tracer's injection, the minimal flow rates of circulating FFA into the lipid compartments of white adipose tissue could be determined by a digital computer program (*Koblet* 1971; *Sheppard* 1962; *Welch* et al. 1972) using a two-compartmental model (*Schenk* et al. 1974).

Student's *t*-test was applied for the statistical evaluation of the results.

Results

Body weight and the mass of white adipose tissue (Table I)

Body weight without stomach and bladder content of 7-day-old well-fed rabbits kept at thermoneutrality (35° C) was 109 ± 3.9 g (Group I in Table I).

In rabbits reared at T_a 20° C from the 4th to the 7th day of life practically the same growth rate was observed. Their body weight amounted to 114 ± 4.3 g on day 7 (Group II, Table I). Animals in the latter group, however, ate much more than those raised at T_a 35° C. This was reflected by the stomach content: Group I: 10.9 ± 3.9 g ($n=19$), and Group II: 19.6 ± 5.1 ($n=21$) ($p=0.10$), and body weight including stomach contents on the 7th day was 120 ± 3.8 in Group I and 133 ± 4.5 in Group II ($p < 0.05$).

The body weight of rabbits in Groups II, III and IV was practically identical at the beginning of cold exposure or starvation (see Methods).

Starvation at T_a 20° C for 48 hr resulted in a weight reduction of the same magnitude (Group IV in Table I) as starvation at T_a 35° C for 72 hr (Group III in Table I). Obviously the increased energy metabolism during cold exposure accelerated weight loss.

In another series of experiments ($n=6$) up to 80 minutes after ^{14}C-1-palmitate injection no radioactivity was found in the stomach contents. Therefore the latter was deleted from the reference body weight in all groups of experimental animals.

By the 7th day of life a greater amount of WAT was seen in well-fed animals reared at T_a 35° C (Group I in Table I), than in well-fed animals raised at T_a 20° C (Group II in Table I).

During starvation at T_a 35° C, WAT diminished (Group III in Table I) and an even more pronounced weight reduction was observed in animals starved at T_a 20° C (Group IV in Table I).

Concentration of lipids in WAT (Table II)

Triglycerides. The concentrations of gliceride fatty acids (G-FA) were the same in Groups I and II, reflecting that the relative participation of lipids was not materially affected by prolonged cold exposure of well-fed animals.

Starvation at T_a 35° C (Group III) or T_a 20° C (Group IV) somewhat reduced the TG content of WAT. The differences were, however, not significant statistically.

Phospholipid concentration in well-fed rabbits raised at T_a 35° C was 8.8 ± 1.01 μM/g wet tissue (Group I in Table II) and was not influenced by cold exposure (Group II in Table II) or starvation (Groups III and IV in Table II).

Free fatty acid concentration in well-fed animals raised at T_a 35° C or T_a 20° C was consistently between 2—3 μM/g tissue (Groups I and II in Table II) and did not increase significantly during starvation for 72 hr at T_a 35° C (Group III in Table II).

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Table I

Body weight without stomach and bladder content and mass of white adipose tissue of newborn rabbits between 4th and 7th day of life. Group I: well-fed rabbits, reared—in doe's nest (32—35 °C); Group II: well-fed rabbits, kept in individual cages at an ambient temperature of 20 °C for N2 hours; Group III: rabbits starved for 72 hours in a thermoneutral environment of 35 °C; Group IV: rabbits starved for 48 hours at 20 °C; n = number of animals (M ± S.E. of the mean)

Groups	I	II	III	IV
Body weight without stomach and bladder content on 7th day of life, g	(n = 42) 109 ± 3,9 *** (III, IV)	(n = 21) 114 ± 4.3 **** (III, IV)	(n = 39) 78 ± 2.0 **** (I, II)	(n = 32) 84 ± 2.4 **** (I, II)
Change in body weight during starvation (precent of original body weight)	–	–	20.3	17.6
White adipose tissue, mg/100 g body weight	(n = 30) 1954 ± 102 **** (II, III, IV)	(n = 18) 1094 ± 86 **** (I, IV)	(n = 20) 1079 ± 97 *** (IV) **** (I)	(n = 25) 750 ± 72 **** (I, II)

Inter-group differences: *** $p < 0.01$; **** $p < 0.001$

Table II

Concentration of lipids in white adipose tissue in micromole fatty acid per gram wet weight
Groups as in Table I
($M \pm S.E.$ of the mean)

Groups	I	II	III	IV
Number of animals	10	7	8	5
Free fatty acids (FFA), μM/g	2.40 ± 0.25 ** (IV)	2.95 ± 0.38 ** (IV)	3.09 ± 0.46 ** (II)	5.38 ± 1.08 ** (I, II)
Phospholipid fatty acids (PL-FA), μM/g	8.84 ± 1.01	10.90 ± 0.42 * (III)	9.04 ± 0.64 * (II)	11.28 ± 2.44
Glycerid fatty acids (G-FA), μM/g	1680 ± 61	1600 ± 131	1510 ± 82	1300 ± 187
Total amount of esterfied and non-esterfied fatty acids (M_F), μM/g	1691 ± 62	1614 ± 132	1522 ± 83	1617 ± 191

Inter-group differences: * $p < 0.05$; ** $p < 0.02$

Combination of starvation and cold-exposure (Group IV in Table II) resulted in a significant increment in tissue free fatty acids ($p < 0.05$) reflecting either an increased rate of lipolysis or a decreased utilization.

Pool size of esterified and non-esterified fatty acids in WAT (Table III)

Glyceride fatty acids (G-FA) amounted to 99% of the total (esterified and non-esterified) fatty acid pool.

G-FA pool of WAT was significantly ($p < 0.001$) reduced in well-fed animals raised at T_a 20° C (Group II in Table III) indicating either a diminished deposition or an increased lipolysis.

Similar results were found in starving animals at T_a 35° C (Group III in Table III).

Starvation combined with cold exposure (Group IV in Table III) reduced the glyceride FA pool drastically at T_a 35° C to less than one third of that of the well-fed littermates ($p < 0.001$) (Group I in Table III).

Phospholipid fatty acid (PL-FA) pool amounted to 0.5% of the total FA pool of WAT per 100 g body weight.

PL-FA pool of well-fed newborn rabbits kept at T_a 35° C (Group I in Table III) was 17.3 ± 1.9 μM/100 g body weight and exhibited a significant reduction during prolonged exposure to cold (Group II in Table III) and/or starvation (Groups III and IV in Table III).

Free fatty acid (FFA) pool is the smallest fraction of the total FA pool of the animal.

Alterations of the FFA pool in WAT did not exert any influence on the change in total fatty acid pool during cold exposure and/or starvation (Groups II, III and IV in Table III).

Specific radioactivities (Table IV)

Free fatty acids. Specific radioactivity (SA) was the largest in the FFA pool of WAT since—although quantitatively the smallest—it constitutes the metabolically most active fraction of the total FA pool.

During prolonged exposure to cold FFA SA increased twofold ($p < 0.01$) in WAT of well-fed newborn rabbits (Group II in Table IV).

After prolonged starvation at T_a 35° C or T_a 20° C, acute cold exposure resulted in a 6-fold and 12-fold increase of tissue FFA SA, respectively (Groups III and IV in Table IV).

Phospholipid FA. There was no significant difference in the SA of tissue PL of well-fed animals kept at T_a 35° C and at T_a 20° C (Groups I and II in Table IV).

Prolonged starvation either at T_a 35° C or at T_a 20° C resulted in a significant increase in SA of PL-FA (Groups III and IV in Table IV).

Table III

Pool size of lipids of white adipose tissue in micromole fatty acid per 100 g body weight (bwt), i.e. total amount of esterified and non-esterified fatty acids in the three main lipid classes of white adipose tissue. Groups as in Table I (M ± S.E. of the mean)

Groups	I	II	III	IV
Number of animals	10	7	8	5
Free fatty acids (FFA), μM/100 g b.w.	4.68 ± 0.48 ** (III) *** (II)	2.80 ± 0.36 *** (I)	3.33 ± 0.43 ** (I)	4.03 ± 0.81
Phospholipid farry acids (PL-FA), μM/100 g b.w.	17.3 ± 1.97 *** (II, III, IV)	10.35 ± 0.40 *** (I)	9.75 ± 0.68 *** (I)	8.45 ± 1.83 *** (I)
Glyceride fatty acids (G-FA), μM/100 g b.w.	3280 ± 119 **** (II, III, IV)	1520 ± 124 **** (I. IV)	1630 ± 89 **** (I, IV)	975 ± 140 **** (I, II, III)
Total amount of esterified and non-esterified fatty acids (m_F), μM/100 g b.w.	3302 ± 234 **** (II, III, IV)	1533 ± 167 * (IV) **** (I)	1643 ± 151 **** (I, IV)	987 ± 194 * (II) **** (I, III)

Inter-group differences: * $p < 0.05$; ** $p < 0.02$; *** $p < 0.01$; **** $p < 0.001$

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Table IV

Specific radioactivity of lipids in white adipose tissue (cpm per micromole fatty acid). Groups as in Table I (M ± S.E. of the mean)

Groups	I	II	III	IV
Number of animals	10	7	8	5
Free fatty acids (FFA), cpm/μM	1650 ± 278 * (II, IV)	4400 ± 914 * (I)	10,610 ± 2620 ** (II)	20,050 ± 8110 * (I)
Phospholipid fatty acids (PL-FA), cpm/μM	675 ± 96 * (IV) *** (III)	652 ± 75 * (IV) ** (III)	1,340 ± 120 ** (II) *** (I)	1,480 ± 272 * (I, II)
Glyceride fatty acids (G-FA), cpm/μM	63 ± 10	74 ± 19	77 ± 15	192 ± 102

Inter-group differences: * $p < 0.05$; ** $p < 0.002$; *** $p < 0.01$

Table V

Flow rate of free fatty acids into the lipid compartments of white adipose tissue; m_{SF} = absolute flow rate; $\frac{m_{SF}}{m_s} \cdot 100$ = relative flow rate of FFA, i.e. the percentage of turnover rate of circulating FFA (m_s) into white adipose tissue. Groups as in Table I (M ± S.E. of the mean)

Groups	I	II	III	IV
Number of animals	10	7	8	5
Flow rate of FFA from plasma into white adipose tissue (m_{SF}), μM/100 g body weight	0.059 ± 0.012 * (III) *** (II)	0.011 ± 0.003 * (III) ** (IV)	0.027 ± 0.007 * (I, II, IV)	0.131 ± 0.043 * (III) ** (II)
Absolute turnover rate of plasma FFA (m_s), μM/min/100 g body weight (*Schenk* et al. 1975a)	5.84 ± 0.77 **** (II, III)	2.26 ± 0.35 **** (I, IV)	2.14 ± 0.25 **** (I, IV)	5.02 ± 0.70 **** (II, III)
Relative flow rate of plasma FFA into white adipose tissue $\left(\frac{m_{SF}}{m_s} \cdot 100\right)$, per cent	1.01 ± 0.35	0.49 ± 0.24	1.27 ± 0.47	2.68 ± 1.27

Inter-group differences: * $p < 0.05$; ** $p < 0.02$; *** $p < 0.01$; **** $p < 0.001$

Glyceride fatty acids. Specific radioactivity of FA was the smallest in the glyceride pool of WAT containing, of course, by far the largest amounts of the three tissue lipid classes. Accordingly, even a considerable increase in the influx of labelled FA will cause only a minimum change in the SA of WAT. Therefore, a small and statistically not significant increase in the SA of G-FA might imply a substantial increase of FFA incorporation into the glyceride pool.

There was no significant difference in the specific radioactivities of G-FA pools in the four groups (Groups I—IV in Table IV).

Flow rates of plasma FFA into the lipids of WAT (Table V)

The flow rate of FFA from plasma into WAT (m_{SF}) of well-fed animals reared at T_a 35° C was 0.059 μM FFA/min which amounted to 1.01% of the plasma FFA turnover rate.

Incorporation of plasma FFA into WAT was the smallest ($p < 0.01$) in animals kept at T_a 20° C (Group II in Table V). It was even lower ($p < 0.05$) than that of the basal flow in animals starved for 72 hr at T_a 35° C (Group III in Table V).

In contrast to these groups (Groups I, II, III in Table V), it was after starvation for 48 hr at T_a 20° C (Group IV in Table V) that the highest influx of plasma FFA into WAT (0.131 ± 0.043 μM/min) was observed. It must be noted that the total FA pool was the smallest in this group of animals (Group IV in Table III).

The turnover of circulating FFA (m_s) was significantly diminished in well-fed animals reared at T_a 20° C as well as in those starved at T_a 35° C (Groups II and III in Table V). During starvation and cold exposure, however, the FFA turnover in plasma remained at the level of well-fed animals (Groups I and IV in Table V).

The participation of FFA flux into brown adipose tissue in the absolute turnover of circulating FFA ($m_{SF}/m_S \cdot 100$) was somewhat diminished in animals kept at T_a 20° C (Group II in Table V) and increased in rabbits starved and exposed to T_a 20° C (Group IV in Table V) but neither of the differences was significant statistically.

Discussion

Body weight and the mass of white adipose tissue

The growth rates of well-fed animals kept at T_a 35° C (Group I in Table I) or at T_a 20° C for days (Group II in Table I) were identical, although the amount of WAT was significantly smaller in the latter group.

Hardmann et al. (1970) found that the growth of the newborn rabbits reared for the first two weeks of life in a cold environment of 25° C was delayed, but at the age of one month they reached the body weight of littermates raised in a thermoneutral environment of 35° C. Since in our experiments the milk intake of animals kept at T_a 20° C was much greater than that of their littermates kept

at thermoneutrality (T_a 35° C) and the length of cold exposure (T_a 20° C) was much shorter than that applied by *Hardmann* et al. (1970) the equal growth rate in our Groups I and II is easily explained (see Methods).

Similar in the two sets of experiments was, however, the profound effect of prolonged cold exposure on the amount of WAT. Two weeks after the rabbits had been reared in a cold environment a significant reduction in WAT was found by *Hardmann* et al. (1970) similarly as observed by us after 3 days of exposure to cold.

The smaller mass of WAT in rabbits raised at T_a 20° C (Group II in Table I) might be the consequence of retarded proliferation of preadipocytes or of deposition of fat in them, since at the same time the concentration of lipids (Group II in Table II) did not differ in the WAT of the two groups of animals indicating that the existing lipid depots were not materially influenced by the length of cold exposure applied by us. The fact that the amount of WAT of 7-day-old rabbits kept at T_a 20° C for 3 days remained the same as in 4-day-old rabbits (*Heim* et al. unpublished) might indicate that prolonged exposure to cold from the 4th to the 7th day of life prevented the accumulation of lipids in WAT rather than increased their mobilization.

Since the gastric contents in Groups I and II were variable but contained no radioactivity, it was deemed advisable to use body weight without gastric contents for the standardization of the injected label, pool size and flow rates.

The diminution of the mass of WAT in starving animals at T_a 35° C (Group III in Table I) could be explained by an augmented mobilization of lipids.

Starvation with prolonged cold exposure (Group IV in Table I) further reduced the mass of WAT and resulted in a slight diminution of its glyceride concentration (Group IV in Table II). These results imply either that not only starvation but also exposure to cold enhances lipolysis in WAT (*Novák* and *Morkers* 1972, 1973; *Novák* et al. 1965/66, 1969, 1971, 1972a, b) or that the pronounced weight reduction of WAT might be due in part to a greater loss of tissue water.

Concentrations of esterified and non-esterified fatty acids of WAT and their pool size per 100 g body weight

The largest lipid pool of WAT is that of glycerides. Concentrations expressed as μM glyceride-fatty acid per g wet weight did not decrease significantly during starvation at either T_a 35° C or T_a 20° C (Groups III and IV in Table II). The most probable explanation might be that the greater loss of other tissue constituents, e.g. water, glycogen, masked the decrease in glycerides. The latter explanation is supported by the data on pool size (μM FA/100 g body weight) which showed a significant reduction in both the glyceride-FA and the total-FA pool during starvation at T_a 35° C and T_a 20° C (Groups III and IV in Table III).

This explanation might also be valid for the practically identical PL con-

centrations in the WAT of all four groups of animals (Groups I—IV in Table II). The changes in the pool size of PL-FA in Groups II—IV (Table III) might, however, imply that exposure to cold and/or starvation somewhat reduced the total amount of structural lipids.

The concentrations of tissue FFA in the rabbits of Groups I, II and III (Table II) were similar (2—3 μM/g tissue) as that found in WAT of the rat (*Angel* et al. 1971). The significantly higher tissue FFA concentration in Group IV (Table II) might indicate either an increased rate of lipolysis or a reduced utilization in animals starved and exposed to cold for 48 hr (Group IV in Table II).

It is interesting to note that *Angel* et al. (1971) found that noradrenaline increased the concentration of FFA in isolated white adipocytes. Since the triggering mechanism for cold-induced thermogenesis is the release of noradrenaline in brown adipose tissue (*Smith* and *Horwitz* 1969), application of noradrenaline *in vitro* might imitate the effect of cold exposure on white adipose tissue.

The phenomenon does not, however, seem to be reflected in the pool size of tissue FFA (Groups I—IV in Table III). The difference between the two results can be explained by the fact that *in vivo* the local increase of lipolysis and the FFA release in the adipose cell is immediately compensated or rather equilibrated by the parallel increase in utilization and/or elimination by the increased blood flow (*Bullard* and *Funkhauser* 1962; *Nielsen* et al. 1968; *Ballard* 1973) and cardiac output (*Dawes* and *Mestyán* 1963).

Specific radioactivities (SA) and flow rates (m_{SF})

The absolute values for SA in the various lipid classes of WAT (Table IV) differed according to their pool size and resulted in the highest SA in the FFA fraction followed by the PL-FA and G-FA. Thus a minor absolute increase in labelled FFA inflow considerably enhances the SA of FFA while the label is immensely diluted in the G-FA pool, and effects only a minor change in its SA.

The SA of tissue FFA reflected, however, very sensitively that during cold exposure and starvation not only lipolysis (*Angel* 1968; *Angel* et al. 1971; *Novák* and *Monkus* 1972; *Novák* et al. 1965/66, 1971, 1972a, b) but the exchange rate between plasma and tissue FFA was also enhanced.

Flow rates of plasma FFA into the lipid classes of WAT provide a more quantitative account of the incorporation of plasma FFA into adipose tissue lipids at a steady state (*Heim* et al. 1974; *Schenk* et al. 1974; 1975a).

In our experiments the greatly diminished flow rate of plasma FFA in well-fed animals kept at T_a 20° C (Group II in Table V) might have been the consequence of increased lipolysis.

Zierler et al. (1965) observed in an *in vitro* system that FFA influx into WAT is probably a passive process, slowed down by starvation and low glucose levels.

In our experiments *in vivo*, the flow rates of circulating FFA into WAT lipids

of animals starved for 72 hr at 35° C were less than 50% of those in well-fed littermates.

Based on earlier observations (*Heim* et al. 1970), animals starved for 72 hr at T_a 35° C (Group III) might be assumed to have low blood glucose and liver glycogen levels, consequently one of the most important substrates for triglyceride synthesis in WAT, namely alpha-glycerophosphate, may have been diminished during starvation. Low levels of the latter were found by *Ballard* (1972) in WAT of starving adult rats, in agreement with the above concept. Thus substantial evidence is available to support the assumption that the reduced FFA influx into WAT in Group III (Table V) was the consequence of hypoglycaemia and impaired production of alpha-glycerophosphate in white adipose tissue.

It is tempting to regard the FFA flux into any organ, especially into adipose tissue, as an indicator of its general metabolic activity. This assumption seems to be particularly valid for the newly born, since little FFA synthesis from glucose, acetate and other precursors was found in the weaned newborn rat (*Hahn* et al. 1965/66; *Hahn* 1970). Therefore, any increase in tissue FFA or its incorporation into triglycerides originates at this age from the uptake of plasma FFA.

The hypothesis that a slower rate of FFA flux into WAT might be an indicator of decreased cellular metabolism in general is further supported by the findings of *Braun* et al. (1966), who showed that starvation greatly reduced the ribonucleic acid concentration in WAT of the rat, indicating diminished protein glycogen and fat synthesis as well as a depressed metabolism.

During starvation in a cold environment (Group IV in Table V) inflow of plasma FFA increased significantly (see Groups III and IV in Table V) beside increased lipolysis and an increase in the uptake of plasma FFA, i.e. the exchange rate of FFA between plasma and WAT increased.

The present findings indicate that the depressive effect of starvation (Group III in Table V) on FFA influx into WAT can be prevented or abolished by prolonged exposure to cold (Group IV in Table V) probably through its catecholamine and thyroxine mobilizing effect (*Leblanc* et al. 1961; *Leblanc* 1971).

Comparison between the flow rates of plasma FFA into WAT and BAT (*Schenk* et al. 1975b) indicates, however, the existence of an essential difference between the two adipose tissues in respect of the metabolic response to cold exposure. In well-fed newborn rabbits during acute exposure to cold the FFA flux between plasma and BAT was 0.163 ± 0.041 μM/min (*Schenk* et al. 1975b), i.e. considerably more than between plasma and WAT which was 0.011 ± 0.003 μM/min (Table V, Group II).

Forty-eight-hour starvation at T_a 20° C resulted in a substantial increase in FFA flux into both white and brown adipose tissue but the difference still remained fourfold as inflow of FFA into WAT was 0.131 ± 0.043 μM/min (Table V, Group IV) and into BAT 0.515 ± 0.109 μM/min (*Schenk* et al. 1975b). The participation of WAT in the overall turnover of circulating FFA was in this group 2.68 ± 1.27%

(Table V, Group IV), while that of BAT was $10.5 \pm 3.7\%$ (*Schenk* et al. 1975b). These data reflect that, at least in the newborn rabbit, either acute or prolonged cold exposure exerts a much more pronounced influence on FFA metabolism of BAT than on that of WAT. Moreover our tracer kinetic studies revealed that the participation of the same amount of WAT in the metabolism of circulating FFA is about one fifth that of BAT. Therefore the contribution of WAT to cold-induced calorigenesis of the whole animal cannot be denied categorically but must be assumed to play a secondary role compared to that of BAT. Data comparing metabolic parameters of WAT and BAT and assessing their responses to thermoregulatory stimuli in the human neonate would be of great interest.

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ADAPTIVE PROCESSES IN CHILD AND ADOLESCENT SWIMMERS

BLOOD COAGULATION AND VISCOSITY

By

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Multifactorial studies were performed in child and adolescent swimmers in the early period of training and in a submaximum state of training in the year's racing period. Blood coagulation, viscosity, acidity, protein metabolism, muscle enzymes, ions, haematologic, cardiac and endocrine parameters have been investigated.

In the early period of training, physical exercise resulted in fibrinolysis, and a decrease in the fibrinogen and prothrombin levels and the platelet count. The same exercise in fully-trained organisms failed to produce such changes.

Data concerning acidity, protein metabolism, muscle enzymes, ions, haematologic, cardiac and endocrine parameters will be published in subsequent papers.

Training must be begun very early to achieve outstanding successes in sports. Thus adaptation of children and adolescents to increasing requirements has become an important problem. Some pertaining questions to be answered in the near future are as follows:

— is an extreme physical exercise harmful, indifferent or advantageous for the organism of children and adolescents;

— what is the maximum of physical exercise tolerated without irreversible damage;

— is such an extreme physical exercise indeed necessary to achieve outstanding results;

— is there any kind of adaptation on capacity in the young organism that would not be present in adults?

It has been attempted to clarify these questions on the basis of multifactorial studies performed on swimmers. Swimmers have been chosen, swimming being the most physiological basic sport. It results in an increased circulatory and breathing capacity instead of causing an athletoid hypertrophy of the musculature and so its training may be started in childhood.

The changes of blood coagulation following psychical and physical exertion have extensively been studied (*Bingham* and *Roepke* 1945; *Biggs* et al. 1947; *Sherry* et al. 1959; *Ogston* et al. 1962; *Menon* et al. 1967; *Winckelman* et al. 1968; *Whiddon* et al. 1969; *Maxley* et al. 1970; *Paul* et al. 1971; *Poller* et al. 1971; *Metze* et al. 1973; *Morley* and *Linnerad* 1973; *Shapiro* et al. 1973; *Karp* and *Bell* 1974).

These reports described an increase in plasma fibrinolytic activity following mental and physical exercise and some of the results (*Whiddon* et al. 1969) support *Cannon*'s finding of enhanced clotting in emergency situations. Physical exercise, however, causes an emergency reaction only in the untrained organism.

Controversial reports have been published concerning coagulation parameters (platelet count, recalcination time, prothrombin level, thrombin time, platelet aggregation, prothrombin consumption, etc.) after physical exercise. A common finding of these reports is an increased fibrinolytic activity following physical exercise. The mechanism of increase is not fully understood, since there are no data which would mention a significant decrease in the blood fibrinogen level. In healthy subjects the blood fibrinogen content is stable; its half-life was found between 4.5 and 6.4 days (*Zetterquist* 1969).

Blood corpuscles as well as plasma proteins, mainly fibrinogen, are involved in the regulation of blood viscosity (*Bingham* and *Roepke* 1945; *Rosenblatt* et al. 1965; *Begg* and *Hearns* 1966; *Ditzel* and *Kampmann* 1971). There are several problems in the evaluation of blood viscosity (*Copley* 1968; *Chisolm* and *Gainer* 1971), but it has been accepted that a decrease in blood pH results in increased viscosity (*Hardaway* 1968), and it is well known that physical exercise induces acidosis.

The controversy in the literature concerning the changes of coagulation following physical exercise might be due to the differences in age of the experimental subjects or to the different phase and duration of training. The present findings may offer some data to clarify these problems and it may prove a useful model for the prolonged observation of adaptive processes.

Methods

The experimental subjects were nine healthy volunteer swimmers of both sexes of a sports club. Their main data are shown in Table I.

The subjects had a standard protein-rich diet of more than 3000 cal/day during the whole training period.

Two experimental series were performed, the first in the early, preparing period of the training season and the tests were repeated in the fully-trained state of the subjects in the racing period. The second investigation was done at least one and half months after the first one. Both series included determinations before and after the daily training.

The subjects visited the laboratory before the training, where blood was withdrawn. The blood samples were obtained from the cubital vein and stored in ice in native form or with citrate, oxalate, EDTA or heparin as anticoagulant.

After this procedure the subjects had their usual daily afternoon training (5000 m) in a swimming pool near the laboratory. Subsequently, the same investigations were repeated and the swimmers had milk to drink.

Determinations were done within 5 hours after sampling, by routine methods as described by *Bauer* et al. (1968), *Richterich* (1969) and *Pálos* and *Sas* (1973).

The laboratory tests included peripheral blood smear, platelet count (in procaine solution under phase-contrast), fibrinogen (biuret reaction), prothrombin level, prothrombin consumption, euglobulin lysis time, recalcination time, thrombin time (with Topostasin), fibrin degradation products (with Sta-Fi test, Galenopharm), viscosity [in heparinized blood and plasma with Oswald's viscosimeter, calculated by comparison of the flow-out time (sec) measured always with the same tube, in a 37° C water bath], serum proteins (total) and alkaline phosphatase.

The corresponding normal values for coagulation parameters in our laboratory are as follows:

platelet count:	150,000—250,000
prothrombin level:	90—110%
fibrinogen level:	300—500 mg/100 ml
euglobulin lysis time:	180—240 min
thrombin time:	15—25 s
prothrombin consumption:	30—50 s
recalcination time:	120—180 s

Results and discussion

Since our results were obtained in subjects of both sexes, with a different training scale (Table I), evaluation of the data will be individual rather than statistical. Subjects Nos 1, 2, 4 and 6 formed a comparatively homogeneous group according to the opinion of the trainers and investigators. Subjects Nos 3, 5 and 8 were different from the previous group, and subjects Nos 7 and 9 had an intermediate position.

The data of blood clotting and of viscosity measured prior to and after the training in the early period of training procedure are summarized in Tables II and III. Increased fibrinolysis and prothrombin consumption, decreased levels of fibrinogen, prothrombin and a low platelet count were measured after training.

The increased thrombin time in subjects Nos 1, 6 and 7, measured in the posttraining period, indicates the presence in blood of heparin-like substances, since this change had normalized on the addition of toluidine blue to the samples and no fibrin degradation products (Sta-Fi test) could be shown in these blood samples either prior to or after the training.

There was a marked change in the platelet distribution on the blood smears. Prior to the training they were aggregated in clumps, while after training they were dispersed with the single platelets situated far from each other.

Blood viscosity decreased after the training in each case (in some cases the volume of blood samples was not enough to perform this measurement).

Data of the same subjects before and after training in the fully-trained state are summarized in Tables IV and V. The exertion was the same as one and a half months earlier, but the changes in blood clotting and in viscosity were different from those measured previously. Only the consequently decreased prothrombin level and the increased prothrombin consumption had persisted. Increased lytic activity was found only in three subjects and a slight decrease in fibrinogen content in two cases, while in the others the fibrinogen content rose after training. The platelet count was higher before the training than in the first investigation and it increased further after the training in correlation with the increased output of blood pools (*Grok* et al. 1974). In three subjects a decrease of the platelet count could, however, be observed after training in this second investigation too. Dispersion of the platelets after training was seen similarly as in the first investigation. In

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Table I

Data of the Experimental Subjects

No.	Age (years)	Sex	Serum alkaline phosphatase[a] (IU)	Body weight (kg)	Body height (cm)	Menstruation	Regular training[b] (month)	Subjective[c] training scale (1—10)	Best swimming results
1.	10	female	150	38	150	–	30	9	800 m sp: 11′ 40″ 200 m br: 3′ 16″
2.	14	male	106	58	168	–	30	10	200 m ba: 2′ 32″ 1500 m sp: 19′ 30″
3.	15	female	99	61	176	+	30	3	100 m br: 1′ 25″
4.	16	male	78	68	184	–	30	10	100 m sp: 1′ 02″ 400 m sp: 4′ 50″
5.	17	female	98	60	176	+	30	4	100 m sp: 1′ 09″ 400 m sp: 5′ 25″
6.	11	female	65	54	164	+	30	9	200 m ba: 2′ 42″ 100 m bu: 1′ 15″
7.	15	female	62	53	162	+	30	6	100 m br: 1′ 23″ 200 m br: 2′ 58″
8.	17	male	52	70	166	–	30	3	100 m br: 1′ 17″
9.	18	male	45	69	184	–	30	6	100 m sp: 59′ 5″

[a]: alkaline phosphatase as an indicator of biological age (normal adults level in our laboratory: 20—50 IU)

[b]: regular training: two trainings daily for 30 months preceding the investigation

[c]: subjective training scale: arbitrary scale based on the trainer's opinion. It includes individual diligence of the subject, absence from training due to illness.

Abbreviations: sp: sprint; br: breast-stroke; ba: back-stroke; bu: butterfly-stroke

Table II

Effect of Training on Clotting Parameters in Early Training Period

No.	Platelet count (1000/μl)			Prothrombin level %			Euglobulin lysis time (min)			Recalcination time (sec)			Thrombin time (sec)			Prothrombin consumption (sec)		
	b	a	%	b	a	%	b	a	%	b	a	%	b	a	%	b	a	%
1.	100	60	−40	78	51	−35	90	70	−23	179	166	− 8	17	300 t	−	47	60	+28
2.	90	100	+11	70	63	−10	100	95	− 5	141	120	−15	19	17.2	−10	54	75	+38
3.	100	90	−10	77	68	−12	290	108	−46	160	137	−15	19.4	14.8	−24	39	12	−70
4.	90	100	+11	86	71	−18	190	136	−29	118	137	+16	21	16.6	−21	51	68	+34
5.	80	150	+87	84	84	−	150	87	−42	161	166	+ 3	18.5	15	−19	36	58	+61
6.	90	100	+11	86	41	−53	190	136	−29	165	285	+72	18	120 t	−	29	32	+10
7.	90	110	+22	92	74	−20	105	87	−18	117	138	+18	18	32	+77	89	119	+33
8.	150	110	−27	80	80	−	240	240	−	100	100	−	18.3	19	+ 3	43	94	+118
9.	140	80	−43	91	71	−22	240	108	−55	120	157	+30	19	15.8	−17	30	78	+160

b: before training

a: after training

%: percentual difference in pre- and post-training values

−: no difference

Table III

Effect of Training on Haematocrit, Serum Protein and Fibrinogen Levels and on Viscosity in Early Training Period

No.	Haematocrit (%)			Serum protein level (total) (%)			Fibrinogen level (mg/100 ml)			Viscosity (flow-out line) (sec)					
										plasma			blood		
	b	a	%	b	a	%	b	a	%	b	a	%	b	a	%
1.	36	36	–	7.32	7.22	−2	398	282	−30	140	80	−43	190	190	–
2.	40	40	–	7.4	6.75	−9	409	316	−23	83	74	−11	No	No	–
3.	38	40	+5	7.7	7.9	+2	559	400	−29	No	No	–	No	No	–
4.	40	39	–	7.7	7.51	−3	726	407	−44	No	No	–	No	No	–
5.	40	38	−5	7.41	7.13	−4	466	376	−20	78	75	− 4	180	154	−15
6.	36	36	–	7.32	7.32	–	568	391	−32	No	No	–	No	No	–
7.	40	39	–	7.1	7.32	+3	506	372	−27	85	81	− 5	217	200	− 8
8.	50	46	−8	8.1	7.6	−7	495	539	+ 8	No	No	–	261	218	−17
9.	42	40	−5	7.2	7.22	–	495	388	−22	No	No	–	No	No	–

No: no determination
Abbreviations: as in Table II

Table IV

Effect of Training on Clotting Parameters in Fully-Trained Subjects

No.	Platelet count (1000/μl)			Prothrombin level (%)			Euglobulin lysis time (min)			Recalcination time (sec)			Thrombin time (sec)			Prothrombin consumption (sec)		
	b	a	%	b	a	%	b	a	%	b	a	%	b	a	%	b	a	%
1.	200	330	+ 65	100	100	0	95	120	+26	152	105	−31	15.1	18	+19	45	45	0
2.	180	220	+ 22	92	76	−18	240	120	−50	184	141	−24	16	16	0	49	62	+26
3.	190	160	− 16	92	61	−34	95	150	+57	173	149	−14	19.5	19	− 3	38	35	− 8
4.	140	110	− 22	92	70	−24	95	120	+26	137	155	+13	16.6	19	+14	45	50	+11
5.	160	310	+ 93	100	79	−21	125	150	+20	183	198	+ 8	17.5	19	+ 8	47	38	−20
6.	130	120	− 8	100	80	−20	240	170	−30	184	152	−18	16	19.2	+22	29	30	+ 3
7.	170	110	− 36	88	62	−30	240	105	−77	137	152	+10	18.8	19.6	+ 4	58	87	+50
8.	140	210	+ 50	61	63	+ 3	95	150	+57	215	164	−24	90	18.8	−80	33	57	+72
9.	120	240	+100	92	72	−22	95	150	+37	153	152	− 1	16.2	19	+17	37	30	−19

Abbreviations: see in Table II

Table V

Effect of Training on Haematocrit, Serum Protein and Fibrinogen Levels and on Viscosity in Fully-Trained Subjects

No.	Haematocrit (%)			Serum protein level (total) (%)			Fibrinogen level (mg/100 ml)			Viscosity (flow-out line) (sec)					
										plasma			blood		
	b	a	%	b	a	%	b	a	%	b	a	%	b	a	%
1.	36	40	+11	6.9	7.25	+5	392	506	+29	117	122	+ 4	226	253	+11
2.	41	40	–	7,05	6,85	−3	380	440	+15	83	92	+10	No	No	–
3.	40	40	–	7.7	7.55	−2	400	561	+40	123	103	+ 3	290	260	−11
4.	42	42	–	7.6	8.0	+5	396	356	−11	121	132	+ 9	245	286	+16
5.	36	38	+5	7.05	7.0	–	409	528	+29	117	120	+ 2	235	252	+ 7
6.	42	39	− 8	7.0	7.1	+1	354	535	+51	85	86	+ 1	No	No	–
7.	40	41	–	7.32	7.32	–	464	422	−10	87	89	+ 2	No	No	–
8.	46	46	–	7.6	7.45	−2	273	464	+69	120	132	+10	275	314	+14
9.	44	43	–	7.4	7.0	−6	383	444	+15	118	118	–	242	272	+12

Abbreviations: see in Table II

subject No. 1, lengthened thrombin time was measured prior to the training and it normalized on the addition of toluidine blue to the sample. No fibrin degradation products were present, as investigated with Sta-Fi test.

Since there is ample evidence of interrelationships between the clotting system, pH and viscosity of the blood, detailed evaluation of the data will be given in forthcoming papers in correlation with the acid—base balance.

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INVESTIGATION OF ADAPTIVE PROCESSES IN CHILD AND ADOLESCENT SWIMMERS

ACID–BASE PARAMETERS OF SWIMMERS AND WEIGHT-LIFTERS

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Acid–base parameters of adolescent swimmers (capacitive sport) and of adult weight-lifters (athletoid sport) were investigated before and after training in the submaximally trained phase of a year-round training period.

In swimmers, training induced incompensated metabolic acidosis, which persisted at 10 min after the training. This acidosis showed no correlation to the blood lactate level.

In weight-lifters, there was only a slight, compensated posttraining acidosis, which tended to decrease 10 min after the training. The weight-lifters had extremely high posttraining ammonia levels. It might be supposed that ammonia-genesis has a role in the compensation of exercise-induced acidosis in weight-lifters.

The differences in acid–base status of swimmers and weight-lifters might be related to the different breathing mechanism needed for the two different sports.

In response to heavy exercise (*Coester* et al. 1973; *Gyódi* 1973; *Jung* 1975; *Torma* 1975) the acid–base equilibrium was shown to shift toward acid. This kind of acidosis is considered metabolic in origin, due to accumulated acid metabolites (*Osnes* and *Hermansen* 1972; *Johnson* 1973; *Jung* 1975).

Intracellular acidosis and the lactate level in muscles have a particular role in exhaustion (*Karlsson* and *Saltin* 1970; *Hermansen* and *Osnes* 1972; *Torma* 1975; *Karlsson* et al. 1975).

The question arose whether there is some correlation between sport exercise and acidosis. Since swimming and Marathon racing are capacitive sports, whereas weight-lifting induces a hypertrophy of the musculature (*Apor* 1971; *Stobey* 1972), it seemed interesting to compare the data of swimmers and weight-lifters. In order to study their acid–base changes before and after training, they were analysed in submaximally trained subjects in the second phase of a year-round training period.

Methods

The experimental subjects were 10 healthy volunteer swimmers and 9 healthy volunteer weight-lifters of a sports club in Pécs. Data for weight-lifters are summarized in Table I, while those for swimmers were published earlier (*Fendler* et al. 1977), except for swimmer No. 10. This subject was a 15-year-old girl of 68 kg body weight and 178 cm height having regular bleedings. She has been participating in training twice daily for 4 years. Her training scale was 7, the best swimming performance in 100 m back-stroke, 1 min 13.4 sec, 200 m back stroke, 2 min 36.8 sec.

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Table I

Characteristic data of weight-lifters

No.	Age	Alkaline phosphatase (IU)	Body weight (kg)	Body height (cm)	Regular training (month)	Subjective* training scale	Best results (kg)
1.	16	85	58.9	156	24	6	175
2.	15	46	65.2	163	14	9	180
3.	17	49	65.8	163	12	7	160
4.	16	116	52.0	157	12	6	132.5
5.	17	93	106.5	184	15	8	175
6.	20	40	62.5	158	24	4	200
7.	18	82	64.7	162	48	9	215
8.	26	20	62.7	162	96	7	207.5
9.	22	43	87.5	179	24	5	230

* Subjective training scale: arbitrary scale based on the trainer's opinion. It includes individual diligence of the subject, absence from training due to illness

The investigations were performed in the submaximal state of training, in the second part of a year-round training period. Pretraining blood and urine samples were obtained in the late afternoon, the posttraining samples were collected immediately and 10 min after the training (for details see *Fendler* et al. 1977).

Acid–base parameters were determined by means of an Astrup micro pH meter (Radiometer, Copenhagen) according to the Siggaard–Andersen equations.

Normal:	blood pH:	7.37—7.44
	base excess (BE):	±1.5 mEq/l
	actual bicarbonate:	20—24 mEq/l
	pCO_2:	30—40 mm Hg.

Blood and urine ammonia levels were determined with the Berthelot-reaction (Hyland kit) after ion-exchange separation.

Normal: blood ammonia: 20—50 μg/100 ml.

Blood lactate level was estimated by colorimetry after deproteinization and removal of carbohydrates.

Normal: 6—20 mg/100 ml.

Urinary acidity measured according to Folin, normal value: 1—20 mEq/24 hr. The 24-hr urinary levels of acidity and ammonia did not give a real basis for comparison since the determinations were made in urine samples collected from a single micturition and not in 24-hr pooled samples.

Results

The acid–base parameters of swimmers are summarized in Table II. In some subjects (Nos 1, 5, 8, 9) a slight pretraining metabolic acidosis could be measured, which may have reflected the effect of the previous training in the same morning. Immediately after training almost every subject had incompensated metabolic acidosis; it improved somewhat but was still incompensated 10 min later. An increase in blood lactate occurred only in the posttraining blood samples of subjects 2 and 10.

In posttraining blood ammonia contents there were no characteristic changes. Subjects 5 and 9 had, however, markedly elevated pretraining blood ammonia levels, which might have been correlated with the compensated pretraining metabolic acidosis, most expressed in the two swimmers Nos 2 and 10. Their posttraining urinary acidity and urinary ammonia values showed the highest increase compared to the pretraining levels.

The acid–base data for weight-lifters are summarized in Table III. A mild, compensated pretraining acidosis could be detected in subjects 3, 4 and 5. Immediately after training, most subjects showed a slight compensated metabolic acidosis disappearing 10 min after the training. Very high lactate and ammonia levels were found in the posttraining samples. Their range ought to be characterized clinically as toxic. There was a similarly high posttraining increase in urinary acidity and ammonia level in most of the weight-lifters.

Table II

Acid-base parameters in swimmers

No.	Blood							Urine			
	pH BE (mEq/l) Actual bicarbonate (mEq/l) pCO_2 (mm Hg)			Lactate (mg/100 ml)		Ammonia (μg/100 ml)		Titratable acidity (mEq)		Ammonia (mg)	
	1	2	3	1	2	1	2	1	2	1	2
1.	.43 − 3.9 28.0 42.5	7.31 −10.8 14.2 29.0	7.23 −10.8 16.4 40.3	7.5	15.6	53	39	4.95	2.7	49.5	11.7
2.	7.36 − 0.5 24.2 43.0	7.31 −13.0 11.4 23.0	7.31 − 8.2 16.9 34.2	14.2	32.2	64	69	5.4	4.9	56.0	39.8
3.	7.4 − 2.0 22.0 36.0	7.26 − 9.3 17.2 39.5	7.24 − 9.8 17.1 41.3	6.1	9.6	51	24	1.6	2.7	4.9	11.4
4.	7.41 + 2.5 26.6 43.0	7.25 −10.4 15.6 36.9	7.24 − 9.1 17.1 41.8	9.8	11.0	49	39	14.5	6.5	123.0	40.5
5.	7.38 − 4.8 18.9 32.8	7.23 −11.1 14.9 37.0	7.22 −12.0 14.7 37.2	12.4	11.1	93	83	3.1	8.6	24.6	41.3

6.	7.39 − 2.0 22.4 37.5	7.27 − 7.0 19.5 44.0	7.33 − 6.0 18.5 37.0	19.0	9.2	56	51	3.15	4.8	14.2	32.7
7.	7.4 − 2.0 22.0 36.0	7.27 −12.6 12.9 29.0	7.32 − 8.0 16.8 33.0	7.0	−	50	46	7.5	7.5	24.8	27.2
8.	7.4 − 3.4 20.3 33.4	7.25 − 9.7 17.1 40.8	7.26 − 8.1 17.6 41.0	12.8	13.3	36	44	2.4	4.0	60.2	73.5
9.	7.38 − 4.0 20.0 34.4	7.24 − 8.5 17.7 43.5	7.25 − 6.8 20.2 48.0	14.4	11.4	85	88	1.1	5.5	12.8	33.1
10.	7.44 + 0.5 23.5 35.5	7.33 − 0.5 19.8 39.0	7.31 − 7.0 18.5 38	5.6	29.5	48	23	2.3	1.7	11.2	6.4

1: before training
2: immediately after training
3: 10 min after training
−: no data

Table III

Acid-base parameters in weight-lifters

No.	Blood							Urine			
	pH BE (mEq/l) Actual bicarbonate (mEq/l) pCO_2 (mm Hg)			Lactate (mg/100 ml)		Ammonia (μ/100 ml)		Titratable acidity (mEq)		Ammonia (mg)	
	1	2	3	1	2	1	2	1	2	1	2
1.	7.36 − 0.5 24.5 44.5	7.33 − 7.2 17.5 33.8	7.41 − 1.0 23.0 37.0	8.9	22.7	40	120	6.3	9.8	74.0	102.6
2.	7.37 0.0 25.3 45.0	7.38 − 5.0 18.9 32.7	7.41 − 3.0 21.0 34.0	13.8	23.3	79	131	5.5	5.3	57.6	38.0
3.	7.36 − 4.5 19.5 35.5	7.36 − 6.0 17.8 32.0	7.38 − 2.8 21.5 37.0	7.3	24.2	54	102	3.8	7.8	41.6	53.6
4.	7.41 − 2.8 19.0 27.0	7.39 − 3.5 20.0 33.5	7.39 − 3.8 20.0 33.5	11.1	16.0	23	84	4.8	4.8	41.2	60.9

5.	7.36 − 3.8 20.5 37.5	7.34 − 8.5 16.5 31.0	7.4 − 6.5 17.0 27.5	9.6	23.6	.45	89	3.5	7.0	37.5	41.0
6.	7.37 0.0 24.6 42.0	7.39 − 4.2 18.8 32.8	7.38 − 3.9 20.2 34.8	10.4	18.8	34	260	3.4	4.9	85.5	70.0
7.	7.42 + 0.5 24.7 39.0	7.45 − 2.0 20.2 29.0	7.45 − 2.1 19.9 29.0	15.1	36.8	40	135	3.8	16.2	46.0	127.5
8.	7.4 − 0.5 23.7 39.0	7.42 − 4.0 19.0 30.0	7.45 − 3.1 19.1 28.0	13.2	31.0	48	240	6.8	8.5	32.0	85.1
9.	7.39 − 0.5 23.9 40.0	7.34 − 5.0 19.8 37.5	7.35 − 1.9 23.0 43.5	12.7	41.4	98	104	1.7	7.7	19.8	112.8

1: before training
2: immediately after training
3: 10 min after training

Discussion

Physical exercise is well known to result in an increase of intracellular and blood lactate levels (*Taylor* and *Rao* 1973; *Torma* 1975). Their interpretation is controversial. *Jung* (1975) and *Torma* (1975) supposed that these were in causal relation to the metabolic acidosis observed after physical exercise. *Osnes* and *Hermansen* (1972) found after maximum exercise a decrease in blood and muscle pH which could not be explained by the increased lactate level alone. *Karlsson* and *Saltin* (1971), *Karlsson* (1971) and *Karlsson* et al. (1975) found that lactate concentration in the working muscles was increased by intermittent exercise and lowered by prolonged exhaustive exercise.

The same authors suggested that the cause of muscular exhaustion was either the increased lactate level or the lactate-induced acidosis (*Karlsson* and *Saltin* 1970), but other investigators denied both possibilities (*Hermansen* and *Osnes* 1972; *Taylor* and *Rao* 1973; *Dunn* and *Critz* 1975).

In the swimmers in our experiments there was no correlation between the blood lactate level and the posttraining acidosis. One cannot exclude, however, that blood lactate level had been high in response to the exhaustive training, but decreased to the normal range during the last 200 m of swimming. This final period was namely one of relaxed swimming, when the time was not measured and the subject could and had to relax. This presumed lactate elimination during the final period of training might have interfered with the normalization of intracellular acidosis, thus an incompensated metabolic acidosis was measured in the posttraining samples.

The same mechanism of lactate elimination may not have been present in the weight-lifters, otherwise the mild, compensated acidosis measured immediately after training ought to have been worse 10 min later.

It might also be supposed that the difference in metabolic acidosis in swimmers and weight-lifters was due to the different breathing mechanisms. Swimming and weight-lifting need entirely different breathing techniques and breathing has a primary role in the elimination of metabolic acidosis.

It is of particular interest that the blood ammonia level was elevated in the posttraining samples of weight-lifters. *Laborit* et al. (1958) observed that physical exercise resulted in increased blood ammonia concentration in rats, and this increase could be antagonized by aspartate plus glutamate treatment. To explain the phenomenon, the questionable theory was offered that the increase in ammonia level under such circumstances is due to the impaired detoxifying function of the liver.

The blood ammonia level may change rapidly, as a conseqence of the rapid renal equilibration (*Van Slyke* et al. 1943; *Hempling* 1971). Thus, ammonia may be involved in various short-term regulatory functions. Ammonia formation has a role in the elimination of acids (*Steiner* et al. 1968; *Gyódi* 1973) and its intravenous infusion produces hyperglycaemia and lipolysis in sheep (*Wiechetek* et al. 1975).

Kazemi et al. (1973), on the other hand, supposed that ammonia might influence the buffer capacity of the brain.

On the basis of these data one might think of a regulatory role of ammonia formation in the acid–base status and intermediate metabolism of weight-lifters. A similar but much slighter increase in the posttraining ammonia level was observed in swimmers in an earlier phase of the year-round training period (unpublished observation).

It has to be clarified whether the posttraining elevation of the blood ammonia level regulates the acid—base status by eliminating H ions, or causes a cellular disturbance which in turn results in early exhaustion of the organism. One should consider both possibilities when administering drugs which are known to inhibit the formation of ammonia, like aspartate and glutamate.

In conclusion, investigation of the whole acid–base status—and not only of the blood ammonia level or of urinary ammonia excretion—seems necessary for characterizing the posttraining metabolic changes in sportsmen.

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EFFECT OF STRESS ON ACTIVITY OF THE SEROTONINERGIC SYSTEM IN LIMBIC BRAIN STRUCTURES AND ITS CORRELATION WITH PITUITARY–ADRENAL FUNCTION IN THE RAT

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Fifteen min after ether stress, the 5-hydroxytryptamine (5-HT) content of the rat hypothalamus decreased, while after electric shock no decline was noted in the mesencephalon and the hypothalamus. At 30 min an increase occurred in the mesencephalon and amygdala and at 60 min in the hypothalamus after both stressors. Fifteen min after electric shock the 5-hydroxyindoleacetic acid (5-HIAA) content was increased in the amygdala, while after both stressors in the mesencephalon and hypothalamus 30 min and after ether stress in the amygdala. In the hippocampus and the septum the 5-HT or 5-HIAA content was unaffected by either type of stress. 5-HT turnover decreased between 10 and 20 min after ether stress in the hypothalamus and after electric shock in the mesencephalon and hypothalamus. Between 50 and 60 min after electric shock, 5-HT turnover increased in the mesencephalon. Other brain areas showed no significant difference from the control 5-HT value. ^{3}H-5-HT uptake decreased in the mesencephalon and hypothalamus 20 min after ether stress, but returned to normal after 60 min. The plasma corticosterone level was highest at 30 min after both stressors and returned to normal 90 min after stress. Therefore, the stress-induced decrease of serotoninergic activity in certain limbic brain structures, especially in the hypothalamus, suggests an inverse relationship with pituitary–adrenal activity. This finding would further support the concept that the serotoninergic system plays an inhibitory role in pituitary–adrenal function.

Reports on the effect of stress on brain 5-hydroxytryptamine (5-HT) content and metabolism are contradictory. Different stressors applied for varying periods of time resulted in conflicting results. Immobilization for 3 hours (*Corrodi* et al. 1968) or 6 hours (*Curzon* and *Green* 1969; *Curzon* 1971) caused a 5-HT depletion in the whole brain. Under similar conditions the opposite was observed by other authors (*Welch* and *Welch* 1968; *De Schaepdryver* et al. 1969). Electric shock applied for three hours (*Thierry* et al. 1968) or swimming until exhaustion increased the 5-HT content (*Barchas* and *Freedman* 1963) 5-HT turnover and 5-hydroxyindoleacetic acid (5-HIAA) content in the brain (*Bliss* et al. 1968; 1972). Other authors could, however, find no alteration (*Toh* 1960; *Levi* and *Maynert* 1964; *Maynert* and *Levi* 1964). Prolonged tumbling stress was reported to decrease hypothalamic 5-HT content (*Riege* and *Morimoto* 1970).

In the present study the effect of ether stress and electric shock on 5-HT, 5-HIAA content, 5-HT turnover and ^{3}H-5-hydroxytryptamine (^{3}H-5-HT) uptake in different regions of the limbic system of male rats was examined in relation to pituitary–adrenal function at different periods of time after stress.

Methods

Male albino adult CFY rats weighing 190—230 g were used. The animals were fed a standard diet and kept for 12 hr in light and 12 hr in the dark. The light period started at 6 a.m., and all experiments were carried out between 8—10 a.m.

Brain 5-HT and 5-HIAA content, 5-HT turnover and ^{3}H-5-HT uptake were measured at different time intervals after exposure to stress. The animals were either placed in ether vapour for 2 min or exposed to electric shock delivered through their paws with 1 mA AC for 5 sec every 15 sec over 2 min. The rats were decapitated after different periods (see Results).

For determination of the 5-HT and 5-HIAA content of brain structures, the brain was removed, immediately frozen and the mesencephalon (40—45 mg), part of the dorsal hippocampus (80—100 mg), the septal region (35—45 mg), the amygdala region (15—25 mg) and the hypothalamus (15—20 mg) were dissected. 5-HT content was measured by the method of *Snyder* et al. (1965), and the 5-HIAA level according to *Cox* and *Perhach* (1973). For 5-HIAA determination, brain tissue obtained from four identical brain areas was pooled.

5-HT turnover was measured according to *Tozer* et al. (1966), by increasing the 5-HT content in the brain by treatment with a monoamino oxidase inhibitor. The animals received 75 mg/kg pargyline (Aldrich) in a volume of 0.1 ml when the ether or electric shock was applied and were killed 10 and 20 min thereafter. In another series of experiments the same dose of pargyline was given 40 min after stress and the animals were killed at 50 or 60 min. The control animals received the same volume of propylene glycol used for diluting the pargyline. 5-HT turnover was studied in the mesencephalon, dorsal hippocampus, septum, amygdala and hypothalamus of individual animals.

Tritiated 5-HT uptake was estimated according to *Blackburn* et al. (1967) as modified by *Telegdy* and *Vermes* (1975).

Plasma corticosterone was measured in trunk blood by the method of *Zenker* and *Bernstein* (1958) as modified by *Purves* and *Sirett* (1965).

Statistical analysis of the data was done by Student's *t*-test. Analysis of the 5-HT turnover rate and comparison of the curves were made by the method of *Finney* (1964), and the synthesis rate was calculated according to the procedure of *Tozer* et al. (1966).

Results

Fifteen minutes after exposure to ether, the hypothalamic 5-HT content decreased ($p < 0.001$) but after 30 min the 5-HT level increased in the mesencephalon ($p < 0.01$) and the amygdala ($p < 0.001$), and after 60 min in the hypothalamus ($p < 0.05$). By 90 min all values had returned to normal. Maximum 5-HIAA levels were observed 30 min after ether stress in the mesencephalon ($p < 0.001$), amygdala ($p < 0.01$) and hypothalamus ($p < 0.01$). Neither the septum nor the hippocampus showed a change in 5-HT or 5-HIAA content (Fig. 1). Plasma corticosterone was highest at 30 min ($p < 0.001$) and returned to normal at 90 min.

Fifteen min after electric shock, the 5-HT level declined in the mesencephalon ($p < 0.05$) and hypothalamus ($p < 0.001$). After 30 min 5-HT values increased in the mesencephalon ($p < 0.001$) and the amygdala ($p < 0.01$) and after 60 min also in the hypothalamus ($p < 0.01$). 5-HIAA levels were increased in the amygdala ($p < 0.01$) at 15 min and in the mesencephalon ($p < 0.01$) and hypothalamus ($p < 0.01$) at 30 min after electric stress. Septum and dorsal hippocampus values did not change. The plasma corticosterone level followed the same pattern as after ether stress (Fig. 2).

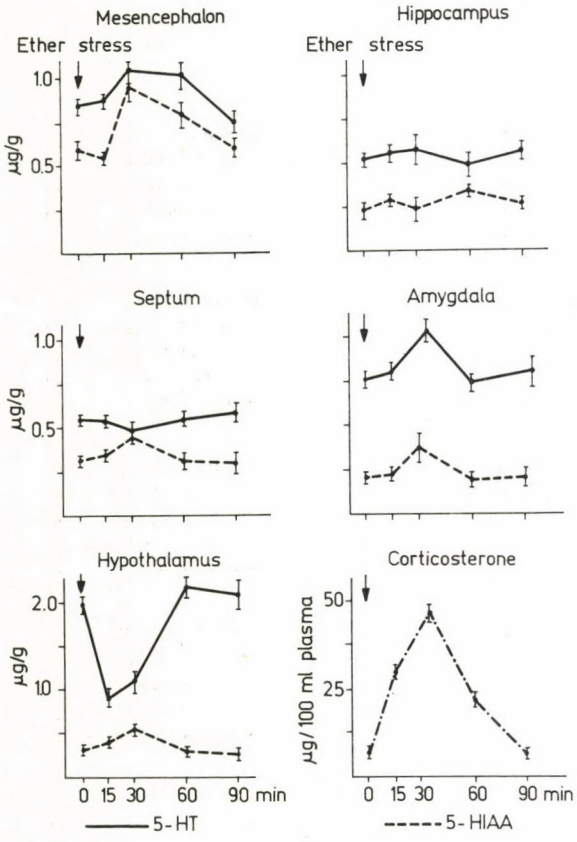

Fig. 1. Effect of ether stress on serotonin (5-HT) and 5--hydroxyindoleacetic acid (5-HIAA) content in limbic brain structures and plasma corticosterone level. Each point represents the mean ± S.D. of 26—52 values

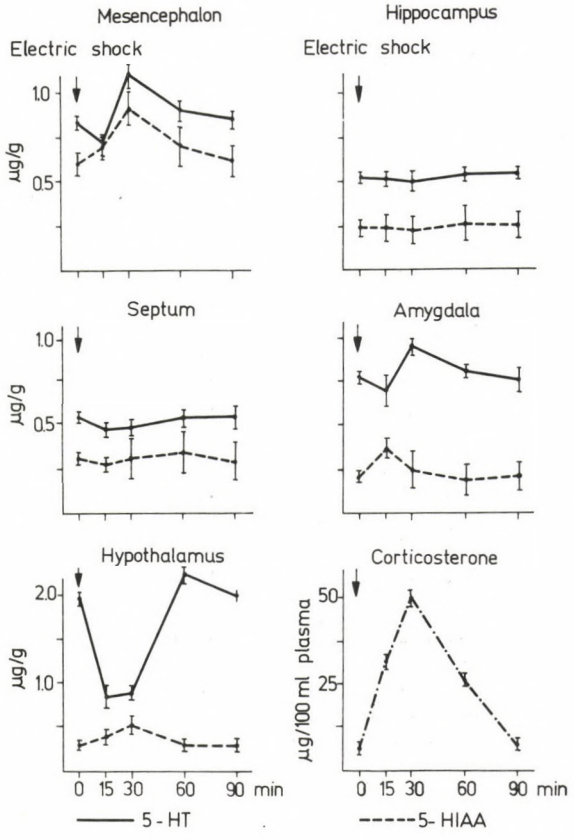

Fig. 2. Effect of electric shock on serotonin (5-HT) and 5--hydroxyindoleacetic acid (5-HIAA) content in limbic brain structures and plasma corticosterone level. Each point represents the mean ± S.D. of 28—52 values

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Table I

Effect of ether stress and electric shock on the synthesis rate of serotonin in different parts of the limbic system calculated according to Tozer et al. (1966)

No.	Groups	N	Equation of regression line as a function of time $y=a\,t+b$	Standard deviation of the slope	5-HT synthesis rate, nmoles/g/h	
			Control			
1.	Hypothalamus	48	$y = 0.0262t + 1.405$	0.151	8.93 ± 2.55	
2.	Amygdala	37	$y = 0.0308t + 0.936$	0.143	10.50 ± 2.81	
3.	Septum	36	$y = 0.0239t + 0.566$	0.103	8.04 ± 2.06	
4.	Hippocampus	36	$y = 0.0216t + 0.564$	0.107	7.36 ± 2.14	
5.	Mesencephalon	50	$y = 0.0346t + 0.910$	0.121	11.79 ± 2.04	
			10—20 min after ether stress			
6.	Hypothalamus	36	$y = 0.0030t + 1.321$	0.144	1.02 ± 2.88	a)
7.	Amygdala	36	$y = 0.0278t + 0.924$	0.135	9.47 ± 2.70	
8.	Septum	37	$y = 0.0232t + 0.587$	0.107	7.90 ± 2.14	
9.	Hippocampus	36	$y = 0.0197t + 0.564$	0.094	6.71 ± 1.88	
10.	Mesencephalon	36	$y = 0.0274t + 0.906$	0.121	9.34 ± 2.42	
			50—60 min after ether stress			
11.	Hypothalamus	27	$y = 0.0430t + 1.673$	0.145	14.65 ± 3.32	
12.	Amygdala	27	$y = 0.0248t + 0.948$	0.084	8.45 ± 1.93	
13.	Septum	27	$y = 0.0183t + 0.559$	0.065	6.23 ± 1.50	
14.	Hippocampus	27	$y = 0.0189t + 0.510$	0.136	6.44 ± 3.13	
15.	Mesencephalon	27	$y = 0.0279t + 0.934$	0.062	9.51 ± 1.43	
			10—20 min after electric shock			
16.	Hypothalamus	24	$y = 0.0040t + 1.359$	0.150	1.36 ± 3.75	a)
17.	Amygdala	24	$y = 0.0296t + 0.927$	0.122	10.09 ± 3.05	
18.	Septum	24	$y = 0.0190t + 0.539$	0.108	6.47 ± 2.70	
19.	Hippocampus	24	$y = 0.0174t + 0.548$	0.093	5.93 ± 2.32	
20.	Mesencephalon	24	$y = 0.0155t + 0.945$	0.110	5.28 ± 2.75	b)
			50—60 min after electric shock			
21.	Hypothalamus	24	$y = 0.0373t + 1.717$	0.161	12.71 ± 3.02	
22.	Amygdala	24	$y = 0.0250t + 0.930$	0.126	9.52 ± 3.15	
23.	Septum	24	$y = 0.0204t + 0.571$	0.097	6.95 ± 2.42	
24.	Hippocampus	24	$y = 0.0136t + 0.584$	0.098	4.63 ± 2.45	
25.	Mesencephalon	24	$y = 0.0491t + 0.955$	0.100	16.73 ± 2.50	c)

Footnotes: N = Number of cases, a): $p < 0.001$ vs. Group 1, b): $p < 0.01$ vs. Group 5, c): $p < 0.05$ vs. Group 5.

Table II

Effect of ether stress on hypothalamic and mesencephalic ^{3}H-5-hydroxytryptamine uptake in normal animals at 20 and 60 min after stress

No.	Groups	Total uptake		5-HT		5-HIAA	
		DPM/100 mg tissue	significance (p)	DPM/100 mg tissue	significande (p)	DPM/100 mg tissue	significance (p)
		Hypothalamus					
1.	Normal (0 time) (10)	202,000 ± 16,000**		100,000 ± 7000		99,000 ± 7,000	
2.	20 min after stress (10)	137,000 ± 10,000	< 0.001 vs. 1.	65,000 ± 3000	< 0.001 vs. 1.	52,000 ± 4,000	< 0.001 vs. 1.
3.	60 min after stress (10)	234,000 ± 20,000	NS vs. 1.	108,000 ± 6000	NS vs. 1.	90,000 ± 9,000	NS vs. 1.
		Mesencephalon					
4.	Normal (0 time) (10)	171,000 ± 18,000		87,000 ± 6000		98,000 ± 10,000	
5.	20 min after stress (10)	102,000 ± 10,000	< 0.001 vs. 4.	53,000 ± 3000	< 0.001 vs. 4.	49,000 ± 4,000	< 0.001 vs. 4.
6.	60 min after stress (10)	170,000 ± 15,000	NS vs. 4.	81,000 ± 6000	NS vs. 4.	72,000 ± 7,000	< 0.001 vs. 4.

*: Number of animals
**: Mean ± S.D.
NS: Non-significant

The turnover rate of 5-HT between 10 and 20 min after ether stress was lower in the hypothalamus ($p < 0.001$), while there was no alteration in the mesencephalon, dorsal hippocampus, septum and amygdala (Table I).

Fifty and 60 min after ether stress no deviation from the control values was noted in any of the brain areas studied (Table I).

Following electric shock a depression in 5-HT turnover occurred in both the mesencephalon ($p < 0.01$) and the hypothalamus ($p < 0.001$) between 10 and 20 min, but the rate in the hippocampus, septum and amygdala remained unchanged (Table II). There was a slight increase in the mesencephalon ($p < 0.05$) after 50 and 60 min but in the hippocampus, septum, amygdala and hypothalamus the turnover rate was unaltered (Table I).

^{3}H-5-HT uptake was studied *in vitro* in the mesencephalon and hypothalamus of rats stressed with ether. There was a significant decline in total tritium uptake and of 5-HT and 5-HIAA in animals sacrificed 20 min after ether stress. After 60 min the uptake was again at the normal level (Table II).

Discussion

The present data agreed well with our earlier observations (*Telegdy* and *Vermes* 1973; *Vermes* and *Telegdy* 1973; *Vermes* et al. 1973) and clearly show that the changes induced by different stressors in cerebral serotonin metabolism are time related. The most significant alteration occurred 20—30 min after exposure to stress. Involvement in these changes of the limbic structures was not uniform. It seemed that the dorsal hippocampus and septal region were not involved in changes of 5-HT metabolism caused by stress. The 5-HT depletion after exposure to ether was noted only in the hypothalamus, and only between 20 and 30 min after stress. Considering the small proportion of the brain which the hypothalamus represents, it is only natural that this change was not reflected in the 5-HT content of the whole brain (*Toh* 1960; *Levi* and *Maynert* 1964; *Maynert* and *Levi* 1964).

After electric shock not only the hypothalamus, but also the mesencephalon showed a depletion of 5-HT content, indicating that in this type of stress more neural structures are involved. Since 5-HIAA content changes only slightly during the time of depletion, this was not caused by an increase in 5-HT metabolism. The lower 5-HT turnover during the time of hypothalamic depletion indicates a low functioning of the serotoninergic system. Since 5-HT uptake is also low, synaptic transport is affected by stress-induced changes. At this time the hypothalamo–pituitary–adrenal system is activated, and plasma corticosterone levels increase.

After ether stress the 5-HT and 5-HIAA contents were increased in the mesencephalon and amygdala at 30 min and also in the hypothalamus at 60 min. After electric shock, however, in the amygdala the 5-HIAA increased first and this increase was at 15 min associated with a slight, non-significant decline in 5-HT content.

However, at 60 min the 5-HIAA content was again normal in the hypothalamus and the amygdala. 5-HT turnover between 50 and 60 min had normalized in the affected brain areas or even increased after electric shock in the mesencephalon. At this time, 5-HT uptake returned to normal in the hypothalamus and in the mesencephalon.

Since the mesencephalon and the amygdala show similar, while the septum and hippocampus different, changes in serotonin metabolism the amygdala and the mesencephalon appear to be part of the same inhibitory pathway in which the transmitter material is serotonin.

The data presented support the concept formulated on basis of our earlier data (*Vermes* and *Telegdy* 1972; *Telegdy* and *Vermes* 1973; *Vermes* and *Telegdy* 1973; *Vermes* et al. 1973) that the serotoninergic system exerts an inhibitory influence on the pituitary–adrenal axis. During activation of the latter, serotoninergic inhibition is diminished by depletion of the hypothalamic 5-HT content and by decreased 5-HT turnover and uptake. After an increase in plasma corticosterone, the activity of the serotoninergic system returns to normal or to a slightly higher level. Restoration of or an increase in the activity of the serotoninergic system partially results from the increased plasma corticosterone (*Telegdy* and *Vermes* 1975), suggesting that the negative feed-back action of corticosteroids is mediated in part by serotoninergic inhibition. These data agree with the observations of *Vernikos-Danellis* et al. (1973) and *Berger* et al. (1974) in that a low brain 5-HT content facilitates the stress response and diminishes the negative feed-back action of steroids.

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LOCAL BLOOD FLOW OF THE HYPOTHALAMUS IN HAEMORRHAGIC HYPOTENSION

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Local blood flow of the hypothalamus (HBF) was measured by the hydrogen clearance method in anaesthetized dogs. The average value for HBF in normal controls was 0.64 ± 0.05 ml/g/min, which compares favourably with values available in the literature. During haemorrhagic hypotension induced by a modified *Wiggers* method there occurred a marked reduction of HBF to 52% of the initial control value at a mean arterial blood pressure (MABP) of 55—60 mm Hg and to 44% at 35—40 mm Hg.

Prevention of concomitant extracellular acidosis by infusion of sodium bicarbonate solution during the hypotensive period resulted in a significantly smaller decrease in HBF as compared to an untreated control group, and a significant protection of HBF was also found at 55—60 mm Hg MABP in the bicarbonate treated group, but not in controls treated with physiologic saline. Hypothalamic tissue hypoxia was inevitable in untreated bled animals due to insufficient circulatory transport, while evidence suggested that the metabolism might have remained unaltered in the group protected against acidosis during haemorrhage.

Analysis of the control of local hypothalamic blood flow revealed a significant correlation ($r = 0.7026$, $p < 0.001$) between HBF and arterial blood pH in severe hypotension which is outside the autoregulatory blood pressure domain.

In a series of previous studies (*Maklári* and *Kovách* 1968; *Maklári* et al. 1973a), we were able to demonstrate an elevated tissue carbon dioxide content in the brain due to haemorrhagic hypotension. There was a difference in the accumulation of tissue CO_2 with respect to various brain regions. Alterations were the most pronounced in the hypothalamus and the frontal cortex, whereas the thalamus and the medulla oblongata were the least affected. The infusion of buffer solution (sodium bicarbonate) during haemorrhage prevented the increase of CO_2 content and subsequent tissue acidosis in the brain (*Maklári* et al. 1973b). Nevertheless, we are aware of the fact that the role of correcting extracellular acidosis in determining the final outcome of shock is not yet clarified, neither is there a consensus in the literature with regard to this question (for discussion see *Maklári* et al. 1973b). Our approach to this end was to evaluate first the local haemodynamic component in the beneficial effect of preventing tissue acidosis during haemorrhage, then to draw conclusion concerning the possible metabolic involvement. One may sp
that with administering solutions of any kind a diminished blood viscosity a
haemodilution is inherent, which in itself increases cerebral blood flow (*Hägge*

et al. 1966; *Johansson* and *Siesjö* 1974) and the correction of extracellular acidosis might prevent an increased catecholamine mobilization (*Fiorica* et al. 1969) sufficiently improving microcirculation. It has been demonstrated (*Baeckström* et al. 1971) that acute blood loss can produce micro-plugging of the vascular bed, triggered by circulating catecholamines. The aim of this study was to repeat our preceding control and acidosis-corrected haemorrhagic shock experiments, completing them by the simultaneous measurement of local blood flow in the hypothalamus, which proved to be the region most susceptible to tissue hypercapnia.

Methods

The experiments were carried out on mongrel dogs of both sexes, weighing between 7 and 20 kg. The animals were anaesthetized with chloralose [100 mg/kg alpha-D(+)-glucochloralose, Merck, Darmstadt] and breathed room air spontaneously.

Prior to starting the measurements 1000 IU/kg heparin (Richter, Budapest) was given intravenously to prevent blood clotting; anticoagulation was maintained by the subsequent administration of heparin in a dose of 500 IU/kg. Arterial blood pressure was continuously monitored via a polyethylene catheter placed into the left femoral artery. Haemorrhagic shock was induced by a modified *Wiggers* method (*Wiggers* 1950). The animals were bled into a pressurized reservoir (*Engelking* and *Willig* 1958) through a short, thick cannula inserted into the right femoral artery. Mean arterial blood pressure (MABP) was kept at 55—60 mm Hg for 90 min, then at 35—40 mm Hg for another 90 min. By the end of the hypotensive period the shed blood was reinfused. Arterial blood samples were taken from the left carotid artery through a T-shaped cannula and cerebral venous samples were drawn from the sagittal sinus. Sampling was carried out before bleeding, at 30 and 90 min of each bleeding period as well as 30 and 60 min after reinfusion. A Beckman type 160 blood gas analyser was used for pH and pCO_2 determination, while the values for actual bicarbonate and BE were taken from the Siggaard—Andersen alignment nomogram. O_2 saturation of the blood was measured with an oxymeter (Elema-Schönander), the haemoglobin content of blood was assessed by the cyan-methaemoglobin method (*Bakos* 1963). Haematocrit was determined using a microhaematocrit centrifuge (MSE).

After completing cannulation, the head of the anaesthetized animal was fixed in a stereotaxic head-holder. The skin and muscles were removed on both sides of the central crista while bleeding was controlled carefully. A burr hole was made in the skull by means of a dental drill, the dura was dissected and a platinum electrode (0.12—0.80 mm in diameter) was placed into the lateral hypothalamus. The trephination hole was closed with bone wax. The coordinates of the electrode tip were: R:19, V:6 mm (*Lim* et al. 1960). At the end of each experiment the animals were killed by an intravenous injection of KCl solution, the brains were perfused with 8% formalin, removed and processed for histological analysis.

Hypothalamic blood flow (HBF) was measured making use of the hydrogen clearance method as described by *Aukland* et al. (1964). The Pt-electrodes were connected to a measuring bridge and a non-polarizing Ag/AgCl electrode (Radelkis OP 8201) was used as reference. The output signals from the measuring bridge (0.1 mV or less) were directly registered on a compensograph (Kipp Zonen BD 3) and the, mostly monoexponential, washout curves obtained were analysed graphically (20 from a total of 196 analysed curves were biexponential; 10%). In the case of biexponential washout the graphic "peeling off" technique was applied to resolve the components. Mean blood flow was calculated according to the equation (*Fieschi* et al. 1969)

$$\bar{f} = w_1 f_1 + w_2 f_2.$$

Table I

Hypothalamic blood flow (ml/g/min) in haemorrhagic hypotension

	Bleeding I			Bleeding II		After reinfusion	
		30′	90′	30′	90′	30′	60′
Control (n = 7)	0.61 ± 0.10	0.32 ± 0.06**	–	0.27 ± 0.08**	–	0.49 ± 0.10 n = 3	–
NaCl-treated (n = 6)	0.66 ± 0.06	0.36 ± 0.05**	0.36 ± 0.05*	0.35 ± 0.06**	0.38 ± 0.6*	0.40 ± 0.08*	0.34 ± 0.09*
Bicarbonate-treated (n = 8)	0.65 ± 0.07	0.63 ± 0.08++=	0.52 ± 0.06++=	0.48 ± 0.08+*	0.42 ± 0.07**	0.53 ± 0.11	0.55 ± 0.15

The values are means ± SEM.

* significant difference as compared with prebleeding values
\+ significant difference as compared with the control group
= significant difference as compared with the NaCl-treated group

single symbol $p < 0.05$; double symbol $p < 0.01$

A total of 32 animals was studied. Data obtained from 11 animals were excluded from further analysis because of either imperfect electrode placement (verified in the histological sections) or unsuitable condition due to the surgical intervention. The remaining animals were divided into three groups.

Group I (n=7): untreated controls.

Group II (n=8): sodium-bicarbonate infusion was given to this group throughout the bleeding period to prevent acidosis. The rate of infusion was set just to restore control extracellular acid–base balance, the amount of bicarbonate to be infused was calculated on the basis of intermittent arterial standard bicarbonate determinations (for methodological details see *Maklári* et al. 1973b).

Group III (n=6): this group received the same volume of solution under identical experimental conditions as Group II without correcting acidosis (physiological saline-treated group).

Student's *t*-test was used to assess statistical significance and *Dixon*'s test (*Dixon* and *Massey* 1957) was applied to judge outstanding values in the samples.

The figures presented in this paper are means ± S.E.M.

Results

Mean local blood flow in the dog's hypothalamus was 0.64±0.05 ml/g/min under control conditions (n=21, MABP 118±5 mm Hg, P_aCO_2 35±1.5 mm Hg, arterial pH 7.34±0.02). Following haemorrhage there was a significant drop in HBF in the control group from the initial value of 0.61±0.10 ml/g/min to 0.32±0.06 ml/g/min in bleeding I and to 0.27±0.08 ml/g/min in bleeding II (Table I). The shock model used in our experiments resulted in a substantial reduction of local HBF, since flow decreased to 52% of the prebleeding level at a MABP of 55—60 mm Hg and to 44% at 35–40 mm Hg. Similar changes were found in the saline-treated group (corresponding figures: 0.66±0.06 ml/g/min control value, 0.36±0.06 ml/g/min and 0.35±0.06 ml/g/min during bleeding, respectively). HBF in the bicarbonate-treated group displayed much less of a reduction (0.65±0.07 ml/g/min before bleeding, 0.63±0.08 ml/g/min at 30 min of bleeding I and 0.48±0.08 ml/g/min at 30 min of bleeding II) and was significantly higher in both bleeding periods as compared to the control group, whereas the difference in the first bleeding was significant only with respect to the saline-treated group (Table I, Fig. 1).

Changes in the blood parameters displayed the same alterations as reported previously (*Maklári* et al. 1973a, b). We do not intend to repeat them except for the variables of arterial blood summarized in Table II, to demonstrate identity with the preceding studies and to serve as direct reference for later discussion.

Circulatory oxygen transport (the product of blood flow and arterial oxygen content) displayed an even more pronounced difference in favour of the treated groups than the difference with regard to their HBF. Tissue oxygen delivery remained significantly elevated even in bleeding II in the bicarbonate- and saline-treated groups as compared to the control (Table III).

Table II

Arterial blood parameters in haemorrhagic shock

		Before bleeding	Bleeding I	Bleeding II	After reinfusion
			30′	30′	30′
pH (pH units)	Control	7.34 ± 0.02	7.32 ± 0.02	7.07 ± 0.07**	7.21 ± 0.06
	NaCl-treated	7.39 ± 0.02	7.36 ± 0.03*	7.27 ± 0.04**	7.22 ± 0.03***
	Bicarbonate-treated	7.39 ± 0.02	7.47 ± 0.02*** = = +	7.43 ± 0.02+ + + = =	7.28 ± 0.02**
pCO_2 (mm Hg)	Control	40 ± 1.4	33 ± 3.5*	25 ± 4.3**	20 ± 2.4**
	NaCl-treated	30 ± 2.5	25 ± 2.4**	24 ± 2.5**	26 ± 2.4*
	Bicarbonate-treated	32 ± 2.6	28 ± 2.4*	27 ± 2.2	29 ± 2.3+
O_2 content (ml/100 ml)	Control	19.2 ± 1.1	15.7 ± 1.6*	14.3 ± 2.2*	20.9 ± 1.1
	NaCl-treated	19.8 ± 1.3	18.5 ± 1.1*	17.6 ± 0.83*	17.2 ± 0.77*
	Bicarbonate-treated	19.6 ± 0.82	17.9 ± 0.77**	17.1 ± 0.65**	17.6 ± 0.63*
Haemoglobin (g per cent)	Control	16.2 ± 0.3	14.1 ± 0.5	14.9 ± 0.4	16.1 ± 1.1
	NaCl-treated	16.8 ± 0.9	15.3 ± 0.7**	14.6 ± 0.5*	15.1 ± 0.8*
	Bicarbonate-treated	16.0 ± 0.7	14.1 ± 0.6**	13.4 ± 0.5+*	13.9 ± 0.5**

The values are means ± SEM.

* significant difference as compared with prebleeding values
\+ significant difference as compared with the control group
= significant difference as compared with the NaCl treated group
single symbol $p < 0.05$; double symbol $p < 0.01$; triple symbol $p < 0.001$

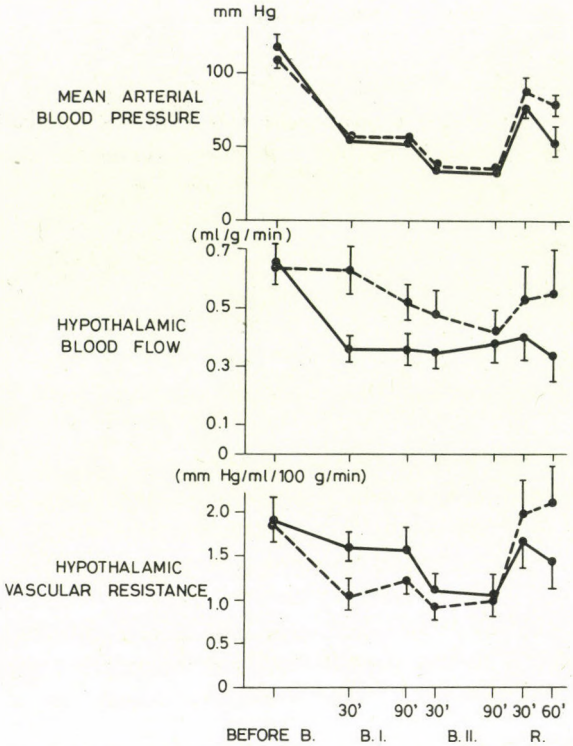

Fig. 1. Mean arterial blood pressure, hypothalamic blood flow and hypothalamic circulatory resistance[1] in haemorrhagic shock. The values are means ± S.E.M. ●——●: NaCl-treated; ●– – –●: bicarbonate-treated. B: bleeding, B.I.: Bleeding I, B.II.: Bleeding II; R: after reinfusion

Table III

Circulatory oxygen transport (ml/100 g/min) in hypothalamus

	Before bleeding	Bleeding I	Bleeding II
		30′	30′
Control	11.5 ± 2.21	5.51 ± 1.35*	2.73 ± 0.71**
NaCl-treated	13.0 ± 1.37	7.00 ± 0.58**	6.15 ± 0.94**++
Bicarbonate-treated	13.6 ± 1.47	12.1 ± 1.43==++	8.36 ± 1.21**++

The values are means ± S.E.M.

* significant difference as compared with prebleeding values
\+ significant difference as compared with the control group
= significant difference as compared with the NaCl-treated group
single symbol $p < 0.05$; double symbol $p < 0.01$

[1] Hypothalamic venous pressure is not accessible, neither is consequently the hypothalamic perfusion pressure, thus resistance calculated from arterial blood pressure alone is an overestimate of the true value.

Discussion

Fieschi et al. (1969), and more recently *Pásztor* et al. (1973) and *Symon* et al. (1973) have shown that the clearance of the diffusible indicator hydrogen is a suitable measure of local cerebral blood flow even in acute experiments. Tissue destruction, inevitable with this method, may be considered negligible from a functional point of view because the characteristic control of cerebral circulation prevails and flow data obtained are comparable within the range of statistical error with the values of parallel measurements assessed by less invasive methods (*Fieschi* et al. 1969).

Table IV offers a compilation of HBF values available in the literature. The data of *Rosendorff* and *Cranston,* (1969) show the strongest deviation. Even the authors themselves do not recommend their own method for the determination of absolute flow values and considered only the relative changes. The figures obtained by the tissue-clearance detection method, used by *Rosendorff* and *Cranston,* depend strongly on the value of the hypothalamic tissue-blood partition coefficient of the indicator (^{133}Xe in this case), which in turn is very difficult to measure. This uncertainty is reflected by the fact that in the cited paper (*Rosendorff* and *Cranston* 1969) $\lambda_{Xe} = 0.63$ was applied, whereas, in a more recent publication (*Rosendorff* and *Cranston* 1971) $\lambda_{Xe} = 0.74$ was used for blood flow calculations. *Goldman* and *Sapirstein* (1973) pointed out the possibility of species specific difference in explaining why they found for deep brain structures including the hypothalamus 15–20% higher flow values systematically, along with normal figures for cortical areas as compared with data reported previously by other authors (*Freygang* and *Sokoloff* 1959; *Reivich* et al. 1969). The average control HBF of 0.64 ± 0.05 ml/g/min observed in this study compares very well with HBF values measured by autoradiographic techniques (Table IV).

Previous experiments using heated thermocouples for blood flow measurement have revealed a substantially reduced HBF in haemorrhagic hypotension (*Kovách* et al. 1973). The diminution of HBF, to 44% of the initial value in bleeding II, seems to be more marked than the reduction of the whole brain blood flow, viz., 74% under similar conditions (*Reivich* et al. 1973), provided that data obtained on dogs (*Kovách* et al. 1973) are comparable with those obtained on artificially ventilated primates (*Reivich* et al. 1973). This might explain the augmented CO_2 accumulation in the hypothalamus during hypotension as compared with other brain regions such as frontal and occipital cortex, thalamus, pons, medulla oblongata (*Maklári* et al. 1973a). Our hypothesis considering the role of local blood flow is supported by the findings of this study, since HBF decreased to a lesser extent in the bicarbonate-treated group, while a previous study (*Maklári* et al. 1973b) revealed that CO_2 accumulation in the hypothalamus disappeared as a result of bicarbonate infusion. When discussing this effect of bicarbonate treatment other factors preventing extracellular acidosis should, however, also be considered.

Table IV

Author	Hypothalamic blood flow (ml/g/min)	Animal	Method
Freygang et al. 1959	0.84 ± 0.05 (n = 10)	Conscious cat	$CF_3{}^{131}I$ autoradiography
Freygang et al. 1959	0.55 ± 0.06 (n = 11)	Anaesthetized cat	
Reivich et al. 1969	0.68 ± 0.06 (n = 6)	Conscious cat	^{14}C – antipyrine autoradiography
Rosendorff et al. 1969	0.31 ± 0.06 (S.D.)	Conscious rabbit	^{133}Xe washout
Goldman et al. 1973	0.82 ± 0.03	Conscious rat	Indicator fractionation
	0.85 ± 0.02	Anaesthetized rat	
Present study	0.64 ± 0.05 (n = 21)	Anaesthetized dog	H_2 washout

The oxygen-binding capacity of blood decreases in haemorrhagic shock because of the ensuing haemodilution and the shift to the right of the oxygen dissociation curve (*Naylor* et al. 1972), though this tendency is somewhat counterbalanced by increased arterial oxygen saturation associated with inherent hyperventilation. The shift to the right of the oxygen-binding curve, a mechanism that generally results in an improved oxygen delivery to the tissues, might have a reverse effect and contribute to further deterioration when accompanied by low flow. This is apparent from Table III which shows the changes of circulatory oxygen transport in the three groups.

The normal average oxygen consumption of the brain is about 3.5 ml/100 g/min, while that of the grey matter is considerably higher, 7—8 ml/100 g/min (*Gleichmann* et al. 1962; *Lübbers* et al. 1964). There are no available data concerning the regional oxygen consumption of the hypothalamus, though it can be estimated if we adopt the hypothesis of direct coupling between metabolism and flow, viz., that the flow of blood complies with the requirement of local tissue metabolism. This widely accepted assumption has recently been proven (*Reivich* et al. 1974; *Raichle* et al. 1976). *Gleichmann* et al. (1962) have measured simultaneously the local blood flow (0.65 ± 0.05 ml/g/min) and the metabolic rate of oxygen consumption [7.1 ± 2.8 (S.D.) ml/g/min] of the sensorimotor area (postsigmoid gyrus) in anaesthetized dogs. On the basis of these data, we may assume that oxygen consumption of the hypothalamus would fall in the same degree at a control mean blood flow of 0.64 ± 0.05 ml/g/min. It is clear now that the circulatory transport in the bicarbonate-treated group would provide enough oxygen to maintain unaltered aerobic metabolism even in deep hypotension, while tissue hypoxia is inherent in the control group even at a total extraction of arterial oxygen content (Table III), which in itself would also be deleterious in view of the zero venous pO_2 and concomitant diffusion problems.

The regulation of HBF under normal conditions displays the same features which are characteristic of the cerebral cortex, viz., autoregulation (*Cranston* and *Rosendorff* 1971) and CO_2 responsiveness of the vascular bed (*Schmidt* 1934). It is well demonstrated for cortical blood flow that the effectiveness of these factors diminishes in deep hypotension [the question has extensively been reviewed by *Purves* (1972) in his monograph], therefore, on the ground of reciprocity the same may hold true for the hypothalamus. In order to investigate the problem of HBF regulation under pathologic conditions, we carried out a multiple correlation analysis of the data obtained during hypotension, where HBF was considered to be a dependent variable, while MABP, arterial pCO_2, arterial pH, the amount of shed blood and haematocrit were independent variables. Table V contains the correlation matrix and the regression coefficients. It is evident from Table V that arterial pH was the sole parameter showing a significant correlation ($p < 0.001$) with HBF under the previously described experimental conditions. Fig. 2 shows the scattergram when HBF was plotted against arterial pH. The relationship is not quite linear, the function

best fitting the data points follows a second polynomial order. Curve-fitting was done by means of the least squares method ($p < 0.01$, F ratio test). At a normal MABP and 30—40 mm Hg arterial pCO_2 level cortical blood flow proved to be invariable with wide alterations of arterial pH (*Harper* and *Bell* 1963). It is not clear whether the observed pH-dependence of blood flow was a consequence of the pathologic conditions in general or a characteristic of the hypothalamic region.

Table V

Bled n = 33	y	x_1	x_2	x_3	x_4	x_5	Regression coefficients
y	1.000						
x_1	0.0050	1.0000					−0.0032
x_2	0.2012	0.3548	1.0000				0.0784
x_3	0.7026***	0.3317	0.2044	1.0000			0.0032
x_4	0.3548	0.0017	0.2000	0.2473	1.0000		0.3247
x_5	0.2557	0.3253	0.3208	0.0098	0.1880	1.0000	−0.0686

Multiple correlation coefficient r = 0.6341***

y = hypothalamic bloow flow (ml/g/min)
x_1 = arterial blood pressure (mm Hg)
x_2 = arterial pCO_2 (mm Hg)
x_3 = arterial pH (pH units)
x_4 = haematocrit (per cent)
x_5 = corrected exsanguinated volume (ml/kg); referring to the correction see *Maklári* et al. 1973a
*** $p < 0.001$

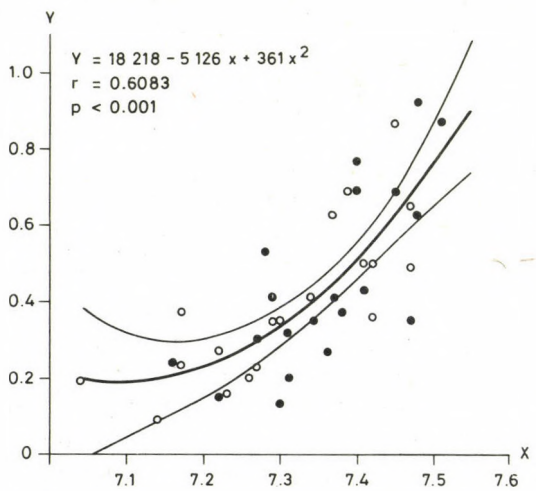

Fig. 2. Hypothalamic blood flow in hypotension plotted against arterial pH. x: Arterial pH (in pH units); y: hypothalamic blood flow (ml/g/min); •: MABP: 55 ± 0.74 mm Hg, P_aCO_2: 26 ± 1.5 mm Hg; o: MABP: 33 ± 0.71 mm Hg, P_aCO_2: 23 ± 1.5 mm Hg. Drawn lines represent the least squares polynomial best fit of data with 95% confidence limits

This problem warrants further study. It is noteworthy, too, that MABP has a minor role in determining blood flow, in contrast to what would be expected outside the autoregulatory blood pressure domain, but the fact must not be disregarded that data were obtained at a narrow blood pressure range of 30—60 mm Hg.

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AUTOREGULATION OF RENAL CIRCULATION IN THE DOG UNDER FREE FLOW AND STOP FLOW CONDITIONS

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The relation of renal functional parameters such as renal blood flow (RBF), glomerular filtration rate (GFR), glomerular capillary pressure (GCP), deep venous pressure (DVP), ureteral pressure (UP), and of series connected intrarenal resistances to arterial (perfusion) pressure was investigated in anaesthetized dogs under diverse experimental conditions.

1. In normohydrated dogs under free flow conditions, RBF and GFR rise steeply in the hypotensive range (40 to 80 mm Hg) and are constant in the normotensive range (90 to 150 mm Hg); the same applies to GCP and DVP. Autoregulation, i.e. constancy of RBF and GFR, is due to an appropriate increase in afferent resistance, while efferent and venular resistances are unaltered.

2. In normohydrated dogs autoregulation is abolished by ureteral occlusion: RBF, GFR, GCP, DVP and UP increase in direct proportion to arterial pressure in the whole pressure range investigated. Pressure-dependent changes in total vascular and series connected intrarenal resistances are negligible.

3. In mannitol-loaded dogs under free flow conditions, autoregulation persists. The slight increase in GCP is compensated by a similar increase in DVP. The brunt of the autoregulation is borne by the afferent resistance. Efferent resistance is somewhat decreased, venular resistance increased as compared with the normohydrated dogs. Pressure-dependence of postglomerular resistance is negligible.

4. In mannitol-loaded dogs autoregulation is completely abolished by ureteral occlusion: all functional parameters increase in direct proportion to arterial pressure. Vascular resistance is increased due to the enhancement of afferent and venular resistances.

The smooth muscle of the vascular walls possesses an intrinsic contractility which is independent of its innervation. As long ago as 1902 *Bayliss* suggested that the blood pressure might act as a mechanical stimulus to the smooth muscle of the vascular wall. The kidney belongs to the organs in which the pressure-dependent contraction of vascular muscle is very pronounced: owing to appropriate changes in vascular tone, i.e. vascular resistance, the kidney is capable to keep its blood flow fairly constant over an arterial pressure range from about 80 mm Hg to 180 mm Hg. This phenomenon is usually designated as autoregulation of renal circulation.

Autoregulation is not confined to renal blood flow (RBF). Another basic parameter of renal function, glomerular filtration rate (GFR), is also autoregulated: its pressure-dependence is negligible beyond 80 mm Hg arterial pressure. Owing to the special vascular architecture of the kidney vessels, GFR may be controlled

independently of renal blood flow. Other factors remaining unaltered, constriction of the afferent and/or dilatation of the efferent arterioles tend to lower filtration; on the other hand, afferent dilatation and/or efferent constriction may increase filtration even if total renal vascular resistance is constant or elevated.

Autoregulatory behaviour of in situ kidneys is determined by measuring RBF and/or GFR at various arterial pressure levels decreased stepwise by an adjustable clamp placed on the aorta above the origin of the renal arteries. It is customary to construct flow/pressure curves which consist of three phases. In the autoregulatory range, i.e. above 80 to 90 mm Hg in the dog, flow/pressure relation is linear and the slope of the line is slight or absent. Below the autoregulatory range, between about 40 and 80 mm Hg, the flow vs. pressure relation is linear, with a more or less steep slope. The two rectilinear curves are connected by a short curvilinear portion concave towards the pressure axis (see Fig. 1). It follows from elementary considerations that a constancy of RBF and/or GFR notwithstanding variations in arterial pressure indicates inverse changes in renal vascular resistance. On the other hand, linear proportionality of RBF and/or GFR to arterial pressure below the autoregulatory range speaks for constancy of renal vascular resistance (see Fig. 4). With a further reduction of arterial pressure, renal vascular resistance rises steeply and at about 15 mm Hg blood flow stops entirely (so-called critical closing pressure; *Selkurt* 1946).

Circumstantial evidence speaks strongly in favour of the view that autoregulatory adjustments in renal vascular resistance are localized almost entirely at the preglomerular segment (*Selkurt* et al. 1949; *Shipley* and *Study* 1951; *Thurau* 1964; *Liebau* et al. 1968; *Navar* 1970; *Renkin* and *Robinson* 1974; etc.). This view has been challenged by *Abe* et al. (1970) who published results according to which, in contradistinction to RBF, GFR declines significantly between pressures of 100 and 75 mm Hg, secondary to a decrease in efferent arteriolar resistance. As changes in efferent resistance seem to play a role in the pathogenesis of acute renal failure (*Bálint* et al. 1973), we aimed at the elucidation of the role of series connected intrarenal resistances in the regulation of GFR in the normal canine kidney.

The afferent arteriole can vary its resistance to flow over wide range: when fully dilated, i.e. below the autoregulatory range, its resistance falls to about one-tenth of its value in the upper autoregulatory range. Quite apart from their doubtful role played in the autoregulation of RBF and/or GFR, efferent arterioles can decrease their resistance to about four-tenths of their normal value (*Renkin* and *Robinson* 1974). Resistance in the renal venules contributes little to total renal vascular resistance and plays no active role in the haemodynamic responses of renal circulation.

Haemodynamic alterations in renal circulation can be brought about by ureteral obstruction or by elevating ureteral pressure. With a partially or completely obstructed ureter, RBF may increase and GFR may also increase or remain at control levels (*Nash* and *Selkurt* 1964; *Gilmore* 1964; *Navar* and *Baer* 1970; etc.). Changes in renal vascular resistance are similar to those obtained on decreasing

arterial (perfusion) pressure. Afferent dilatation seems to be predominant but an increase in the resistance of the efferent arterioles cannot be excluded. Flow/pressure relationships are seriously altered by ureteral obstruction, i.e. under stop flow conditions. Autoregulatory capacity is diminished or abolished and the flow/pressure relation becomes rectilinear both below and within the autoregulatory pressure range.

According to *Navar* (1970) afferent resistance is equally at minimum under both conditions of decreased arterial pressure and of elevated ureteral pressure at normal arterial pressure levels. We aimed at the verification of this conception by establishing flow/pressure relationships both under free flow and under stop flow conditions.

A further possibility of influencing flow/pressure relationships consists in establishing a brisk osmotic diuresis. At normal arterial pressures renal vascular resistance is identical in normohydrated and in mannitol-loaded dogs under free flow conditions; apart from negligible differences in postglomerular resistance, even series connected intrarenal resistances are partically equal (*Bálint* et al. 1975). Under stop flow conditions, however, the influence of mannitol-loading on total and series connected resistances is by no means negligible. Increase in plasma osmolality above a certain limit brings about an increase in RBF associated with a decreased GFR, and flow/pressure curves become linear in the entire range above about 40 mm Hg (*Navar* et al. 1966; *Kiil* et al. 1969).

In order to elucidate the influence of (i) arterial pressure and/or (ii) ureteral pressure and/or (iii) osmotic loading on flow/pressure relationships, RBF and GFR were determined and series connected resistance was calculated at various arterial pressure levels both in normohydrated and in mannitol-loaded dogs under free flow and stop flow conditions.

Methods

The observations were made on mongrel dogs of both sexes. They were kept on a protein-rich diet and fasted 16 hours prior to the experiment; water was allowed ad libitum. Under pentobarbital (0.03 g/kg) anaesthesia the aorta above the renal arteries was approached by laparotomy, an adjustable clamp placed above the origin of the renal arteries and the renal vein connected by means of a polyethylene tube with the external jugular vein. Surgical procedures and chemical determinations were performed as described in our previous paper (*Bálint* et al. 1975).

Renal blood flow (RBF) was determined directly by measuring renal venous effluent. Clearance of inulin (=GFR) was calculated by the formula:

$$c_{in} = RBF\left(\frac{100-Ht}{100}\right)E_{in}+V. \qquad (1)$$

Arterial pressure (AP), deep venous pressure (DVP, so-called wedged pressure) and renal venous pressure (RVP) were measured. Extraction ratio of inulin and PAH was determined by analysing arterial and simultaneously drawn renal venous blood plasma. Urine was collected by a ureteral catheter.

Experimental procedures. Four types of experiment were performed.

I. Normohydrated, free flow series. Urine was collected for 10—15 min. During this period two arterial and renal venous blood samples were obtained and RBF was determined. Pressure values (AP, DVP, RVP) were read repeatedly. This was followed by a stepwise reduction of femoral (=renal) arterial pressure by appropriate adjustment of the aortic clamp to about 100 (if at the beginning arterial pressure was above 120 mm Hg) and 80, then 60 and 40—50 mm Hg. All pressure levels were kept constant for 10—15 min and the above mentioned parameters were determined. Experiments with spontaneous hypotension (initial arterial pressure below 95 mm Hg) were discarded.

II. Normohydrated, stop flow series. The ureter was occluded by connecting the outer end of the catheter with an electromanometer (Elema-Schönander). Ureteral pressure (UP) rose quickly and reached a constant value in 10—15 min. Subsequently the experiment was performed as described in the above paragraph.

III. Mannitol-loaded, free flow series. A priming dose (8 ml/kg) followed by a maintenance solution (0.08 ml/kg/min) of 20% mannitol dissolved in water were infused intravenously. After about 15 min the experiment was performed as described above.

IV. Mannitol-loaded, stop flow series. After loading with mannitol as described above, ureteral occlusion was performed. Within some minutes ureteral pressure reached a constant value and the experiment was carried out with stepwise decreasing arterial pressure.

Calculations were done as described previously (*Bálint* et al. 1975). Proximal tubular pressure (PTP) was assumed to equal deep venous pressure (DVP) under free flow, and ureteral pressure (UP) under stop flow, conditions. Glomerular capillary pressure (GCP) was calculated by taking mean colloid osmotic pressure of the plasma proteins (COP_m) into consideration and under the assumption that glomerular filtration coefficient k = 3 ml/min/100 g kidney/1 mm Hg effective filtration pressure (EFP). Evaluation of results was done by applying Student's unpaired *t*-test and calculating regression equations, as described by *Cavalli-Sforza* (1965).

Results

Results were grouped into six arterial pressure ranges; means (±S.E.M.) of the four experimental series are presented in Tables I to IV. In order to facilitate comparison of the influence of interventions (ureteral occlusion; loading with mannitol) on the autoregulatory process, regression lines of various parameters vs. arterial pressure are compiled in Figures 1 to 4. Simple continuous lines stand for the normohydrated, free flow series, with dots for the stop flow series; mannitol-loading is indicated by broken lines (free flow), supplemented with circles for the stop flow series. Regression lines for the hypotensive periods (AP below 90 mm Hg) and for the autoregulatory range (AP above 90 mm Hg) were calculated and presented separately.

In statistical evaluation the following aspects have been considered. (i) Significance of difference of the regression coefficient against zero, i.e. the slope of the regression line; (ii) significance of difference between regression coefficients in the hypotensive and in the normotensive range; (iii) accidental differences between regression coefficients of the various experimental series, i.e. free flow vs. stop flow or normohydrated vs. loaded.

Renal blood flow (Fig. 1). Under free flow conditions both normohydrated and mannitol-loaded dogs show complete autoregulation, i.e. regression lines rise significantly ($p < 0.001$) in the hypotensive range, and run parallel to the abscissa

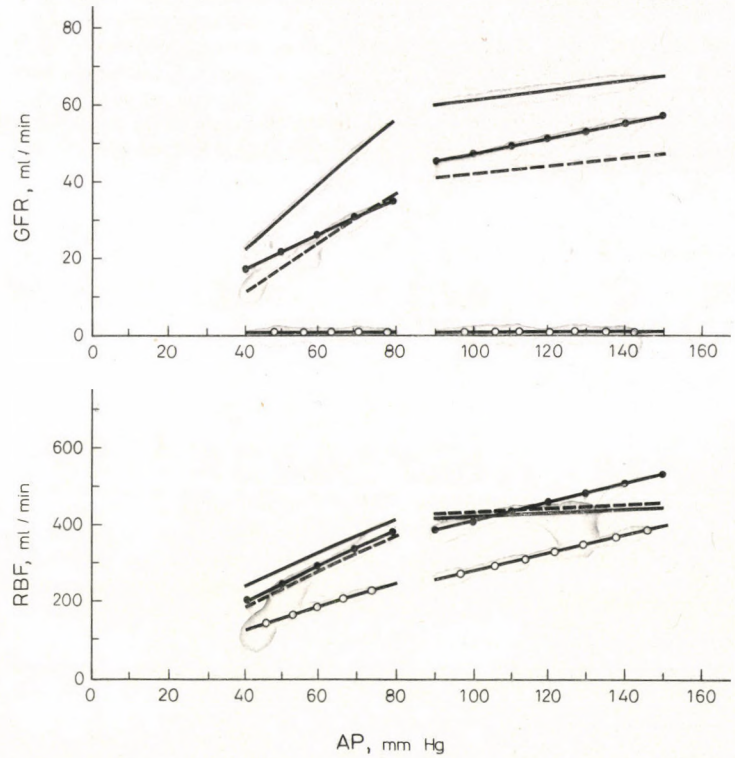

Fig. 1. Upper part: Relation of glomerular filtration rate (GFR) to arterial pressure (AP). Lower part: Relation of renal blood flow (RBF) to arterial pressure (AP). Normohydrated, free flow series: continuous line. Normohydrated, stop flow series: continuous line with dots. Mannitol-loaded, free flow series: broken line. Mannitol-loaded, stop flow series: broken line with circles

in the normotensive range. The difference of regression coefficients in the hypotensive and normotensive range is significant ($p < 0.01$). In sharp contrast to free flow conditions regression lines under stop flow conditions show a definite slope both in the hypotensive ($p < 0.001$) and in the normotensive range ($p < 0.05$ and < 0.001 in normohydrated and mannitol-loaded dogs, respectively). Whereas mannitol-loading does not influence average RBF in the autoregulatory range under free flow conditions (433 ± 12 and 442 ± 16 ml/min), in the normotensive range under stop flow conditions average RBF amounts to 464 ± 13 in normohydrated and to 318 ± 12 ml/min ($p < 0.001$) in mannitol-loaded dogs.

Glomerular filtration rate (Fig. 1). Regression lines rise steeply in the hypotensive range; their slope in the normotensive range is, however, moderate and the difference of the regression coefficients against zero is not significant. In contrast to ureteral occlusion, in both free flow series the difference between regression coefficients in the hypotensive and normotensive ranges is significant ($p < 0.001$). In the autoregulatory range the average GFR is much higher in normohydrated dogs (64 ± 2) than

in mannitol-loaded ones (44 ± 2; $p < 0.001$). In dogs loaded with mannitol after ureteral occlusion GFR becomes zero and proximal tubular pressure equilibrates the ureteral pressure (*Selkurt* et al. 1965).

Glomerular capillary pressure (upper part of Fig. 2). In the hypotensive range the slope of the regression line is very steep in all experimental series. In the autoregulatory range the mild slope of both free flow sets differs definitely from the steep rise of the regression lines in the stop flow sets of experiments; the difference in regression coefficients is highly significant ($p < 0.001$). The significant difference between regression coefficients of the hypotensive vs. normotensive range ($p < 0.001$ for normohydrated and $p < 0.05$ for mannitol-loaded dogs) speaks strongly in favour of the view that GCP is autoregulated. The regression lines of the stop flow series are continuous, i.e. pressure dependence is pronounced and constant in the whole pressure range examined.

Table I

Parameters of renal function at various arterial pressure levels.
1. Normohydrated, free flow series

$\bar{x} \pm s_x$

AP-range, mm Hg	≦ 50	51—70	71—90	91—110	111—130	≧ 131
n	29	14	25	22	32	17
AP, mm Hg	47 ± 1	60 ± 1	80 ± 1	105 ± 1	125 ± 1	142 ± 1
GCP, mm Hg	40 ± 1	49 ± 2	57 ± 2	62 ± 2	63 ± 1	65 ± 2
DVP, mm Hg	15 ± 1	18 ± 1	21 ± 1	23 ± 1	23 ± 1	21 ± 1
COP_m, mm Hg	16 ± 1	18 ± 1	18 ± 1	17 ± 1	20 ± 1	21 ± 1
RVP, mm Hg	4 ± 1	5 ± 1	6 ± 1	7 ± 1	7 ± 1	5 ± 1
RBF, ml/min	261 ± 16	356 ± 29	401 ± 25	438 ± 26	426 ± 16	458 ± 24
Ht, per cent	39 ± 1	41 ± 1	38 ± 1	38 ± 1	38 ± 1	38 ± 1
GFR, ml/min	28 ± 3	40 ± 5	55 ± 2	64 ± 3	62 ± 2	66 ± 4
$E_{in} \cdot 100$	18 ± 1	19 ± 2	24 ± 1	25 ± 1	25 ± 1	23 ± 1
$E_{PAH} \cdot 100$	78 ± 2	76 ± 2	75 ± 2	75 ± 2	75 ± 2	76 ± 2
V, ml/min	0.01 ± 0.01	0.08 ± 0.03	0.60 ± 0.10	1.62 ± 0.26	1.41 ± 0.18	0.95 ± 0.18
TRR	1.08 ± 0.07	1.01 ± 0.09	1.22 ± 0.08	1.46 ± 0.09	1.77 ± 0.06	1.86 ± 0.07
IRR	0.81 ± 0.05	0.77 ± 0.07	0.97 ± 0.06	1.22 ± 0.08	1.53 ± 0.05	1.65 ± 0.09
AR	0.19 ± 0.04	0.23 ± 0.06	0.40 ± 0.04	0.65 ± 0.06	0.93 ± 0.04	1.06 ± 0.06
ER	0.62 ± 0.04	0.54 ± 0.04	0.57 ± 0.03	0.57 ± 0.03	0.60 ± 0.03	0.59 ± 0.04
VR	0.27 ± 0.03	0.24 ± 0.03	0.25 ± 0.02	0.24 ± 0.02	0.24 ± 0.02	0.21 ± 0.02

Renal vascular resistances in R-units: mm Hg/ml/sec/kg-kidney

Renal vascular resistances (Fig. 4) were calculated by the formula

$$\frac{RAP-RVP}{RBF}=\frac{RAP-GCP}{RBF}+\frac{GCP-DVP}{RBF}+\frac{DVP-RVP}{RBF}, \tag{2}$$

where RAP stands for renal arterial pressure which is assumed to equal systemic arterial pressure (AP). The left-hand side of Eq. (1) stands for total renal resistance (TRR), whereas the members on the right-hand side stand for afferent (AR), efferent (ER) and venular (VR) resistances. All resistances are calculated in R-units: mm Hg/ml/sec/kg kidney. Glomerular capillary pressure (GCP) is calculated by the formula

$$GCP = PTP + COP_m + GFR/3. \tag{3}$$

Table II

Parameters of renal function at various arterial pressure levels.
2. Normohydrated, stop flow series
$\bar{x} \pm s_{\bar{x}}$

AP-range, mm Hg	≧ 131	≦ 50	51—70	71—90	91—110	111—130	a131
n		8	9	8	6	19	10
AP, mm Hg		41 ± 1	61 ± 1	80 ± 1	103 ±	122 ± 1	140 ± 1
GCP, mm Hg		36 ± 2	51 ± 2	61 ± 3	76 ± 3	93 ± 3	101 ± 3
DVP, mm Hg		16 ± 3	20 ± 2	30 ± 2	36 ± 4	47 ± 2	52 ± 3
COP_m, mm Hg		15 ± 2	16 ± 2	16 ± 1	15 ± 1	18 ± 1	18 ± 1
RVP, mm Hg		4 ± 1	4 ± 1	5 ± 1	7 ± 3	7 ± 1	6 ± 1
UP, mm Hg		16 ± 1	25 ± 2	34 ± 2	45 ± 3	58 ± 2	66 ± 3
RBF, ml/min		197 ± 11	296 ± 19	382 ± 19	435 ± 47	465 ± 19	492 ± 17
Ht, per cent		38 ± 2	38 ± 2	39 ± 2	35 ± 2	38 ± 1	37 ± 1
GFR, ml/min		16 ± 2	31 ± 4	33 ± 3	49 ± 3	53 ± 3	52 ± 5
$E_{in} \cdot 100$		14 ± 2	16 ± 2	13 ± 1	18 ± 1	20 ± 2	18 ± 1
$E_{PAH} \cdot 100$		63 ± 5	64 ± 4	65 ± 4	66 ± 1	73 ± 3	76 ± 2
TRR		1.21 ± 0.10	1.18 ± 0.08	1.20 ± 0.07	1.39 ± 0.14	1.53 ± 0.06	1.65 ± 0.05
IRR		0.85 ± 0.11	0.85 ± 0.08	0.81 ± 0.06	0.95 ± 0.05	1.00 ± 0.04	1.08 ± 0.05
AR		0.16 ± 0.05	0.23 ± 0.05	0.30 ± 0.04	0.37 ± 0.04	0.38 ± 0.03	0.51 ± 0.05
ER		0.69 ± 0.11	0.62 ± 0.06	0.51 ± 0.06	0.58 ± 0.06	0.62 ± 0.03	0.57 ± 0.05
VR		0.36 ± 0.05	0.33 ± 0.03	0.39 ± 0.05	0.44 ± 0.10	0.53 ± 0.04	0.57 ± 0.04

Renal vascular resistances in R-units: mm Hg/ml/sec/kg-kidney

Deep venous pressure (lower part of Fig. 2) *and ureteral pressure* (Fig. 3). Regression lines are continuous, i.e. pressure dependent, in both sets of experiments following ureteral occlusion ($p < 0.001$). On the other hand, DVP in the normohydrated, free flow series is constant over the whole autoregulatory range. The moderate slope of the regression line in the mannitol-loaded, free flow series is on the border of significance ($p \sim 0.05$).

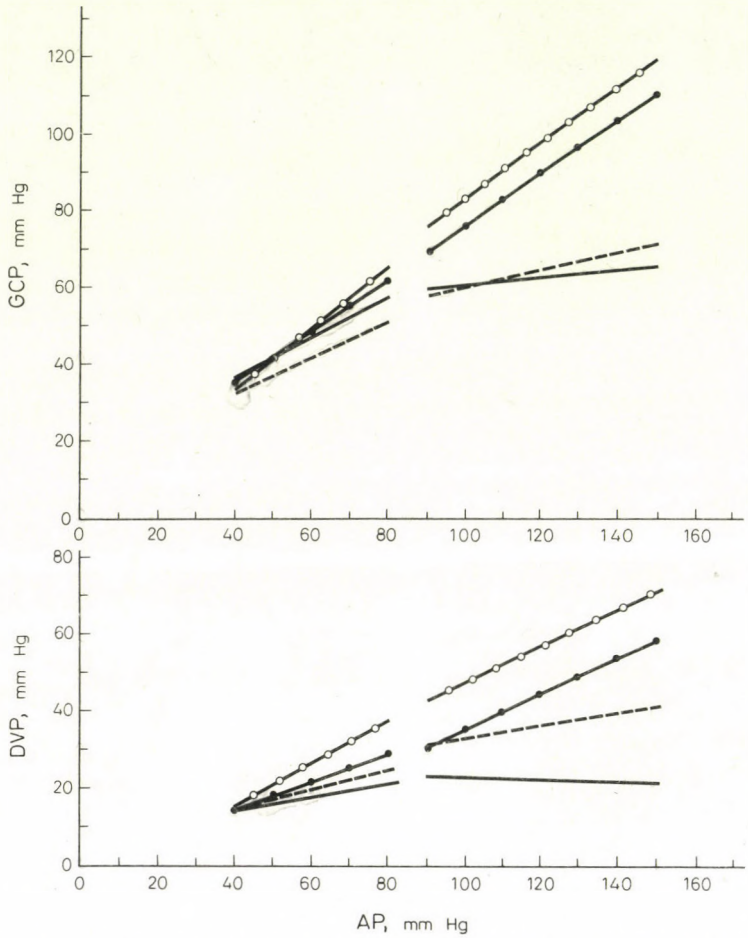

Fig. 2. Upper part: Relation of glomerular capillary pressure (GCP) to arterial pressure (AP). Lower part: Relation of deep venous pressure (DVP) to arterial pressure (AP). For symbols, see Fig. 1

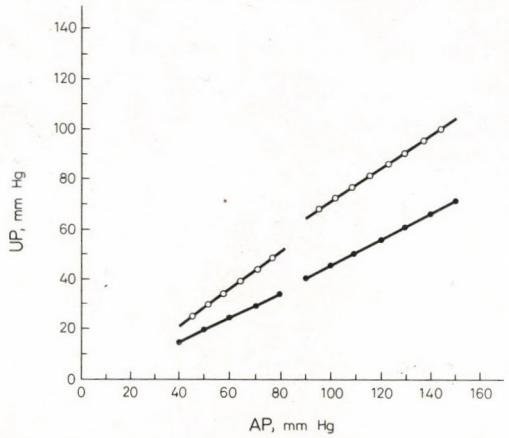

Fig. 3. Relation of ureteral pressure (UP) to arterial pressure (AP). For symbols, see Fig. 1

Table III

Parameters of renal function at various arterial pressure levels.
3. Mannitol-loaded, free flow series
$\bar{x} \pm s_x$

AP-range, mm Hg	≦ 50	51—70	71—90	91—110	111—130	≧ 131
n	12	11	16	18	14	17
AP. mm Hg	43 ± 1	61 ± 1	82 ± 2	102 ± 1	124 ± 1	145 ± 2
GCP, mm Hg	34 ± 1	42 ± 1	51 ± 2	59 ± 3	66 ± 3	72 ± 3
DVP, mm Hg	16 ± 1	20 ± 1	24 ± 1	32 ± 2	38 ± 2	41 ± 3
COP, mm Hg	14 ± 1	14 ± 1	15 ± 1	13 ± 1	14 ± 1	15 ± 1
RVP, mm Hg	5 ± 1	6 ± 1	7 ± 1	7 ± 1	8 ± 1	10 ± 1
RBF, ml/min	208 ± 18	298 ± 24	389 ± 22	425 ± 29	450 ± 25	455 ± 24
Ht, per cent	32 ± 2	33 ± 2	35 ± 2	32 ± 1	30 ± 1	33 ± 1
GFR, ml/min	12 ± 3	26 ± 3	36 ± 2	44 ± 3	41 ± 2	47 ± 2
$E_{in} \cdot \cdot 100$	9 ± 2	13 ± 1	13 ± 1	19 ± 2	13 ± 1	16 ± 1
$E_{PAH} \cdot 100$	76 ± 2	75 ± 2	62 ± 7	66 ± 3	63 ± 3	69 ± 2
V, ml/min	0.63 ± 0.20	1.68 ± 0.40	4.79 ± 1.00	5.43 ± 0.74	7.21 ± 0.52	8.21 ± 0.78
TRR	1.16 ± 0.10	1.15 ± 0.08	1.22 ± 0.07	1.42 ± 0.09	1.63 ± 0.11	1.87 ± 0.12
IRR	0.83 ± 0.09	0.87 ± 0.07	0.94 ± 0.07	1.06 ± 0.08	1.22 ± 0.10	1.44 ± 0.11
AR	0.29 ± 0.05	0.40 ± 0.05	0.51 ± 0.05	0.65 ± 0.07	0.84 ± 0.08	1.02 ± 0.09
ER	0.54 ± 0.05	0.47 ± 0.03	0.43 ± 0.03	0.41 ± 0.03	0.38 ± 0.02	0.42 ± 0.02
VR	0.33 ± 0.04	0.28 ± 0.03	0.28 ± 0.02	0.36 ± 0.04	0.41 ± 0.03	0.43 ± 0.05

Renal vascular resistances in R-units: mm Hg/ml/sec/kg-kidney

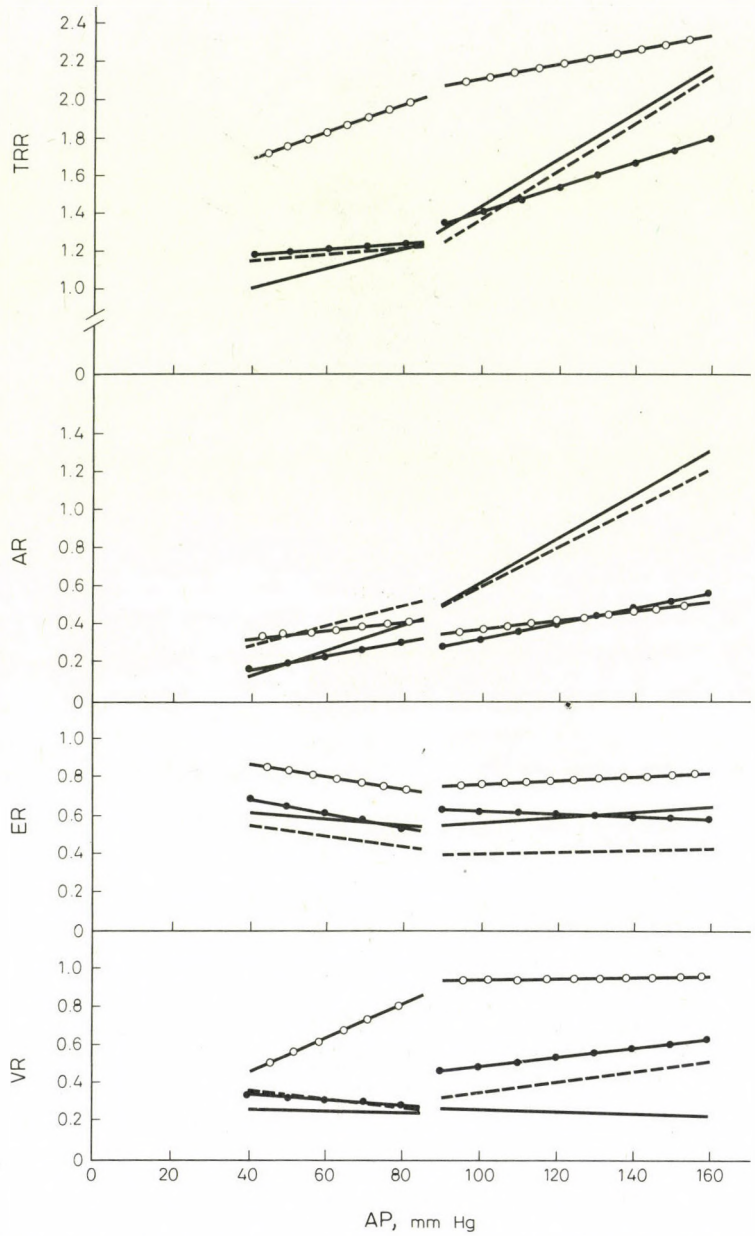

Fig. 4. Relation to arterial pressure (AP) of total renal resistance (TRR), afferent resistance (AR), efferent resistance (ER), and venular resistance (VR). For symbols, see Fig. 1

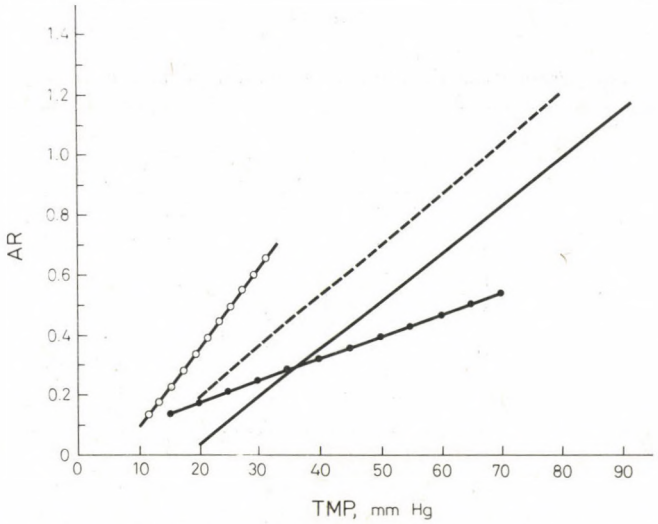

Fig. 5. Relation of afferent resistance (AR) to transmural pressure (TMP). For symbols, see Fig. 1

Table IV

Parameters of renal function at various arterial pressure levels.
4. Mannitol-loaded, stop flow series
$\bar{x} \pm s_{\bar{x}}$

AP-range, mm Hg	≦ 50	51—70	71—90	91—110	111—130	≧ 131
n	8	8	10	14	10	7
AP, mm Hg	40 ± 0	60 ± 0	82 ± 1	102 ± 2	123 ± 2	142 ± 3
GCP, mm Hg	34 ± 2	47 ± 2	67 ± 3	85 ± 3	101 ± 5	111 ± 5
DVP, mm Hg	17 ± 2	25 ± 3	39 ± 4	49 ± 3	53 ± 3	69 ± 7
COP_m, mm Hg	13 ± 1	13 ± 1	13 ± 1	12 ± 1	13 ± 1	15 ± 1
RPV, mm Hg	6 ± 1	6 ± 1	6 ± 1	7 ± 1	7 ± 1	8 ± 1
UP, mm Hg	22 ± 2	34 ± 2	57 ± 3	72 ± 3	88 ± 4	97 ± 4
RBF, ml/min	130 ± 9	186 ± 12	244 ± 18	277 ± 12	327 ± 17	385 ± 27
Ht, per cent	34 ± 3	34 ± 3	33 ± 2	30 ± 2	34 ± 1	37 ± 1
$E_{PAH} \cdot 100$	34 ± 6	44 ± 5	51 ± 5	49 ± 5	57 ± 3	59 ± 3
TRR	1.67 ± 0.14	1.81 ± 0.13	1.98 ± 0.16	2.11 ± 0.11	2.21 ± 0.14	2.17 ± 0.19
IRR	1.17 ± 0.19	1.21 ± 0.19	1.15 ± 0.17	1.15 ± 0.12	1.26 ± 0.10	1.21 ± 0.18
AR	0.28 ± 0.10	0.42 ± 0.08	0.40 ± 0.08	0.38 ± 0.05	0.43 ± 0.08	0.50 ± 0.09
ER	0.89 ± 0.20	0.79 ± 0.16	0.75 ± 0.13	0.77 ± 0.12	0.83 ± 0.10	0.71 ± 0.17
VR	0.50 ± 0.09	0.60 ± 0.08	0.83 ± 0.10	0.96 ± 0.06	0.95 ± 0.08	0.96 ± 0.09

GFR = 0 and $E_{in} = 0$ in all columns
Renal vascular resistances in R-units: mm Hg/ml/sec/kg-kidney

Table V

Parameters of glomerular dynamics in various experimental conditions

AP-range, mm Hg	40—50	51—70	71—90	91—110	111—130	131—160
	Normohydrated, free flow					
GFR, ml/min	28	40	55	64	62	66
GCP, mm Hg	40	49	57	62	63	65
PTP, mm Hg	15	18	21	23	23	21
ΔP, mm Hg	25	31	36	39	40	44
COP_m, mm Hg	16	18	18	17	20	21
EFP_m, mm Hg	9	13	18	22	20	23
	Normohydrated, stop flow					
GFR, ml/min	16	31	33	49	53	52
GCP, mm Hg	36	51	61	76	93	101
PTP, mm Hg	16	25	34	45	58	66
ΔP, mm Hg	20	26	27	31	35	35
COP_m, mm Hg	15	16	16	15	18	18
EFP_m, mm Hg	5	10	11	16	17	17
	Mannitol-loaded, free flow					
GFR, ml/min	12	26	36	44	41	47
GCP, mm Hg	34	42	51	59	66	72
PTP, mm Hg	16	20	24	32	38	41
ΔP, mm Hg	18	22	27	27	28	31
COP_m, mm Hg	14	14	15	13	14	15
EFP_m, mm Hg	4	8	12	14	14	16
	Mannitol-loaded, stop flow					
GFR, ml/min	0	0	0	0	0	0
GCP, mm Hg	34	47	67	85	101	111
PTP, mm Hg	22	34	53	72	88	97
ΔP, mm Hg	12	13	14	13	13	14
COP_m, mm Hg	12	13	14	13	13	14
EFP_m, mm Hg	0	0	0	0	0	0

It is assumed that under free flow conditions PTP = DVP and under stop flow conditions PTP = UP

It is assumed that PTP equals DVP under free flow and UP under stop flow conditions; glomerular filtration coefficient, k = 3 (*Bálint* et al. 1975). The regression lines of TRR can be regarded as the consequence of what was said about the relation of RBF to arterial pressure in the hypotensive and in the autoregulatory range. In the hypotensive range, regression coefficients do not differ significantly

from zero. The slope of the regression lines is equally negligible in the autoregulatory range under stop flow conditions. Under free flow conditions, however, there is a steady and significant increase in TRR in both normohydrated and mannitol-loaded dogs in the autoregulatory range.

As for the series connected intrarenal resistances, the most conspicuous changes in afferent resistance (AR) are to be stressed. Under free flow conditions there is a steady increase in afferent resistance over the whole pressure range examined. The increase in AR is about tenfold from AP = 40 mm Hg to 150 mm Hg. Under stop flow conditions, alterations are negligible and AR can be considered constant in spite of changes in arterial (perfusion) pressure.

Changes in efferent resistance (ER) do not seem to play a role in autoregulatory adjustments. The slightly decreasing slope of the regression lines in the hypotensive range is not significant. The role of venular resistance (VR) in autoregulatory adjustment seems to be negligible under free flow conditions. After ureteral occlusion, however, increased venular resistance plays a decisive role in the enhancement of total renal resistance, especially in dogs loaded with mannitol.

Discussion

Reliability of our data concerning renal blood flow and glomerular filtration rate need not be commented; the pressure drop along the branches of the renal artery is neglected and it is assumed that renal arterial pressure (RAP) equals systemic arterial pressure (AP). Circumstantial evidence concerning proximal tubular pressure (PTP), i.e. its assumed identity with DVP under free flow and with UP under stop flow conditions, was presented in our previous paper (*Bálint* et al. 1975). The same applies to glomerular capillary pressure (GCP), the calculation of which is based on PTP, COP_m, i.e. mean colloid osmotic pressure in glomerular capillary plasma, GFR and the glomerular filtration coefficient (k). Our assumption that k = 3 represented an average of data published in literature is likewise based on circumstantial evidence.

Most recently our assumptions which hitherto could be regarded as a working hypothesis only, have been verified by micropuncture experiments performed in the dog (*Knox* 1975); glomerular capillary pressure averaged 63.2 mm Hg and proximal tubular pressure averaged 21.0 mm Hg, yielding a net hydrostatic pressure difference of 41.8 mm Hg. Average mean colloid osmotic pressure (COP_m) calculated from the arterial plasma protein concentration and the filtration fraction, i.e. the extraction ratio of inulin, amounted to about 20 mm Hg in the normotensive phase of our normohydrated, free flow series (Table V). By collating the latter results with the data won by micropuncture, effective filtration pressure, i.e. $EFP = GCP - PTP - COP_m$, amounts to about 22 mm Hg in the canine kidney.

It is generally accepted that the number of glomeruli in the kidney of the adult dog amounts to about 10^6 per 100 g kidney weight; for data in the literature, see *Bálint* et al. (1975). In the present paper all parameters of renal function (RBF, GFR, V) have been calculated for 100 g kidney weight. By assuming a uniform distribution among the nephron population, mean nephron (MN) values for RBF and GFR are numerically equal to data presented in Tables I to IV; the dimensions, however, have to be converted to nl/min/nephron (nanolitres!) as compared to ml/min/100 g total kidney. MNGFR in our present normohydrated, free flow series averaged 64 nl/min/nephron. Mean glomerular filtration coefficient amounted to GFR/EFP, i.e. $64/22 = 2.91 \sim 3$.

In contradistinction to the rat kidney, in the canine kidney a "filtration disequilibrium"exists even at the distal end of the glomerular capillaries, i.e. net hydrostatic pressure difference exceeds colloid osmotic pressure along the whole length of the glomerular capillaries. It follows that filtration rate is much less dependent on renal plasma flow in the dog than in the rat (*Knox* et al. 1975). Parameters of glomerular dynamics of our present experimental series are presented in Table V; until proven to the contrary it is assumed that glomerular filtration coefficient (k) is independent of the actual perfusion (arterial) pressure and/or mannitol loading and/or ureteral obstruction.

I. Normohydrated, free flow series. RBF/pressure relationship compares well with similar data in literature (*Gilmore* 1964; *Navar* et al. 1966; *Kiil* et al. 1969; *Abe* et al. 1970; *Baer* et al. 1970; *Navar* and *Baer* 1970; *Navar* 1970; *Gassée* et al. 1974; etc.). In the autoregulatory range, i.e. beyond AP = 80 mm Hg, the regression line parallels the abscissa; in the hypotensive range the slope of the straight regression line corresponds to the approximate constancy of vascular resistance; in the normotensive range a rise in vascular resistance ensures the constancy of RBF.

GFR is somewhat less autoregulated than RBF. Our results are in accordance with *Navar*'s (1970) data but differ from those of *Abe* et al. (1970) who found that although autoregulation of RBF is complete above AP = 75 mm Hg, GFR declines significantly between pressures of 100 and 75 mm Hg. In conformity with micropuncture data in the rat (*Thurau* and *Wober* 1962) and in the dog (*Liebau* et al. 1968) glomerular capillary pressure (GCP) and deep venous pressure (DVP), assumed approximately to equal proximal tubular (PTP) and peritubular capillary pressure (PTCP), are well autoregulated, i.e. their value is practically constant over the whole range of autoregulation.

It follows that the brunt of autoregulation is borne by the afferent (preglomerular) arterioles. While efferent and venular resistances (ER and VR) are constant over the whole autoregulatory range, afferent resistance rises steadily in both the hypotensive (non-autoregulated) and the normotensive (autoregulated) pressure range. Its minimum amounts to about 0.12, its maximum to about 1.20 R-units at arterial pressures of 40 mm Hg and 150 mm Hg, respectively. The change in afferent resistance is about tenfold.

II. Normohydrated, stop flow series. Ureteral obstruction in nondiuretic conditions leads to partial suspension of autoregulation: RBF/pressure relations no longer show a plateau and appear almost passive in nature (Fig. 1, lower part). Mean RBF does not, however, differ significantly under free flow and stop flow conditions (433 ± 12 and 464 ± 13, respectively). The comparatively high values for GFR after ureteral occlusion seem rather surprising. Pressure dependence is slightly more pronounced as compared to free flow conditions (Fig. 1, upper part); averages amount to 64 ± 2 and 52 ± 2, respectively, in the autoregulatory range. The same was found by *Navar* and *Baer* (1970).

Following ureteral occlusion, stabilized ureteral pressure is definitely arterial pressure dependent (Fig. 3). Its value equals proximal tubular pressure (*Selkurt* et al. 1965; *Allison* et al. 1972). We have found that glomerular filtration proceeds at a reduced rate in spite of decreased arterial and increased ureteral pressures. The explanation of this remarkable phenomenon is provided by Fig. 2: glomerular capillary pressure (GCP) is pressure dependent and rises parallel with ureteral pressure (Fig. 3), i.e. proximal tubular pressure. The fact that net hydrostatic pressure difference (ΔP) within the glomeruli is only slightly less after ureteral occlusion than under free flow conditions (Table V) is due to the sharp increase in GCP.

As compared with free conditions, pressure dependence of afferent resistance after ureteral occlusion is less over the whole arterial pressure range examined: threefold vs. tenfold increase between arterial pressures of 40 mm Hg and 150 mm Hg, respectively. Afferent resistance reaches its minimum at about 40 mm Hg arterial pressure both under free flow conditions and following ureteral occlusion. In other words, total relaxation of the afferent arteriole can be brought about by decreasing perfusion (arterial) pressure and no further relaxation ensues on ureteral occlusion. On the other hand, at normal arterial pressure, e.g. 120 mm Hg, afferent resistance can be halved by obstructing the ureter in the non-diuretic dog.

As the pressure drop along the efferent arteriole and RBF do not change significantly after ureteral obstruction, no alterations in efferent resistance may be expected. This applies equally to the averages and to pressure dependence. Augmentation of intrarenal pressure caused by ureteral occlusion leads to compression of the thin-walled intrarenal veins with a consequential increase in DVP and a very pronounced increase in calculated venular resistance (VR). The pressure dependence of the latter is, however, negligible. The accidental role played by the passive increase in postglomerular resistance in maintaining glomerular filtration cannot be excluded with any certainty.

III. Mannitol loaded, free flow series. RBF/pressure relations are identical under free flow conditions in both normohydrated dogs and those loaded with mannitol; the same applies to mean values in the autoregulatory range: 433 ± 12 and 442 ± 16 for the normohydrated and mannitol loaded, respectively. The increasingly passive nature of the pressure-flow curves, as described by *Navar* et al. (1966) and *Kiil*

et al. (1969), was not observed in our experiments; the difference was due to the much higher doses of mannitol applied in their experiments.

Autoregulatory behaviour of GFR is not influenced by mannitol. Nevertheless, averages are significantly lower in the mannitol series: 44 ± 2 as contrasted to 64 ± 2 in the normohydrated ones. The significant drop in net hydrostatic pressure is not compensated by the drop in mean colloid osmotic pressure (Table V).

Total vascular resistance is constant in the hypotensive and rises steeply in the normotensive range (Fig. 4). Autoregulation is accomplished essentially by the steady increase in afferent resistance with higher arterial (perfusion) pressure. Pressure dependence of venular resistance is the consequence of pressure-dependent alterations in DVP (Fig. 2); deep venous pressure, assumed to represent both proximal tubular and peritubular capillary pressures, is much less autoregulated in mannitol-loaded than in normohydrated dogs. While total postglomerular resistance (ER + VR) seems to be the same in both normohydrated and mannitol-loaded dogs, average venular resistance is somewhat higher and efferent resistance lower in the mannitol-loaded series.

IV. Mannitol-loaded, stop flow series. Ureteral obstruction in osmotically loaded animals leads to a sharp rise in ureteral pressure which reaches much higher values than in nondiuretic preparations ("maximal pressure" of *Schirmeister* et al. 1962). Ureteral pressure is highly responsive to arterial pressure (Fig. 3). Net hydrostatic pressure equilibrates with colloid osmotic pressure and GFR becomes zero (Table V). In complete agreement with evidence published by others (*Nash* and *Selkurt* 1964; *Gilmore* 1964; *Kiil* et al. 1969; *Navar* 1970; etc.) pressure dependence of all renal parameters (RBF, GCP, DVP, UP) was found both below and within the autoregulatory range. Thus, autoregulation is completely abolished by the simultaneous application of mannitol loading and ureteral obstruction.

Neither total vascular nor series connected intrarenal resistances are pressure dependent (Fig. 4). Increase in postglomerular (ER + VR) resistances dominates over decreased afferent resistance (AR) with a resultant increase in total vascular resistance (TRR) and diminution of renal blood flow. According to *Navar* (1970) under such conditions afferent resistance is minimal and is the same under conditions of decreased arterial pressure and elevated ureteral pressure. Our data do not support this concept. Minimum afferent resistance, i.e. total relaxation of the afferent arterioles can be reached by decreasing arterial (perfusion) pressure to about 40 to 50 mm Hg under free flow or in nondiuretic preparations under stop flow conditions; it amounts to 0.12—0.20 R-units. In mannitol-loaded preparations ureteral occlusion lowers afferent resistance prevailing in osmotically loaded free flow preparations; at normal arterial pressure (about 120 mm Hg) it averages about 0.40 R-units, i.e. more than twice the minimum.

V. Additional evidence in favour of the myogenic hypothesis of autoregulation. According to the myogenic theory the muscular tone of the arterioles is determined by the transmural pressure gradient: its increase elicits vasoconstriction and a reduc-

tion in pressure gradient results in decreased renal vascular resistance (*Thurau* and *Kramer* 1959; *Nash* and *Selkurt* 1964; etc.). Relaxation of the afferent arterioles can be brought about by two different means: (i) reduction in arterial (perfusion) pressure and/or (ii) elevation of ureteral (intrarenal) pressure. Diminution of transmural pressure (TMP) is the common denominator in both types of experiment: while in the former intravascular pressure is decreased towards extravascular (intrarenal) pressure, in the latter extravascular (intrarenal) pressure is increased towards intravascular (arterial) pressure.

Regression equations between afferent resistance (AR) and transmural pressure (TMP mm Hg) were calculated and regression lines of the four experimental series are presented in Fig. 5. TMP was calculated by the formula

$$TMP = \frac{AP + GCP}{2} - DVP. \tag{4}$$

The difference of regression coefficients against zero is highly significant in all series ($p < 0.001$). The difference between the individual regression coefficients is not significant ($p > 0.05$) with the exception of the normohydrated stop flow series, the regression coefficient of which is significantly smaller ($p < 0.001$) than any other.

The conclusion that transmural pressure is one of the factors determining afferent resistance seems justified; these results can be regarded as an additional argument in favour of the myogenic theory of autoregulation.

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CHARACTERISTICS OF BLOOD PRESSURE AND NICTITATING MEMBRANE REFLEXES ELICITED BY ELECTRIC STIMULATION OF SCIATIC NERVE IN CONSCIOUS AND IN ANAESTHETIZED CATS

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Blood pressure (BP) reflexes and contractions of the nictitating membrane (NM) elicited by sustained (60 sec) stimulation of the sciatic nerve have been simultaneously recorded in gallamine immobilized conscious cats and following the administration of chloralose–urethane (50 and 300 mg/kg, respectively). By varying the stimulation parameters in a wide range, voltage and frequency characteristics of BP and NM reflexes have been plotted. In order to reduce the great number of possible BP-characteristics, the "dose" of stimulation (the product of voltage, impulse duration, frequency and of the stimulation period) has been introduced to plot "dose"–response curves. Differences in the BP and NM responses, and between awake and anaesthetized states as well as their probable causes are discussed. On the basis of the characteristic curves, a complex consisting of two facilitatory and at least one inhibitory mechanism has been supposed to be involved in the CNS integration of somato-sympathetic reflexes.

Electric stimulation of spinal afferents elicits somato-vegetative reflexes which show qualitative and quantitative changes varying the parameters of the stimulation. The changes are different in conscious and in anaesthetized animals (*Gutman* et al. 1961; *Johansson* 1962; *Katz* and *Perryman* 1965; *Khayutin* 1966; *Fedina* et al. 1966; *Bergmann* and *Gutman* 1966; *Molnár* et al. 1969).

In the present paper, experiments performed on conscious and on anaesthetized cats are described, in which the effects of serial stimulations of a mixed spinal nerve upon blood pressure and upon the activity of the nictitating membrane have been investigated simultaneously. Discussing the characteristic curves displaying the dependence on the stimulation parameters of the magnitude of the responses, an attempt has been made to elucidate some details of the reflex organization.

Some of the results have already been published (*Pavlik* et al. 1970; *Erdélyi* et al. 1971; *Mitsányi* et al. 1971b).

Methods

The experiments were performed on 60 cats of either sex, weighing between 1.9 and 4.6 kg. In superficial ether anaesthesia the trachea, one of the common carotid arteries, the right femoral vein and the left sciatic nerve were dissected free. The animals were immobilized by 5 mg/kg gallamine triethiodide (Flaxédil^{R}), and ventilated artificially throughout. Immobilization was maintained by additional doses of gallamine.

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Square wave electric stimulation of the sciatic nerve was performed by means of bipolar platinum electrodes. The following stimulation parameters were used. Strength: 1, 2, 4, 8, 16, 32 V; frequency: 1, 2, 4, 8, 16, 32, 64, 128 and in a few cases 256, 512 and 1024 imp/sec; duration: 0.1, 1.0 and 10.0 msec. Two of the parameters having been fixed, the third was varied in monotonously increasing, decreasing or in randomized sequence. The length of a train amounted to 60 sec.

Arterial blood pressure (BP) was measured by a mercury manometer in the carotid artery and recorded continuously. In this paper, the dependence of the maxima upon the stimulation parameters will only be presented; the magnitude of the response is given in per cents of the pre-stimulation value.

Nictitating membrane (NM) contractions on one side (usually the right) were recorded by means of a light isotonic lever. The magnitude of the responses is given either in absolute values (mm excursion on the record), or in per cent of the maximum attainable under the given experimental conditions.

The points in the Figures represent mean values of 10—15 corresponding responses recorded in different animals. The pressor and depressor phases of biphasic reactions were evaluated together with the points of the corresponding monophasic responses. Pressor reactions with post-stimulatory depression have not been considered as biphasic ones. The methods of statistical evaluation of the data are referred to in the corresponding paragraphs.

The experiments were started 30—60 min following the completion of all operative procedures. The responses of the conscious animals having been recorded, the cats were anaesthetized with chloralose–urethane (50 and 300 mg/kg, respectively) and the stimulation program was repeated.

Results

Blood pressure responses as a function of the strength of stimulation (Fig. 1)

In conscious animals pressor responses could only be recorded. In general, the magnitude of the reactions increased proportionally to the logarithm of stimulation voltage. In a few cases, two segments could be distinguished on the curves.

In contrast to the 100% responsiveness in the awake state, after anaesthesia sciatic nerve stimulation elicited BP responses in 77.9% of the trials. The reactions were pressor, biphasic and depressor in the proportion of 48.1, 12.5 and 17.3%, respectively.

Under anaesthesia, the pressor reactions were weaker than in the awake state, and the two segments on the curves were distinguishable more frequently.

Clear-cut depressor reactions were seen in response to weak stimuli only. Elevation of the stimulation voltage diminished or did not influence the size of the depressor response.

Blood pressure responses as a function of the stimulation frequency (Fig. 2)

Elevation of the stimulation frequency resulted in pressor responses of increasing magnitude. A characteristic feature displayed by all the curves was that the size of the reactions increased at first gradually, this was followed by a break or plateau after which a second increase was seen upon further elevation of the stimulation frequency up to 32—64 imp/sec. At the highest frequencies a diminution of the response occurred.

The depressor responses showed an opposite behaviour. The greatest reactions were usually recorded at 1—2 imp/sec, their magnitude and frequency of occurrence displayed a gradual decrease upon the elevation of the repetition rate.

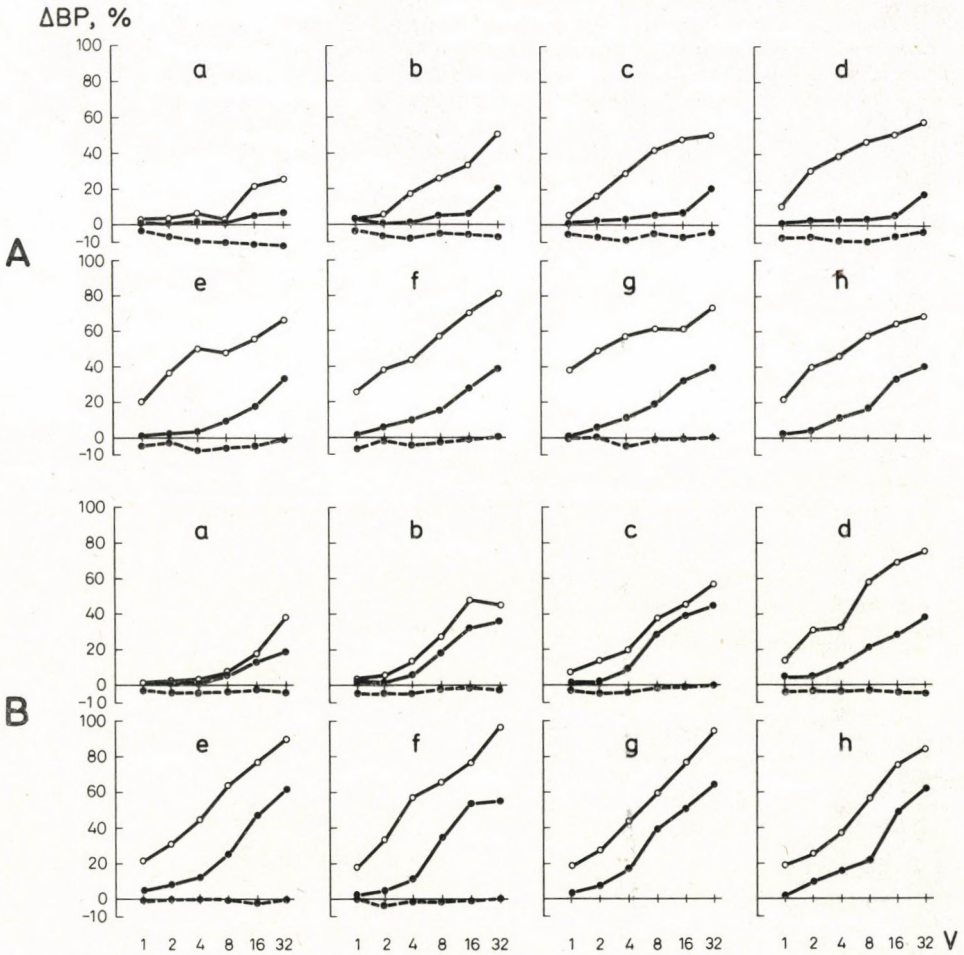

Fig. 1. Voltage characteristics of blood pressure reflexes elicited by electric stimulation of the sciatic nerve. Impulse duration: A: 0.1 msec; B: 1.0 msec. Repetition rate: 1, 2, 4, 8, 16, 32, 64, and 128 imp/sec in a, b, c, d, e, f, g and h, respectively. Stimulation period: 60 sec. Open circles: unanaesthetized, gallamine- immobilized cats. Dots: following the administration of chloralose-urethane (50 and 300 mg/kg, respectively). Continuous lines: pressor reflexes; dashed lines: depressor reflexes. All the points represent mean values

Effect of impulse duration on the blood pressure response

The magnitude of pressor reflexes depends on the duration of impulses, too: the longer the duration, the greater the reflexes. As for the depressor reflexes, with impulse durations of 0.1, 1.0 and 10.0 msec, their occurrence was 30.4, 12.1 and 8.0%, respectively; in case of trains consisting of longer impulses their magnitude was negligible if any.

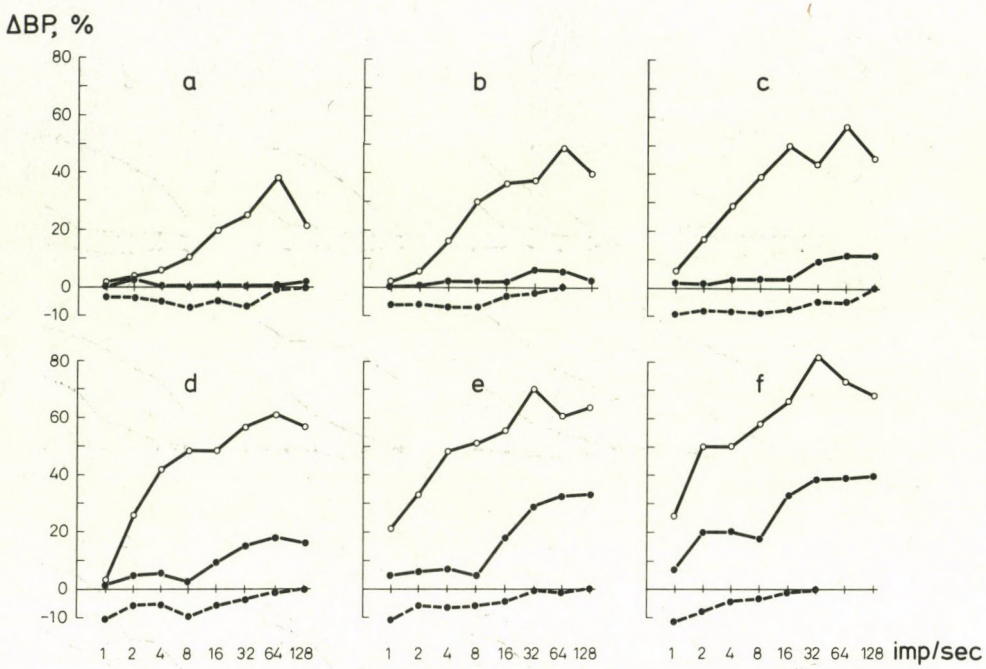

Fig. 2. Frequency characteristics of blood pressure reflexes elicited by electric stimulation of sciatic nerve. Impulse duration: 0.1 msec. Voltage: 1, 2, 4, 8, 16, and 32 V in a, b, c, d, e and f, respectively. Other details as in Fig. 1

Pressor responses as a function of the product of the stimulation parameters

The results demonstrated above clearly showed that the magnitude of the BP reaction depended on all the parameters of stimulation. As any of the latter can assume a large number of independent values, the dependence of the response upon the different parameters has to be plotted by several families of curves. We have therefore attempted to introduce an expression, characteristic of the "dose" of stimulation, by means of which a "dose"–response curve can be plotted. The product of the stimulation parameters seemed to satisfy the needs. Accordingly, the "dose" of stimulation (D) is defined as

$$D = U\tau f T \qquad (Vs)$$

where U is the strength of stimulation (V), τ the duration of the impulses (sec), f the repetition frequency (sec^{-1}) and T the train duration (sec; in our case: T = constant = 60 sec). In this analysis, responses in the voltage range of 1—4 V, and those elicited by higher frequencies which led to less than maximum responses were excluded. No attempt was made to analyze the "dose" dependence of the depressor reactions.

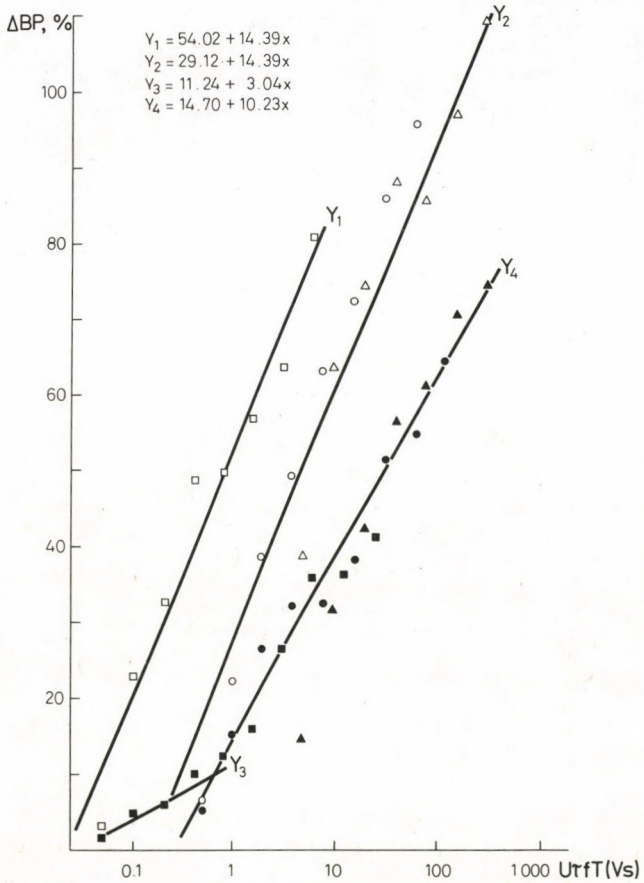

Fig. 3. "Dose"–response characteristics of blood pressure reflexes elicited by electric stimulation of sciatic nerve. The "dose" is defined as the product of voltage (U), impulse duration (τ), frequency (f) and of the stimulation period (T). Squares: products containing $\tau = 0.1$ msec; circles: those containing $\tau = 1.0$ msec; triangles: those containing $\tau = 10.0$ msec. Open symbols: unanaesthetized, gallamine-immobilized cats; filled dots: following the administration of chloralose–urethane (50 and 300 mg/kg, respectively). All the points represent mean values

Statistical evaluation of the data was performed by analysis of variance and of regression (*Brownlee* 1964).

The "dose" dependence of the pressor responses is shown in Fig. 3 and the results of the statistical calculations are presented in Table I. For plotting curves Y_1, Y_2, Y_3 and Y_4, 254, 330, 62 and 547 individual combinations, respectively, were used.

In conscious animals, the responses to 0.1 msec long stimuli and those to 1.0—10.0 msec long ones are represented by separate regression lines (Y_1 and Y_2, respectively). In both cases linearity was proven, the slopes of the two curves were identical, and the parallel shift in their position was significant statistically.

Table I

Main statistical data of the "dose"–BP response curves

	p_r	a	$\bar{b}$	p_b	b	a′	p_a
Y_1	< 0.005	53.55	13.45	> 0.3 (Y_1, Y_2)	14.39	54.02	< 0.001
Y_2	< 0.005	27.94	14.84		14.39	29.12	
Y_3	< 0.05	11.24	3.04	< 0.001 (Y_3, Y_4)	–	–	–
Y_4	< 0.005	14.70	10.23	< 0.001 (Y_{1-2}, Y_4)	–	–	–

Y_1: "dose"-response curve for "doses" consisting of stimuli of 0.1 msec duration; conscious animals

Y_2: "dose"-response curve for "doses" consisting of stimuli of 1.0 and 10.0 msec duration; conscious animals

Y_3: "dose"-response curve for low "doses" consisting of stimuli of 0.1 msec duration; anaesthetized animals

Y_4: "dose"-response curve for "doses" consisting of stimuli of 1.0 and 10.0 msec duration and for higher "doses" consisting of stimuli of 0.1 msec duration; anaesthetized animals

a and b: coefficients of the equation for the straight lines $Y = a + bx$

$\bar{b}$: common slope of parallel lines

a′: y-axis intercepts of parallel lines

Indices r, b and a at the levels of signifiance p refer to testing the existence of linear regression, testing parallelism of lines and to testing horizontal displacement of lines, respectively

Following anaesthesia, the majority of the responses fall on one and the same regression line (Y_4); only responses to small "doses" containing $\tau = 0.1$ msec are positioned differently (Y_3). Curve Y_4, compared to those recorded before anaesthesia, is shifted to the right and its slope is significantly less steep. Curve Y_3 is characterized by a negligible increase of responses on elevation of the "dose" in this range.

Responses of the nictitating membrane as functions of the strength and frequency of stimulation

The dependence of the NM response upon the voltage of stimulation is shown in Fig. 4; the corresponding statistical data are given in Table II.

In the low frequency range, the responses to the three different impulse durations are separated. At 8 imp/sec, however, all the points fall on the same regression line (cf. Table II). No further change can be seen if the frequency of stimulation is increased above this value. The frequency dependence of the reactions, confirming the statements outlined above, is demonstrated at one voltage value (32 V) only (Fig. 5).

After anaesthesia the NM relaxed and no reflex contraction could be elicited by stimulation of the sciatic nerve.

Table II

Main statistical data of the voltage–MN contraction curves

		P_r	a	$\overline{b}$	P_b	$\overline{b}$	a′	P_a
A	Y_1	< 0.005	3.86	6.01	> 0.1 (Y_1, Y_2)	9.01	–1.79	< 0.001
	Y_2	< 0.005	25.41	10.82	> 0.6 (Y_2, Y_3)	9.01	28.88	< 0.001
	Y_3	< 0.005	47.21	9.41	> 0.2 (Y_1, Y_3)	9.01	47.91	
B	Y_1	< 0.005	2.81	11.61	> 0.9 (Y_1, Y_2)	9.97	6.11	< 0.001
	Y_2	< 0.005	29.44	11.55	> 0.1 (Y_2, Y_3)	9.97	32.09	> 0.8
	Y_3	< 0.005	51.03	7.29	> 0.1 (Y_1, Y_3)	9.97	46.55	
C	Y_1	< 0.005	9.66	16.57	>0.8 (Y_1, Y_2)	16.36	10.06	< 0.001
	Y_2	< 0.005	28.81	16.20	—	16.36	28.53	—
	Y_3	< 0.1	—	—	—	—	—	
D	Y_1	< 0.005	28.48	11.61	> 0.7 (Y_1, Y_2)	12.51	26.52	> 0.7
	Y_2	< 0.005	37.47	12.90	> 0.4 (Y_2, Y_3)	12.51	38.16	> 0.9
	Y_3	< 0.025	42.72	9.42	> 0.6 (Y_1, Y_3)	12.51	37.09	

A, B, C and D: stimulations with frequencies of 1, 2, 4 and 8 imp/sec respectively
Y_1, Y_2 and Y_3: voltage-contraction curves to stimulations with 0.1, 1.0 and 10.0 msec impulse durations, respectively
Other details as in Table I

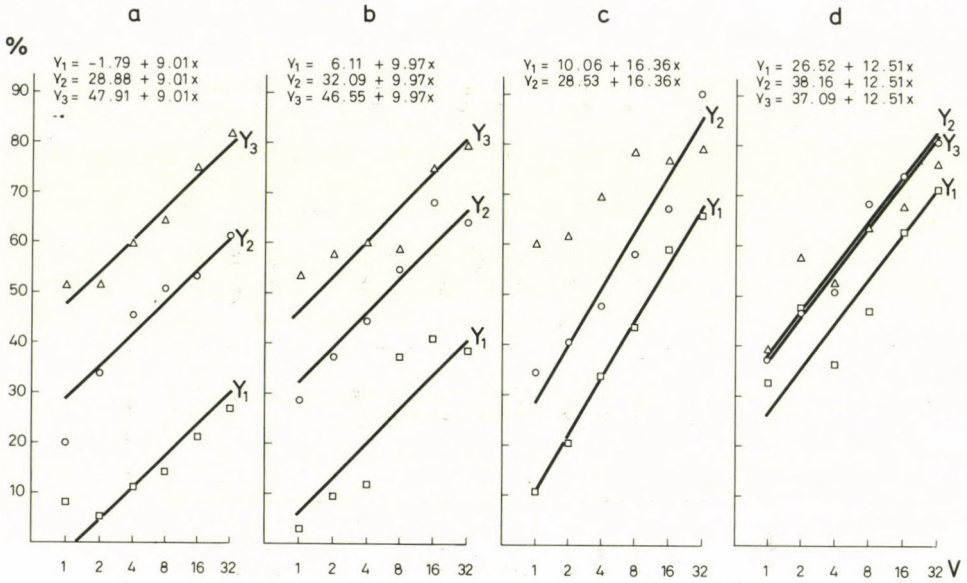

Fig. 4. Voltage characteristics of nictitating membrane contractions elicited by electric stimulation of sciatic nerve in unanaesthetized gallamine-immobilized cats. Repetition rate: 1, 2, 4, and 8 imp/sec in a, b, c and d, respectively. Impulse duration: squares: 0.1 msec, circles: 1.0 msec, triangles: 10.0 msec. Stimulation period: 60 sec. All the points represent mean values

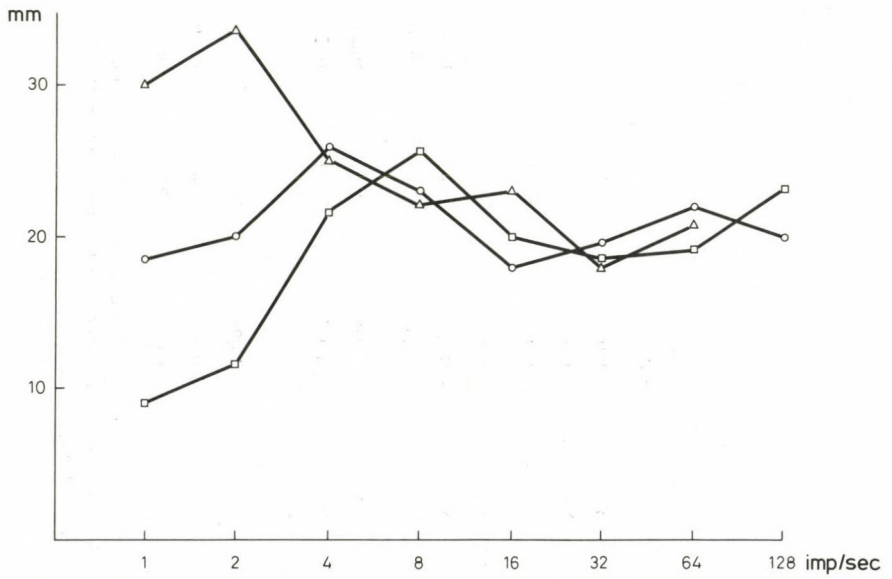

Fig. 5. Frequency characteristics of nictitating membrane contractions elicited by electric stimulation of sciatic nerve in unanaesthetized gallamine-immobilized cats. Voltage: 32 V. Other details as in Fig. 4

Discussion

Two simply registrable components of the complex somato-vegetative reflex following spinal afferent stimulation were investigated in awake and in anaesthetized cats. The starting point of the experiments was the suggestion (*Morison* and *Rioch* 1937; *Molnár* et al. 1969) that these two functions, although controlled partly or entirely by the sympathetic nervous system, may react to the same influence in different ways. We have supposed that the similarities and deviations of the curves displaying the dependence of BP and NM responses upon the stimulation parameters, may give some insight into the details of the central organization of these reflexes.

In the qualitative and quantitative shaping of a reflex response brought about by stimulating a mixed nerve, different modes and ways of summation take place. Increase of the voltage of stimulation or of the duration of impulses leads, on the one hand, to successive activation of various types of nerve fibres and, on the other, sets into activity an increasing number of fibres. The result is a spreading spatial summation of the activity and a gradual increase in the magnitude of the reflex response. Enhancement of the repetition rate — other parameters being fixed — brings about a temporal summation in the central nervous structures, leading similarly to the augmentation of the reflex response.

Low voltage stimulation yields negligible BP reactions in anaesthetized animals. Upon a stepwise increase in the voltage, a gradual augmentation of the response is seen. Reaching a certain voltage level, a sudden increase in the magnitude of the reflex occurs. At 0.1 msec impulse duration, this increase appears at 2 imp/sec and 32 V (Fig. 1Ab). It is seen at 16 V, if the frequency of stimulation is 16 imp/sec, and upon lengthening the impulse duration the threshold drops to about 8 V (Fig. 1B). In the conscious state, the corresponding BP responses are more marked and the curves display a steeper slope. If the two segments can be distinguished, a plateau at the higher voltage end of the first segment can sometimes be observed.

The characteristics outlined above can be explained by peripheral mechanisms. On stimulating the sciatic nerve, the fibre groups of Aα, Aβ, Aδ and C (*Erlanger* and *Gasser* 1937) and also the post-δ fibres described by *Koll* et al. (1961) can participate in the conduction of activity. According to *Brookhart* et al. (1953), *Skoglund* (1960), *Evans* (1961), *Koll* et al. (1961), *Johansson* (1962), *Katz* and *Perryman* (1965) and *Fedina* et al. (1966) low voltage stimulation, especially with short impulse duration, excites fibres belonging to the various A-subgroups only. For the activation of C-fibres an impulse duration of about 0.2 msec and stimulation voltages exceeding 16 V are required; impulses of longer duration (e.g. 1.0 msec) are able to excite C-fibres at correspondingly lower stimulation voltages (*Koslow* et al. 1973). Taking all these into consideration, the voltage dependence of the pressor responses could be explained by supposing that in the case of weak stimulation A-fibres only, and upon higher voltages both A- and C-fibres are involved in the afferentation. Our

results, being in accord with the data of *Koslow* et al. (1973), point, however, to the possibility that even the impulse duration of 0.1 msec is able to excite C-fibres, provided the stimulation voltage is sufficiently high.

The frequency dependent segments of the curves of the pressor responses cannot be explained solely on the basis of peripheral mechanisms, because they manifest themselves in those cases too, in which A-fibres alone are activated: The results can best be understood by postulating the involvement of two different central excitatory mechanisms in the organization of pressor reflexes. One of them is more sensitive and a smaller degree of summation is sufficient for its activation. If the summation reaches a given level, the second mechanism too is set into action. Both these mechanisms can be activated from the periphery via A- and C-fibres as well, although to different extents. Anaesthesia affects the two postulated structures quite differently.

Highest frequencies used bring about less than maximal responses. This might be explained by supposing either the intervention of an inhibitory mechanism, or the impairment of synaptic transmission at these frequencies. The phenomenon observed by *Koizumi* et al. (1970) that "at rates ovér 20/sec, such as 50—100/sec, C-fibres could not be excited readily" might also be taken into account.

Considering the sympathetic output as determined by the balance of central excitatory and inhibitory processes, the usual evaluation of pressor reflexes indicates only that the former outbalance the latter, the pressor characteristics do not offer any relevant information as to their shares.

Some insight into the interplay of facilitation and inhibition is, however, made possible by the analysis of the "dose"–response curves (Fig. 3). As noted earlier, for the construction of the curves both the voltage and frequency ranges were limited. The voltage values selected here were high enough to excite the C-fibres. Correspondingly, an elevation of the stimulation voltage in this range leads to an increase in the total number of fibres involved, but no qualitatively different peripheral structures are recruited. The frequency range taken into consideration contains all the values involved in the activation of the two supposed facilitatory mechanisms, while the highest frequencies producing a diminution of the response are omitted.

As for the meaning of the "dose" introduced, the basis of our reasoning has been that the energy (E) of any series of impulses can be given by the expression

$$E = \int_{t_0}^{t_1} I(t)\,U(t)\,dt,$$

where I is the current, U the voltage and t the time. In case of square wave impulses both I(t) and U(t) are continuous in the interval $[t_0, t_1]$, and provided no polarity reversal occurs, their signs remain unchanged. The mean value theorem for integrals may therefore be applied:

$$\int_{t_0}^{t_1} I(t)U(t)\,dt = I(\vartheta)\int_{t_0}^{t_1} U(t)\,dt \qquad (t_0 < \vartheta < t_1).$$

The energy of the series of impulses is accordingly proportional to the definite integral

$$\int_{t_0}^{t_1} U(t)\, dt$$

which, in case of square wave impulses, is equal to the product $U\tau fT$. Consequently, the "dose" is analogous to the energy of electric stimulation.

Comparison of the lines Y_1–Y_2 and Y_3–Y_4 in Fig. 3 indicates that following anaesthesia the curve is shifted to the right and its slope is less steep. This finding might suggest either a more marked depression of the facilitatory structures or a lesser extent of driving due to the afferent input being closed, or both. The curves point to a further fact. The parallel shift to the right of line Y_2 relative to Y_1 reveals the recruitment of an inhibitory mechanism if the impulse duration is increased from 0.1 to 1.0—10.0 msec. The existence of such an "internal" inhibitory mechanism was indicated in our previous work (*Khayutin* et al. 1969). Taking the position of the points after anaesthesia (lines Y_3 and Y_4) into consideration, one might suppose that for the activation of this inhibitory mechanism "doses" of at least 0.5—1 V are necessary. This suggests that the latter may contribute to the responses of the upper part of line Y_1, the level of its activity might, however, be negligible relative to that of the facilitatory mechanisms.

On the basis of these data, pressor reflexes are postulated to be organized by two facilitatory and at least one inhibitory structure. Activation of the latter needs a higher degree of central impulse summation than that sufficient for the excitation of the first, more sensitive, facilitatory mechanism.

The postulated inhibition is not identical with that mediated by buffer nerves. Our observations (*Mitsányi* et al. 1971a) indicated no qualitative change in the course of the function curves after sectioning the vagi and sinus nerves. Some aspects of the modifications due to baroreceptors have been dealt with in a previous paper (*Khayutin* et al. 1969).

In agreement with *Khayutin* (1966), depressor reflexes have been observed, as a rule, in anaesthetized animals only; they may be due to the lack of certain influences descending from hierarchically higher levels.

The characteristic dependence of NM responses upon the stimulation parameters is in apparent contradiction to the results of *Morison* and *Rioch* (1937). As for their "intensity" characteristics, this contradiction can simply be solved by logarithmic transformation of their data. Nevertheless, it has to be considered that the technical means used at that time did not allow a satisfactory control of the stimulation parameters.

The frequency dependence curves of the NM contractions are distinctly different from those of the BP responses. Increasing the stimulation frequency at a fixed voltage level (Fig. 5) results at first in a gradual increase in the magnitude of the response. Having reached a maximum, further increase of the frequency leads to smaller contractions. The lack of a second ascending segment suggests

that, in contrast to BP (cf. Fig. 2), one facilitatory mechanism is only involved in the integration of NM reflexes. The reduction of the latter at higher frequencies indicates the presence of an active inhibition which, judged by the voltage and frequency characteristics, may be identical with that discussed with the BP reflexes. The observation that following the activation of this inhibitory mechanism the NM responses — independently of the voltage and impulse duration of stimulation — display the same course, points to the self-limiting character of this facilitatory–inhibitory complex.

On elevation of the strength of stimulation, the NM contractions exhibit proportional increments (Fig. 4) resembling the majority of BP *vs.* voltage curves in the awake animals (Fig. 1).

The wide separation of NM responses corresponding to the three impulse durations in the low frequency range (Fig. 4a–c) cannot, however, fit into the self-limiting character of facilitation and inhibition. As an explanation, we might postulate the existence of another, lower threshold inhibition prevailing over the excitation at shorter impulse durations and lower frequencies or a lack or negligible degree of inhibition in this frequency range in the awake state.

The sharpest contrast between the NM and BP reflexes was observed in response to anaesthesia: the former ceased entirely (cf. *Molnár* et al. 1969), while the latter displayed distinctly different characteristic curves (Fig. 1). These changes substantiate the hypothesis that while the BP reactions are integrated, inter alia, by two facilitatory mechanisms, the NM is controlled by that one which is more deeply influenced by the anaesthetics.

The closer identification of the supposed facilitatory and inhibitory mechanisms cannot, however, be approached by the analysis of reflexes in response to stimulation of mixed nerves like the sciatic. In order to obtain finer discrimination in the integrating processes, differentiated selection, as for their origin and destination, of the afferent nerves to be stimulated is required.

Acknowledgement

The authors are indebted to Mrs. *A. Csukás* and Mrs. *R. Mága* for valuable advice on statistical analysis of the results.

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Communicatio Brevis

CORTICAL POTENTIALS ASSOCIATED WITH VOCALIZATION IN THE RHESUS MONKEY

By

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(Received February 2, 1976)

Cortical potentials associated with vocalization have been investigated in two rhesus monkeys. In both animals a slow negative potential shift beginning approximately 1 sec prior to the onset of vocalization has been observed in transcortical recordings from precentral areas. It is suggested that this slow potential change might be the phylogenetic antecedent of the voluntary control of speech in humans.

In recent years, cortical potentials associated with voluntary movements in monkeys have been described by a number of authors (*Vaughan* et al. 1970; *Donchin* et al. 1971; *Rebert* 1973). In self-paced tasks of voluntary hand and foot movements these potential changes present a complex waveform comprising three major components: an early slow shift of negative polarity, a phasic negative wave and a large positive wave. The very existence of these potentials and their striking similarity to the potential changes seen in man during similar behavioural acts (*Arezzo* and *Vaughan* 1975) pose an interesting question with respect to possible brain correlates of vocalization in monkeys. Specifically, it is known from the studies of *Robinson* (1967) and *Ploog* (1967) that expressive calls in monkeys can only be elicited by electrical stimulation of subcortical, limbic areas, and the participation of the neocortex in regulating this behaviour seems negligible. We have therefore investigated the cortical activity in relation to vocalization in rhesus monkeys to clarify whether or not the data on averaged cortical activity would conform to the observations gained by electrical stimulation.

Two young male *Rhesus macacus* monkeys served as subjects. One of them was regularly uttering a fairly uniform musical call ("isolation call") when brought into the experimental room. With the other monkey we used reinforcement to increase the frequency of isolation calls.

The monkeys were placed in a primate chair and their head was immobilized with iron bar restraints (Fig. 1). Vocalizations were picked up by a microphone placed over the head. Platinum electrodes were implanted over the precentral and parietooccipital regions and transcortical recordings were taken from epidural-subcortical white matter pairs. Brain activity was amplified by an EMG 16 channel

EEG apparatus with amplifier time constants set to 3 sec, and was stored on FM magnetic tape (TMC and Philips Analog-7). Averaging was performed off-line on a multichannel digital analyser (NTA-512) with the aid of a delay circuit which allowed to average brain activity 1.5–3 sec before vocalization.

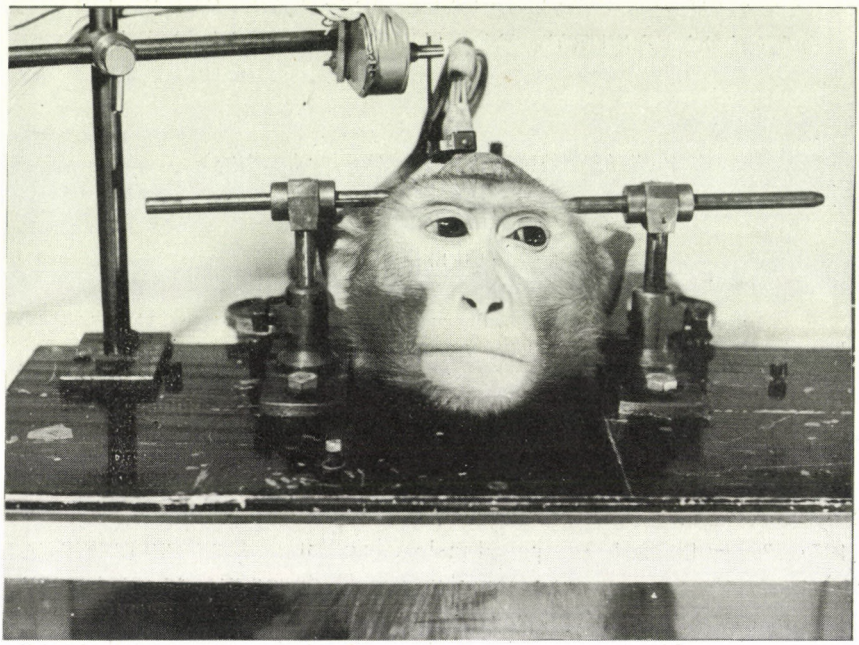

Fig. 1. Immobilization of the monkey's head

Figure 2 shows typical waveforms for the two monkeys. On the left, a record of the "spontaneously" vocalizing monkey is shown, on the right side the averaged tracing of activity in the second monkey who had been conditioned to vocalize. In the left column the largest activity and the most consistently appearing waveform in this monkey was over the precentral area closest to the midline, although the two other precentral leads show a similar pattern; a slow negative shift of the baseline developed approximately 1 sec prior to the onset of vocalization. Occasionally a small positive component beginning 20–60 msec before vocalization could be observed, which, together with the subsequent phasic negative peak, may be related to the activation of neural circuits commanding the vocal tract's musculature. The tracings from the parietooccipital regions showed no sign of comparable slow potential shifts in this monkey. In the averaged EOG record no systematic eye movements were apparent during the interval before vocalization, but simultaneously with vocalization a systematic eye movement could be observed.

In the records from the second monkey, before vocalizations slow negative potential shifts appeared in the two left precentral leads. In this case, activity at the lateral electrode began much earlier than that at the medial one.

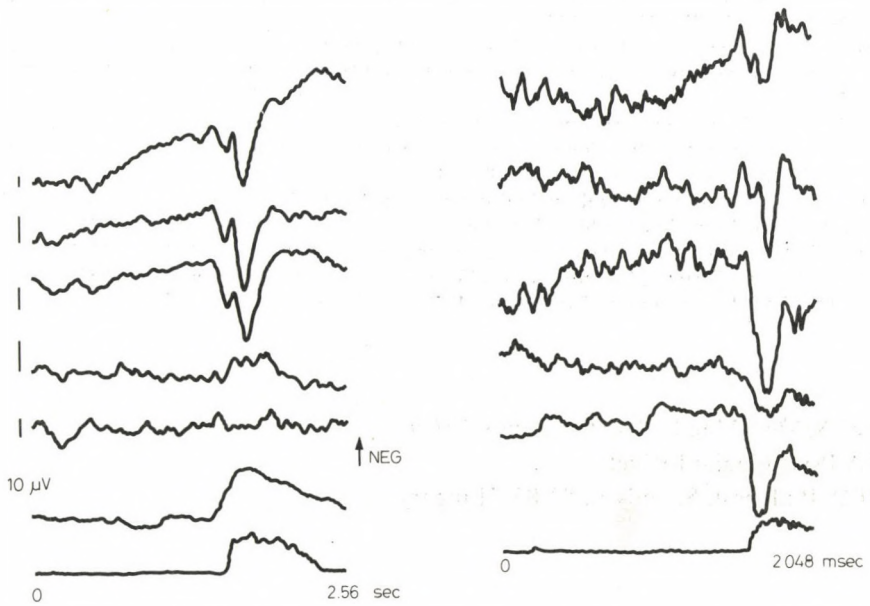

Fig. 2. Left side: from top to bottom, records from precentral medial, middle, lateral regions, parietal, occipital, EOG and rectified voice. Analysis time: 2.56 sec. Nr: 50. Calibration 10 μV. Positivity down. Right side: from top to bottom, left precentral, right precentral medial, left precentral lateral, parietal, averaged output of head movement transducer, rectified voice

The laterally situated electrodes were approximately above those cortical areas which *O'Brien* et al. (1971) described as corresponding to the human Broca area. In the Figures the averaged record of the monkey's head movements is also shown. This was accomplished by connecting a small rod on the monkey's head to a potentiometer, the output of which was amplified and averaged in the same way as was brain activity.

Thus, in two rhesus monkeys we have observed slow negative potential shifts over the precentral cortex preceding "spontaneous" vocalization. It is to be clarified whether these slow potential shifts have a functional role in the communicative vocalizations of these animals or are indirect expressions of motivational states. This neural activity might be the phylogenetic antecedent of voluntary control of human speech.

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Recensiones

M. WEISSBLUTH

Haemoglobin: Cooperativity and Electronic Properties

Molecular Biology, Biochemistry and Biophysics. Vol. 15. Springer-Verlag, Berlin, Heidelberg, New York 1974. VIII + 175 pages with 50 figures. Price: DM 72.–; US $ 29.40

As the author points out in the Preface, haemoglobin is one of the few biogenic molecules the complex function of which has been practically clarified. This work requiring several decades has been done mostly be the Cambridge scientist *Perrutz* and his team, and includes the exploration and exact description of the fine structure of haemoglobin. On this basis, it has become possible to set up plausible hypotheses, explaining the connection between the compound's structure and function.

The author selected two special problems connected with haemoglobin. In the first part of the book, the cooperativity characteristics of haemoglobin are discussed, which contribute to the physiologic function of the molecule, i.e. the oxygenation. The second part gives a quantum-mechanical approach of the iron component, with regard to its rather specific position in the haemoglobin molecule.

A further aim of the book is to illustrate the role of physicists, cooperating with chemical and biological experts in working out multidisciplinary biological problems.

The book is divided into nine chapters.

In Chapter 1, the author summarizes briefly the nomenclature of proteins and their components occurring in the haemoglobin molecule, and clearly demonstrates the primary, secondary, tertiary and quaternary structure of the compound.

Chapter 2 deals concisely with the primary structure of the haemoglobin molecule, it also discusses the similarities and differences in the haemoglobins of different species.

In Chapter 3, the characteristics of oxygen uptake and transport are described, with a quantitative evaluation of the special saturation curve of haemoglobin, as well as the thermodynamic and kinetic properties of the process.

Chapter 4 describes the data obtained with nuclear magnetic resonance and electron spin resonance spectrography, and discusses the possibilities in cooperativity investigations.

Chapters 5 and 6 summarize the present knowledge concerning the electronic state of the haemoglobin molecule and explain how these data help to understand the structure and function of haemoglobin.

In Chapters 7, 8 and 9, the results obtained from optical spectra and Mössbauer spectroscopy are detailed and, on this basis, further possibilities of haemoglobin research are discussed.

The reader familiar with biophysics will enjoy these chapters; they also demonstrate the use of modern physical tools in solving important theoretical and practical problems in biology.

This interesting book is recommended first of all for experts engaged in physical and biophysical experimental work. To read about the application of modern physical methods in research related to a molecule of such great theoretical and clinical importance as haemoglobin, will be impressive also for scientists less experienced in this field.

I. NAGY

D.M. GATES, R.B. SCHMERL (Eds)

Perspectives of Biophysical Ecology

Ecological Studies. Vol. 12. Springer-Verlag, Berlin, Heidelberg, New York 1975. XIII+609 pages with 213 figures and 64 tables. Price: DM 85.30, US $ 36.70

The book offers new aspects concerning the ecological systems, providing insight into the interrelations of the components of ecosystems. Some of these, as the trophic levels, the flow of energy, the flow of cyclic materials, and gains and losses of biomass, are the main factors controlling, regulating and influencing the processes of ecosystems.

The 31 papers presented at a symposium organized by Michigan University at the Douglas Lake Biological Station in 1973, cover several aspects of biophysical ecology. The authors (42 Americans and from other countries) were biologists with physical and mathematical interest and scientist trained primarily in physics and mathematics but interested in ecology.

That the book is not a mosaic of papers scattered all over the field concerned, is due to *D.M. Gates*, the editor and mentor of the symposium. He summarizes in his valuable and detailed introduction the headlines and the present achievements of modern biophysical ecology, based on 15 years work by himself and his research team. The main aims of the field involved are indicated in a physical and chemical approach based on mathematics. Short summaries, and useful evaluations are given by *Gates* for each of the following six parts: Analytical models of plants. Extreme climate and plant productivity. Water transport and environmental control of diffusion. Theoretical models of animals. Observations of animal body temperature. Energy-transfer studies of animals.

All but one of these items cover selected problems on terrestrial ecosystems. Still, the book is of interest for its methodics used, the information on modelling different aspects of ecosystems, for everybody working in ecology.

Finally the results and recommendations of the symposium are summarized in the conclusions. After approaching the questions of biophysical ecology through physical and chemical means using mathematics as a tool, the necessity of the preparation and improvement of mathematical models is underlined, taking the complexity of this branch of science into consideration.

In our times when the solution of ecological problems is more and more imminent, this book may be of interest for biologists as well as for mathematicians, physicists and chemists interested in environmental biology.

B. ENTZ

A.C. NEVILLE

Biology of the Arthropod Cuticule

Zoophysiology and Ecology. Vols 4—5. Springer-Verlag, Berlin, Heidelberg, New York 1975. XVI+448 pages with 233 figures. Price: DM 145.—; US $ 62.40

According to recent information in the Current Contents, one third of the world's agricultural production was destroyed by arthropods. This fact alone suffices to show the importance of research on the life of these animals which compose the most crowded group of the animal world. Considering their agricultural significance as well as their scientific importance, it is not surprising that a number of books and papers have been published on Arthropods in the last decades.

A.C. Neville's monograph appeared in the series of Zoophysiology and Ecology. "The cuticule is commonly regarded as an inert substance", is written in the preface of the book. The author provides data showing that this statement is erroneous. The integument has a multiple role in the life of arthropods and a number of up-to-date complex methods are necessary to clarify the chemical composition, subcellular structure and development of the arthropod cuticule. The book contains 10 chapters: one deals with the general structure of the cuticule, the others describe its molecular and supermolecular architecture, the physical properties as well as the physiological aspects. To illustrate the results 233 photographs and drawings and 1100 references are given. The book will prove useful for both students and research workers.

I. BENEDECZKY

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PHYSIOLOGIA

RECENSIONES

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ACTA PHYSIOLOGICA

ТОМ 49 – ВЫП. 1

РЕЗЮМЕ

IN VIVO ИЗОТОП-КИНЕТИЧЕСКОЕ ИССЛЕДОВАНИЕ МЕТАБОЛИЗМА ЖИРОВЫХ КИСЛОТ В БЕЛОЙ ЖИРОВОЙ ТКАНИ У ОТКОРМЛЕННЫХ И ГОЛОДАЮЩИХ НОВОРОЖДЕННЫХ КРОЛИКОВ ПРИ ОСТРОМ И ДЛИТЕЛЬНОМ ЭФФЕКТЕ ХОЛОДА

Т. ХЕЙМ, Х. ШЕНК, Ф. ВАРГА и Е. ГЁЦЕ

После внутривенозного введения 20×10^6 имп/мин ^{14}C-пальмитиновой кислоты на 100 г веса тела авторы определяли количество и специфическую радиоактивность липидов белой жировой ткани (БЖТ), и сток свободных жировых кислот плазмы в эстерифицированные и неэстерифицированные жировые кислоты БЖТ у 7-дневных кроликов, распределенных на четыре группы: группа I: откормленные животные при термонейтральной окружающей среде; группа II: откормленные животные, воспитанные в холодном окружении; группа III: голодающие животные при 35 °C; группа IV: голодающие животные при 20 °C. Опыты производились у каждой группы при окружающей среде 20 °C.

Количество эстерифицированных и неэстерифицированных жировых кислот БЖТ уменьшалось как у откормленных, но воспитанных в холоде, животных (группа II), так и у голодающих животных (группа III и IV).

Специфическая радиоактивность тканевой свободной жировой кислоты и жировой кислоты, связанной глицеридами и фосфолипидами была самая высокая у группы III и IV; это показывает, что голодание, предшествующее острому эффекту холода, значительно увеличивает метаболизм жировых кислот в белой жировой ткани.

Сток свободных жировых кислот между плазмой и БЖТ повышался в 2 раза у группы IV, но и таким образом это было только 1/5 стока, наблюдаемого между плазмой и бурой жировой тканью, показывая, что участие БЖТ в теплопродукции, вызванной холодом у новорожденных кроликов, имеет второстепенное значение.

АДАПТИВНЫЕ ПРОЦЕССЫ У ДЕТЕЙ-ПЛОВЦОВ И У ВЗРОСЛЫХ ПЛОВЦОВ СВЕРТЫВАНИЕ И ВЯЗКОСТЬ КРОВИ

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Исследовались свертывание, вязкость и кислотность крови, метаболизм протеинов, мышечные энзимы, ионы, гематологические, кардиальные и эндокринные параметры у детей-пловцов и у взрослых пловцов в раннем периоде тренировки и в субмаксимальном состоянии тренировки в течение годового периода соревновании.

В раннем периоде тренировки физическое занятие вызывало фибринолиз и снижение уровня фибриногена и тромбина и число тромбоцитов. Такое же занятие в тренированных организмах не вызывало таких изменений.

КИСЛОТНО-ЩЕЛОЧНЫЕ ПАРАМЕТРЫ У ПЛОВЦОВ И ШТАНГИСТОВ

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Кислотно-щелочные параметры взрослых плавцов и взрослых штангистов исследовались до и после тренировки в течение субмаксимально тренировочной фазы годового периода тренировки.

У пловцов тренировка вызывала некомпенсированный метаболической ацидоз, который наблюдался и через 10 мин после тренировки. Этот ацидоз не показывал никакой корреляции с уровнем лактата крови.

У штангистов наблюдался только слабый, компенсированный ацидоз после тренировки, который через 10 мин показывал тенденцию к уменьшению. У них нашли очень высокий посттренировочный уровень аммония. Авторы предполагают, что генез аммония играет роль в компенсации ацидоза, вызванного занятием, и что разницы кислотно-щелочного состояния у пловцов и штангистов могут находиться в связи с дыхательными механизмами, которые нужны для двух разных видов спорта.

ЭФФЕКТ СТРЕССА НА АКТИВНОСТЬ СЕРОТОНИНЭРГИЧЕСКОЙ СИСТЕМЫ В ЛИМБИЧЕСКИХ МОЗГОВЫХ СТРУКТУРАХ И НА ЕЕ КОРРЕЛЯЦИЮ С ГИПОФИЗАРНО-АДРЕНАЛОВОЙ ФУНКЦИЕЙ У КРЫС

И. ВЕРМЕШ и Г. ТЕЛЕГДИ

Через 15 мин после эфирного стресса содержание серотонина (5—НТ) в гипоталамусе уменьшалось, а после электрического шока не наблюдалось никакого уменьшения в мезенцефалоне и гипоталамусе. Через 30 мин после обоих стрессоров повышение содержания 5—НТ наблюдалось в мезенцефалоне и ядре миндалины, и через 60 мин — в гипоталамусе.

Через 15 мин после электрического шока содержание 5-гидроксииндол-уксусной кислоты (5—HIAA) повышалось в ядре миндалины, через 30 мин после обоих стрессов — в мезенцефалоне и гипоталамусе и после эфирного стресса — в ядре миндалины.

На содержание 5—НТ и 5—HIAA в гиппокампе и перегородке ни один из стрессоров не влиял.

Метаболизм 5—НТ уменьшался в период между 10 и 20 мин после эфирного стресса в гипоталамусе и после электрического шока в мезенцефалоне и гипоталамусе. В период между 50 и 60 мин после электрического шока метаболизм 5—НТ увеличивался в мезенцефалоне. Другие области мозга не показывали значительного отклонения от контрольной величины 5—НТ. Прием ^{3}H—5—НТ уменьшался в мезенцефалоне и гипоталамусе через 20 мин после эфирного стресса и возвращался к нормальной величине через 60 мин.

Уровень кортикостерона в плазме был самым высоким через 30 мин после обоих стрессоров и возвращался к норме через 90 мин. Поэтому, вызванное стрессом уменьшение серотонинэргической активности в некоторых лимбических мозговых структурах, особенно в гипоталамусе, доказывает обратную связь с гипофизарно-адреналовой активностью. Эти данные поддерживают концепцию о том, что серотонинэргическая система играет ингибиторную роль в гипофизарно-адреналовой функции.

ЛОКАЛЬНЫЙ КРОВОТОК ГИПОТАЛАМУСА В ГЕМОРРАГИЧЕСКОЙ ГИПОТЕНЗИИ

И. НЯРИ, Е. МАКЛАРИ, А. Г. Б. КОВАЧ и И. ХАРКА

Измерялся локальный кровоток гипоталамуса методом водородного клиренса у наркотизированных собак. Средняя величина кровотока гипоталамуса у нормальных контролей была $0{,}64 \pm 0{,}05$ мл/г/мин, которая соответствует литературным данным. В течение геморрагической гипотензии, вызванной модифицированным методом Wiggers, кровоток гипоталамуса значительно уменьшался — до 52% исходящей величины при среднем артериальном кровяном давлении 55—60 мм рт. ст., и до 44% при 35—40 мм рт. ст.

Превенция последующего внеклеточного ацидоза с помощью инфузии бикарбоната в течение периода гипотензии имела результатом значительно меньшее уменьшение кровотока гипоталамуса, сравнивая с необработанной контрольной группой. Эта разница в

кровотоке гипоталамуса между группами, которым инфузировали бикарбонат и физиологический солевой раствор, была тоже значительной при кровяном давлении 55—60 мм рт. ст.

У необработанных животных в течение кровоиспускания неизбежно образовывалась гипоксия вследствие недостаточного циркуляторного транспорта кислорода, а у группы, у которой ацидоз был компенсирован, условия аэробного метаболизма контрольного уровня были обеспечены.

Авторы исследовали и факторы, которые влияют на локальный кровоток гипоталамуса при тяжелой гипотензии, и нашли строгую корреляцию между гипоталамическим кровотоком и артериальным pH ($r = 0{,}7026$; $p < 0{,}001$) при среднем артериальном кровяном давлении ниже 60 мм рт. ст.

АВТОРЕГУЛЯЦИЯ ПОЧЕЧНОЙ ЦИРКУЛЯЦИИ У СОБАК ПРИ СВОБОДНОМ ТЕЧЕНИИ МОЧИ И ПРИ ОККЛЮЗИИ МОЧЕТОЧНИКА

П. БАЛИНТ, Е. СЁЧ, Е. ТАРЯН и Й. ЮСКО

Авторами исследовалась связь параметров функции почки [почечный кровоток (RBF), клубочковая фильтрация (GFR), клубочковое капиллярное давление (GCP), глубокое венозное давление (DVP), мочеточниковое давление (UP)] и последовательно включенных внутрипочечных резистенций с артериальным (перфузирующим) давлением у наркотизированных собак при разных экспериментальных условиях.

Установили, что:

1. У нормогидрированных собак при свободном течени мочи RBF и GFR резко повышаются в гипотенсивном периоде (40—80 мм рт. ст.) а константные в нормотенсивном периоде (90—150 мм рт. ст.); то же самое относится к GCP и DVP. Авторегуляция, т. е. постоянство RBF и GFR, является следствием соответствующего повышения афферентной резистенции.

2. Окклюзия мочеточника прекращает авторегуляцию. RBF, GFR, GCP, DVP и UP повышаются пропорционально артериальному давлению при его всех исследованных величинах.

3. После маннитольной нагрузки при свободном течении мочи авторегуляция наблюдается. Она обеспечивается изменением афферентной резистенции. Эфферентная резистенция слабо снижается, венулозная резистенция слабо повышается.

4. Окклюзия мочеточника при маннитольной нагрузке полностью прекращает авторегуляцию. Каждый функциональный параметр повышается пропорционально артериальному давлению. Васкулярная резистенция повышается вследствие повышения эфферентной и венулозной резистенции.

ХАРАКТЕРИСТИКА РЕФЛЕКСОВ КРОВЯНОГО ДАВЛЕНИЯ И МИГАТЕЛЬНОЙ ПЕРЕПОНКИ, ВЫЗВАННЫХ ЭЛЕКТРИЧЕСКИМ РАЗДРАЖЕНИЕМ СЕДАЛИЩНОГО НЕРВА У БОДРСТВУЮЩИХ И НАРКОТИЗИРОВАННЫХ КОШЕК

А. ЭРДЕЙИ, А. МИЧАНИ, И. МОРАВА, Г. ПАВЛИК и А. ТАЛАШИ

У бодрствующих кошек, обездвиженных галламином, а также после внутривенного введения хлоралозы и уретана (50 и 300 мг/кг), одновременно записывались изменения кровяного давления и сокращения мигательной перепонки в ответ на длительное (60 сек) электрическое раздражение седалищного нерва. Изменяя в широком диапазоне параметры раздражения, были составлены вольтажные и частотные характеристики рефлексов кровообращения и мигательной перепонки. В целях сокращения большого числа всевозможных характеристик был введен термин «доза» электрического раздражения (произведение напряжения, длительность стимула, частота и период раздражения), с помощью которого

были изображены кривые «доза»-ответ. Дискутируются разницы в характеристиках ответов кровяного давления и мигательной перепонки, разницы характеристик в бодрствующем и наркотизированном состояниях, а также и их возможные причины. На основе полученных кривых предполагается, что в центральной нервной организации сомато-симпатических рефлексов принимают участие два активирующих и один или два тормозных механизма.

КОРКОВЫЕ ПОТЕНЦИАЛЫ, СВЯЗАННЫЕ С ВОКАЛИЗАЦИЕЙ У МАРТЫШЕК

Й. СИРТЕШ, М. МАРТОН и Й. УРБАН

У двух мартышек при фиксированном состоянии головы регистрировали имплантированными электродами потенциалы, предшествующие и сопровождающие спонтанную вокализацию. При этом регистрировали медленный негативный сдвиг потенциала на фронтальной моторной коре. Этот ответ сходен с изменением потенциала, предшествующего движению и речи человека.

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Physiologia

SOME PROPERTIES OF HUMAN PROGRESSIVE ANTITHROMBIN

By

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(Received February 12, 1976)

The effect of some mono- and divalent cations was examined on thrombin—antithrombin reaction *in vitro*. It was found that 0—0.1 M sodium- or potassium chloride did not affect either thrombin or antithrombin activity; at higher concentrations thrombin activity decreased. Calcium chloride as well as magnesium chloride at concentrations from 0 to 0.05 M increased enzyme activity, whereas at higher concentrations the activity decreased. Thrombin inactivation by antithrombin was also accelerated at calcium or magnesium chloride concentrations above 0.04 M.

Antithrombin was inactivated at pH 7.3 at 65 °C in some minutes and heparin failed to protect it against heat denaturation. Thrombin inactivation by antithrombin did not proceed at 0 °C in 60 min, but the interaction between thrombin and antithrombin was facilitated in the presence of heparin.

Antithrombin-III (heparin cofactor) is one of the most important factor in the regulation of blood coagulation. It forms an inactive complex with thrombin and the complex formation depends on the interaction between the serine active centre of thrombin and an arginine reactive site of antithrombin (*Rosenberg* and *Damus* 1973). This mechanism suggests that all serine proteases in the coagulation process may be inhibited in a similar manner (*Damus* et al. 1973). So far, this hypothesis proved to be correct for thrombin, Factor Xa and Factor XIa, i.e. antithrombin purified from plasma inhibits these factors (*Damus* et al. 1973; *Yin* et al. 1971a). Furthermore, antithrombin-III and Factor Xa-inhibitor proved to be identical molecules (*Yin* et al. 1971b).

Heparin is known to accelerate the rate of thrombin—antithrombin complex formation (*Gerendás* et al. 1949; *Damus* et al. 1973). After some contradictory data (*Porter* et al. 1967; *Damus* et al. 1973) it is also probable that antithrombin-III and heparin cofactor are identical proteins. Some recent data suggest, however, that two distinct heparin cofactors exist in human plasma (*Briginshaw* and *Shanberge* 1974).

With regard to the mechanism of action of heparin on the thrombin–antithrombin reaction, it is assumed that thrombin as well as antithrombin-III may undergo a conformational change in the presence of heparin (*Machovich* 1975; *Machovich* et al. 1975b).

In this paper some properties of human antithrombin-III are presented. The effect of some mono- and divalent cations and heparin has been studied on the thrombin—antithrombin reaction and on the antithrombin stabilization.

Methods

Thrombin activity was measured in a 0.25 ml reaction mixture containing 10 μmoles of Tris-HCl buffer, pH 7.3, 3—4 μg of thrombin protein (200 NIH units per mg protein), and 500 μg of fibrinogen (KABI Grade L, human, lyophilized). Clotting time was recorded with a Hyland Clotek System. Thrombin activity, expressed in NIH units, was calculated from a standard calibration curve determined by bovine thrombin (Topostasin, Hoffman-La Roche).

Thrombin was partially purified from Topostasin as published by *Yin* and *Wessler* (1968).

Antithrombin activity was measured in 0.20 ml of a reaction mixture containing 10 μmoles of Tris-HCl buffer, pH 7.3, 4 μg of thrombin protein, and 50 μg of antithrombin protein. After 3 min incubation at 37 °C, 500 μg of fibrinogen in 0.05 ml volume containing 9 μmoles of sodium chloride was added and clotting time was recorded as mentioned above.

Antithrombin-III was purified and provided by the American Red Cross Blood Research Laboratory.

Protein was measured according to the method of *Lowry* et al. (1951).

Results

Effect of cations on thrombin–antithrombin reaction

The influence of Na^+, K^+, Ca^{++} and Mg^{++} on the thrombin–fibrinogen and thrombin–antithrombin reaction has been examined. Neither sodium chloride nor potassium chloride affected thrombin activity between 0 and 0.1 M concentration (Fig. 1). As the fibrinogen had been dissolved in sodium chloride, the results marked as without sodium chloride only mean that sodium chloride was not added in the reaction mixture. At higher concentrations (up to 0.4 M), the clotting time increased from 10 sec to 40 sec. The effect of Na^+ or K^+ on the thrombin–antithrombin reaction was very similar to their effect on thrombin activity, namely at 0.25 M concentration the clotting time increased from 30 sec to 50 sec and at 0.4 M it was near 100 sec.

In the presence of $1—2 \times 10^{-2}$ M calcium or magnesium chloride, thrombin activity increased (clotting time decreased) (Fig. 2). At higher concentrations clotting times were rapidly prolonged. Above 0.03 M, the effect of Ca^{++} and Mg^{++} was different, the decrease of thrombin activity was more rapid in the presence of Ca^{++} than of Mg^{++}. The antithrombin–thrombin reaction was also influenced by these divalent cations. 0.03 M magnesium chloride decreased clotting time from 30 sec to 18 sec, but at higher concentrations it was prolonged and at 0.18 M it was over 100 sec. Ca^{++} gave a similar result but the decrease of clotting time was more expressed (from 30 sec to 10 sec) and the increase was more rapid.

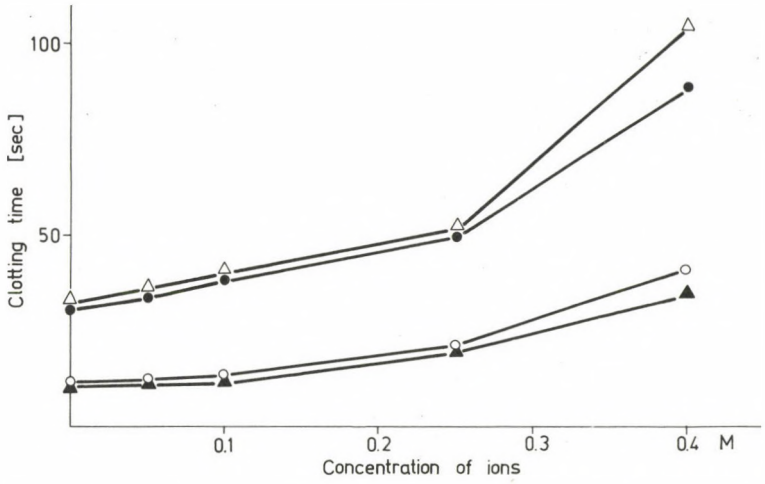

Fig. 1. Effect of sodium chloride and potassium chloride on the thrombin—fibrinogen and thrombin–antithrombin reactions. Thrombin (4 μg protein) was incubated with either sodium chloride (▲—▲) or potassium chloride (○—○) at pH 7.3. The same experiments were carried out in the presence of antithrombin (50 μg of protein). Thrombin and antithrombin with sodium chloride (Δ—Δ), thrombin and antithrombin with potassium chloride (●—●). After incubation at 37 °C for 3 min, fibrinogen solution was added and clotting time measured. For further details, see Methods

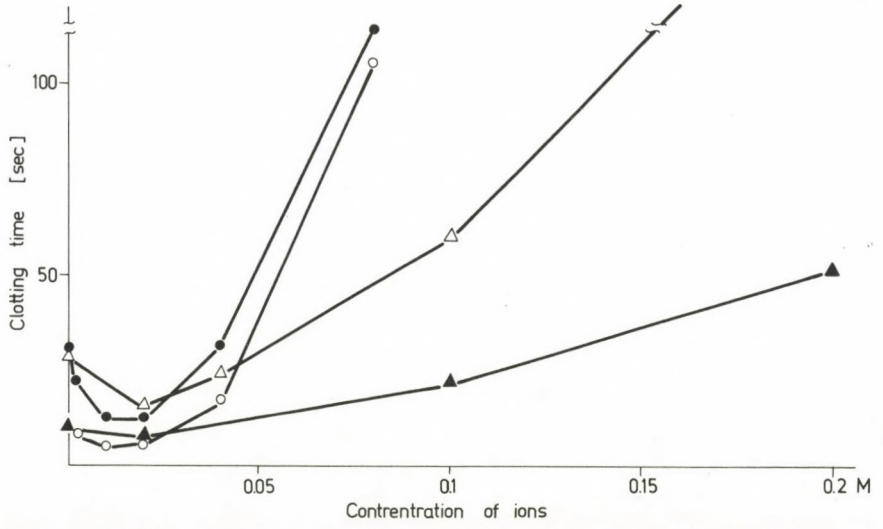

Fig. 2. Effect of calcium chloride and magnesium chloride on the thrombin–antithrombin reaction. Experiments were carried out as detailed in Fig. 1. ○—○: thrombin plus calcium chloride; ▲—▲: thrombin plus magnesium chloride; ●—●: thrombin and antithrombin in the presence of calcium chloride; △—△: thrombin and antithrombin in the presence of magnesium chloride

Heat inactivation of antithrombin-III

Antithrombin is destroyed at 80 °C (*Kekwick* and *Mackay* 1954). According to our experiments presented in Fig. 3, antithrombin incubated at 65 °C was rapidly inactivated; approximately 50% was inactivated in 45 sec and there was no detectable activity after 3 min. It was shown previously that the inactivation of thrombin at 60 °C was protected by heparin (*Pálos* 1949). Therefore, we have examined whether heparin would protect the inactivation of antithrombin at 65 °C. As it can be seen from Fig. 3, heparin had no protecting effect on the heat inactivation of antithrombin.

Thrombin–antithrombin complex formation at 0 °C

Thrombin was preincubated with antithrombin-III at pH 7.3 at 0 °C for 0—60 min. Subsequently, aliquots of the reaction mixture were heated at 37 °C for 30 sec and clotted with fibrinogen. As Fig. 4 shows, clotting time did not change with the duration of preincubation. In the presence of heparin after 5 min incubation the clotting time was, however, prolonged from 30 sec to 45 sec, and during further incubation an additional elevation was observed (after 60 min it was 70 sec).

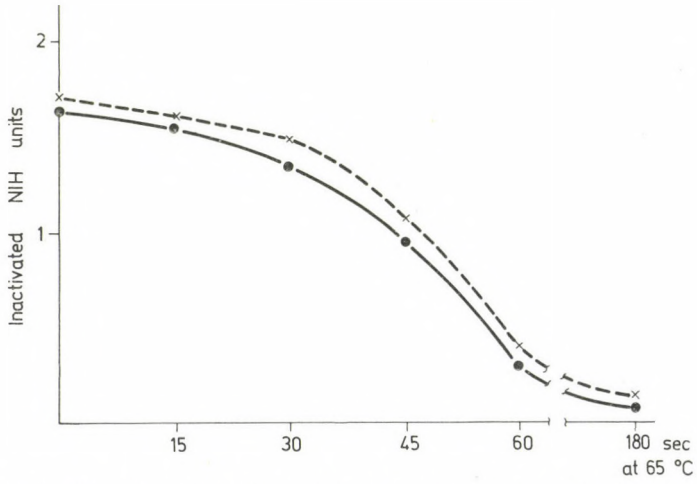

Fig. 3. Heat inactivation of antithrombin. Antithrombin (2 mg per ml) alone or in the presence of heparin (2 mg per ml) was incubated in 0.6 ml final volume at pH 7.3. After incubation at 65 °C for 0, 15, 30, 45, 60 and 180 sec, 0.1 ml aliquot parts were measured with 10 μg thrombin as described in Methods. Antithrombin activity was expressed as NIH units of thrombin inhibited per 5 minutes. ●—●: antithrombin alone; x—x: antithrombin plus heparin

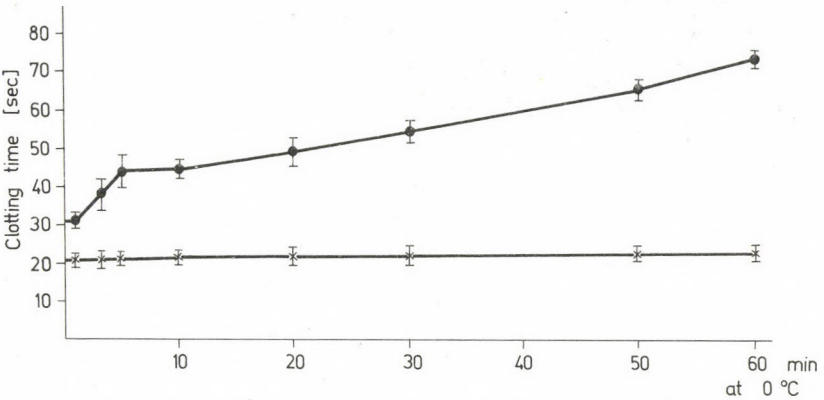

Fig. 4. Preincubation of thrombin with antithrombin at 0 °C in the presence and absence of heparin. 1 ml of thrombin (80 μg/ml) and 1 ml of antithrombin (200 μg/ml) were incubated with 25 μmoles Tris-HCl buffer in a reaction mixture at pH 7.3, or with the same buffer containing 800 μg of heparin at 0 °C. After incubation for 0, 3, 5, 10, 20, 30 and 60 minutes, 0.3 ml aliquots were treated at 37 °C for 30 sec and clotted with 0.1 ml fibrinogen solution (4 mg per ml) at 37 °C. x—x: thrombin and antithrombin without heparin; •—•: thrombin and antithrombin in the presence of heparin

Discussion

The assay of antithrombin activity is accomplished in a complex system, as inactivation of thrombin is measured in a reaction mixture containing thrombin, fibrinogen, antithrombin and in several cases also heparin. It is therefore difficult to determine how the thrombin–antithrombin reaction is affected by some factor. Still, it is important to know the chemical and biological properties of antithrombin and it is of special importance to elaborate and standardize the methods of antithrombin assay as the different laboratories use different ion concentrations, pH, etc.

We have therefore studied the effect of some cations on the thrombin–antithrombin reaction, as well as some properties of the human antithrombin-heparin cofactor.

As to the action of sodium and potassium chloride on the activity of thrombin and antithrombin, it is seen in Fig. 1 that both ions influenced the activity of thrombin in a similar way, i.e. fibrin formation by thrombin was inhibited by sodium or potassium chloride only at concentrations higher than 0.1 M.

Antithrombin activity showed a similar curve in the presence of these ions. It is likely that K^+ or Na^+ do not affect the activity of antithrombin and the alteration of clotting times may be attributed to the inactivation of thrombin.

The effects of calcium or magnesium chloride are more complex. Thrombin is activated by calcium or magnesium chloride in a range of 0.01—0.02 and 0.02—0.04 M, respectively. As to the 0.01 M optimum concentration of calcium chloride on thrombin activity, it correlates well with data in the literature (*Sakuragawa* et al. 1972).

At higher concentrations the enzyme begins to be inactivated (Fig. 2). At high ionic strength, however, the inhibition of fibrin polymerization is also questioned (*Machovich* et al. 1975a).

The effect of ions on antithrombin must be concluded from the mentioned results. It seems to be a secondary one, i.e. a consequence of the effect of ions on thrombin activity.

As to the other characteristics of antithrombin, it can be seen from Fig. 4 that thrombin–antithrombin complex formation does not take place at 0 °C in 60 min. In the presence of heparin, however, a conformational change of thrombin and/or antithrombin is facilitated even at 0 °C. These findings agree with our earlier results and conclusions (*Machovich* et al. 1975b).

Acknowledgements

We are indebted to the Blood Research Laboratory of the American Red Cross for supplies of antithrombin-III. The skilful technical assistance of Mrs. *T. Fazekas* is appreciated.

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EFFECT OF LIPID LOAD AND CARBON TETRACHLORIDE ON THE FATTY ACID RELEASE FROM DOG ADIPOSE TISSUE ISOLATED *IN VIVO*

By

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(Received November 12, 1975)

Under the influence of lipid emulsion (Lipofundin^R) administered into the systemic circulation of dogs, fatty acid release of adipose tissue isolated *in vivo* increased from 0.51 ± 0.096 μEq/100 g/min to 3.18 ± 0.48 μEq/100 g/min. If the lipid emulsion had been injected into the artery of adipose tissue, the fatty acid release was elevated from 0.34 ± 0.053 μEq/100 g/min to 1.67 ± 0.25 μEq/100 g/min. The effect could be observed in denervated adipose tissue too and in this case the fatty acid release increased from 0.40 ± 0.053 μEq/100 g/min to 1.16 ± 0.14 μEq/100 g/min.

If CCl_4 dissolved in lipid emulsion was injected into the systemic circulation, there was no significant change and a value of 0.45 ± 0.063 μEq/100 g/min was measured. However, on administering the CCl_4 dissolved in Lipofundin^R into the artery of adipose tissue, the fatty acid release rose from 0.34 ± 0.053 to 1.01 ± 0.18 μEq/100 g/min. The same manipulation on denervated adipose tissue elevated the fatty acid release from 0.40 ± 0.053 to 0.96 ± 0.016 μEq/100 g/min.

Fatty infiltration is one of the characteristic features of CCl_4-induced liver injury. One of the controversial problems in this connection is the role of fatty acid mobilization from adipose tissue in the development of a fatty liver, the earliest symptom of CCl_4 injury (*Recknagel* 1967). Therefore, we have studied the effect of CCl_4 on fatty acid release of adipose tissue.

A lipid emulsion (Lipofundin^R) was used as a solvent for administering CCl_4 into the blood stream. In the first part of the experiments the effect of the lipid emulsion on the adipose tissue was examined. Subsequently CCl_4 dissolved in the lipid emulsion was injected and its effect on fatty acid turnover was studied.

Methods

The experiments were performed on female dogs weighing 14—18 kg fed with mixed food. After fasting for a day, the dogs were anaesthetized intravenously with 0.1 g/kg chloralose; this dose was repeated whenever necessary. The trachea was cannulated. Blood pressure was measured with a mercury manometer in the femoral artery and registered on a kymograph. At the beginning of the experiment heparin was given intravenously.

In order to study the circulatory and metabolic processes of adipose tissue *in vivo* part of the suprapubic subcutaneous fat was isolated leaving intact its circulation and innervation (*Rosell* 1966).

Into the efferent vein of the isolated adipose tissue a polyethylene cannula was introduced and the quantity of outflowing blood was registered with a drop counter. At short intervals a blood sample of 10 ml was obtained in an ice-cooled plastic tube and the fatty acid concentration was determined. At the same time a blood sample was obtained from the carotid for measuring the arterial fatty acid concentration. The blood lost during the experiment was replaced with the blood of another dog and blood pressure was maintained at a constant level.

The plasma free fatty acid concentration was determined according to *Dole* and *Meinertz* (1960). The number of drops per minute was reckoned over into ml and was related to 100 g of tissue. By multiplying this result with (1 − haematocrit), the quantity of plasma flowing through the tissue was obtained.

Vascular resistance was calculated by dividing the mean arterial pressure with blood flow of adipose tissue.

Arterial and venous free fatty acid concentration was expressed in μEq/ml. The arteriovenous free fatty acid difference was multiplied with the value of plasma flow per minute calculated for 100 g of adipose tissue and so was obtained the fatty acid released from isolated adipose tissue in μEq/100 g/min.

For lipid load and for dissolving the CCl_4, a 20% lipid emulsion (Lipofundin, B. Braun, Melsungen) was used; its composition was: 200.0 g cotton-seed oil; 30.0 g sorbitol, 1.17 g DL-alpha-tocopherol for 1000 ml distilled water. The CCl_4 was dissolved in a tenfold amount of the emulsion.

In one group of 12 dogs, the lipid emulsion and subsequently the CCl_4 was injected into the systemic circulation. In self-control experiments 0.4 ml/kg of the emulsion was administered intravenously to dogs after 3 control fatty acid determinations. Blood samples were taken 3 times for determining the fatty acid concentration during the following 40—60 minutes. Thereafter administration of the emulsion was repeated.

Then 0.04 ml/kg of CCl_4 dissolved in 0.4 ml/kg of the emulsion was injected intravenously and blood samples were taken 3 times for fatty acid determination. Then a further dose of 0.04 ml/kg of CCl_4 was administered and blood samplings were repeated.

Blood flow and vascular resistance were calculated at the moments of fatty acid determination.

In another group of 10 dogs, the lipid emulsion and CCl_4 were administered locally. A thin polyethylene cannula was introduced through the femoral artery to the level of origin of the artery supplying the isolated adipose tissue. Then femoral artery was clamped to ensure that the agent reaches the blood flow of the isolated adipose tissue. 1 ml of the emulsion and 0.1 ml of CCl_4 were injected locally.

In order to complete the latter experiments, the nerve supplying the isolated adipose tissue was divided in 6 animals at the beginning of the experiment.

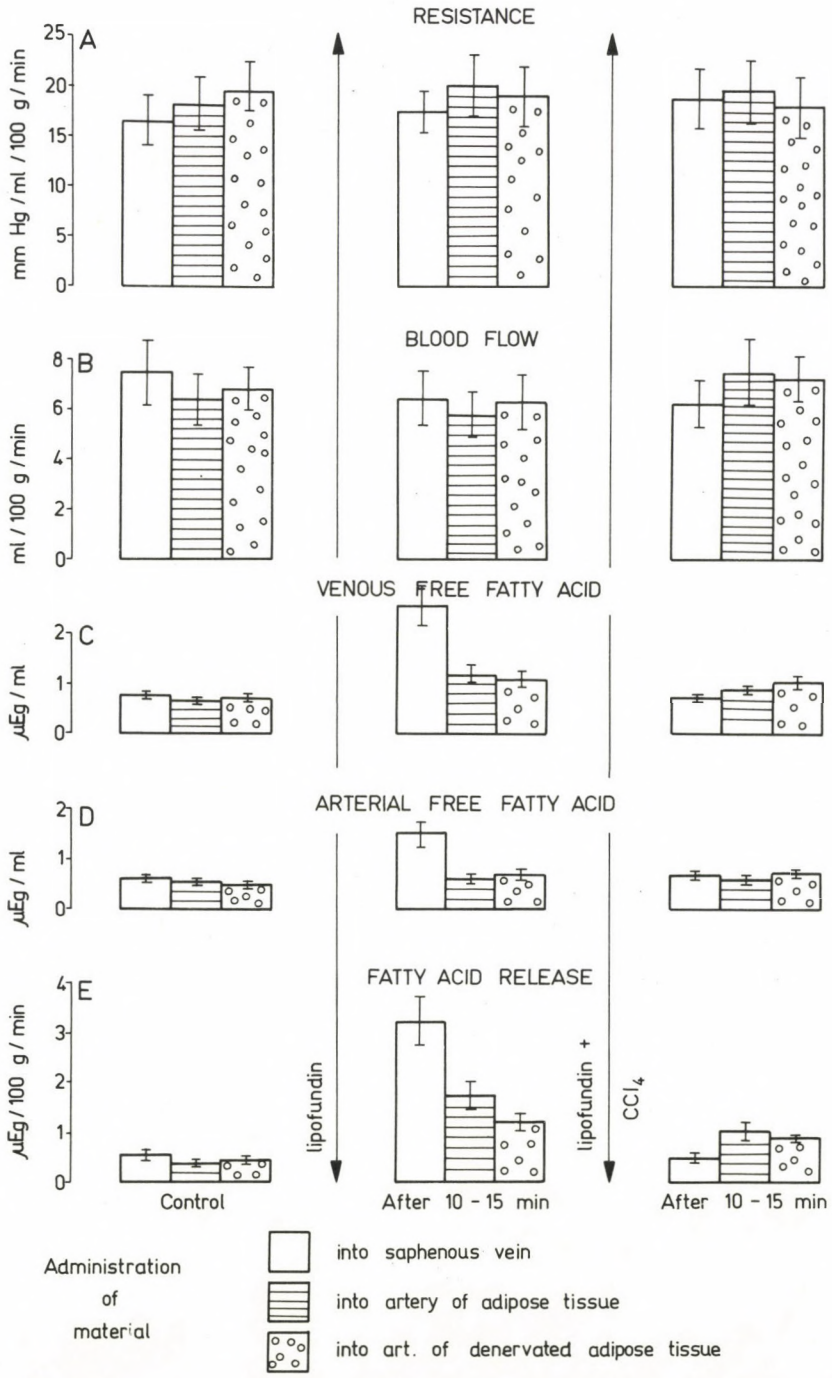
Results

Data for the first and second lipid loads and the first and second CCl_4 doses were evaluated together since the results were identical.

Arterial free fatty acid concentration

Under the influence of the lipid emulsion injected into the saphenous vein, arterial free fatty acid concentration rose from 0.54 ± 0.065 μEq/ml to 1.50 ± 0.23 μEq/ml ($p < 0.001$) in 10—15 minutes and returned to the starting level in 10—15 min (Fig 1D). CCl_4 dissolved in the emulsion caused no significant rise.

Fig. 1. Fatty acid turnover of isolated adipose tissue under the influence of lipid emulsion and CCl_4. A: Vascular resistance of adipose tissue; B: blood flow of adipose tissue; C: FFA concentration of venous effluent of adipose tissue; D: arterial FFA concentration; E: fatty acid release of adipose tissue.


RESISTANCE
mm Hg / ml / 100 g / min
A
BLOOD FLOW
ml / 100 g / min
B
VENOUS FREE FATTY ACID
µEg / ml
C
ARTERIAL FREE FATTY ACID
µEg / ml
D
FATTY ACID RELEASE
µEg / 100 g / min
E
lipofundin
lipofundin + CCl4
Control
After 10 - 15 min
After 10 - 15 min
Administration of material
into saphenous vein
into artery of adipose tissue
into art. of denervated adipose tissue

On injecting the emulsion into the artery of adipose tissue, the control value did not change significantly; arterial FFA concentration was 0.59 ± 0.087 μEq/ml. CCl_4 dissolved in the emulsion caused no significant change.

On administering the emulsion into the artery of denervated adipose tissue, arterial FFA concentration increased from 0.52 ± 0.081 μEq/ml to 0.65 ± 0.096 μEq/ml in 10—15 minutes, but this change was not significant. On injecting CCl_4 dissolved in the emulsion arterial FFA concentration rose from the control value to 0.68 ± 0.076 μEq/ml in 10—15 min ($p < 0.2$).

FFA concentration of the venous effluent of adipose tissue

On administering the lipid emulsion into the saphenous vein, FFA concentration in the vein of adipose tissue (Fig. 1C) rose from 0.71 ± 0.098 μEq/ml to 2.56 ± 0.38 μEq/ml ($p < 0.001$) in 10—15 min and returned to the starting level in 10—20 min. CCl_4 dissolved in the emulsion caused no change as compared to the control.

On injecting the lipid into the artery of adipose tissue, FFA concentration in the venous effluent rose from 0.64 ± 0.085 to 1.16 ± 0.15 μEq/ml ($p < 0.01$) in 10—15 min. CCl_4 dissolved in lipid caused an elevation to 0.88 ± 0.012 μEq/ml ($p < 0.02$) as compared to the control.

On injecting the lipid into the artery of denervated isolated adipose tissue, the FFA concentration in the venous effluent increased from 0.65 ± 0.076 to 1.07 ± 0.14 μEq/ml ($p < 0.05$) in 10—15 minutes. CCl_4 dissolved in lipid caused an elevation of the level to 1.0 ± 0.12 μEq/ml ($p < 0.05$).

Fatty acid release of adipose tissue

On injecting the lipid into the saphenous vein, fatty acid release of isolated adipose tissue increased from 0.51 ± 0.096 to 3.18 ± 0.48 μEq/100 g/min ($p < 0.001$). (Fig. 1E). CCl_4 dissolved in lipid caused no significant change versus the control.

Under the influence of lipid given into the artery of adipose tissue, fatty acid release rose from 0.34 ± 0.053 to 1.67 ± 0.25 μEq/100 g/min ($p < 0.001$), while CCl_4 dissolved in lipid caused an elevation to 1.01 ± 0.18 μEq/100 g/min ($p < 0.01$).

On injecting the lipid into the artery of denervated adipose tissue, fatty acid release increased from 0.40 ± 0.053 to 1.16 ± 0.14 μEq/100 g/min ($p < 0.001$). Then CCl_4 caused it to rise to 0.96 ± 0.016 μEq/100 g/min ($p < 0.001$).

Blood flow and vascular resistance of adipose tissue

Ten to 15 min after the injection of lipid when an increase was observed in fatty acid release, the change of blood flow was not significant statistically (Fig. 1B).

During maximum fatty acid release, the change of vascular resistance was not significant as compared to the control (Fig. 1A).

Mean arterial pressure before administration of lipid was 140 mm Hg and after it, 135 mm Hg. Before CCl_4 injection, mean arterial pressure was 135 mm Hg and during the change of fatty acid release, 125 mm Hg.

Discussion

Injection of lipid emulsion into the saphenous vein caused an elevation of plasma FFA concentration. At the same time a significant elevation of FFA release by the isolated adipose tissue occurred. It is supposed that the increased FFA release was responsible for the elevation of the plasma FFA level following the lipid load. Since in our experiments only the reaction of adipose tissue was examined, it cannot be ruled out that at the same time some other organ too responded with a similar reaction.

FFA release increased also on injecting the lipid emulsion into the artery of adipose tissue. This indicates that the point of attack eliciting the increased FFA release is in the adipose tissue and that the lipid exerts its effect without nervous mediation. *Gordon* and *Cherkes* (1956) noted a plasma FFA elevation following fat consumption.

If CCl_4 dissolved in lipid was injected into the systemic circulation, FFA release fell into the control range but on injecting the CCl_4 into the artery of adipose tissue FFA release increased.

In order to explain these phenomena, the change of activity of lipoprotein lipase and triglyceride lipase has to be taken into consideration. According to some authors (*Garfinkel* et al. 1967), the lipoprotein lipase present in the capillary wall of adipose tissue would play the main role in the deposition of triglycerides. An increase in the activity of this lipase might explain the decomposition of circulating lipids and the elevation of the plasma FFA level. The metabolic state of the fatty cells is influenced mainly by the activity of triglyceride lipase (*Renold* 1966). This enzyme determines the rate of lipolysis. In addition, cellular fat synthesis and resynthesis are also important factors in the FFA release of adipose tissue (*Rosell* 1966). Thus, both an increase of lipolysis inside the fatty cell and the inhibition of synthesis and resynthesis lead to increased FFA release under the influence of lipid emulsion.

A disturbance of these biochemical mechanisms may explain our observation that CCl_4 given into the systemic circulation abolishes the increased FFA mobilization characteristic of lipid loading. As an explanation, an inhibition of the activity increase of lipoprotein lipase is assumed. The toxic agent might restrict the role of enzymes in the metabolism of adipose tissue: under the influence of CCl_4, lipoprotein and triglyceride lipase might not be able to enhance lipolysis.

Adenyl-cyclase is known to be present in adipose tissue. Under the influence of this enzyme, cyclic AMP is formed from ATP (*Sutherland* et al. 1962) which activates the lipolytic enzyme of adipose cells. This nucleotide is the mediator of hormonal effects too. Consequently it might be supposed that in our experiments the change in activity of adenyl cyclase and cyclic AMP had a role in the increase of FFA release coming about under the influence of lipid emulsion. It is possible that the inhibition by CCl_4 of FFA release also takes place by this system.

Although the reserve capacity of adipose tissue decreases under the influence of CCl_4, FFA release is normal under such circumstances. Thus, our experiments support the finding made by other authors (*Recknagel* 1967) that in CCl_4 poisoning hepatic fatty infiltration may ensue in spite of a normal fatty acid flux.

FFA release was found to increase after lipid loading and the injection of the CCl_4 dissolved in lipid into the artery of adipose tissue. This fact and the phenomenon that in the case of systemic CCl_4 administration the adipose tissue does not react upon the lipid load with an increased fatty acid release suggest that in the liver metabolite is produced which is injurious to the adipose tissue. The nephrotoxic effect of CCl_4 is interpreted similarly (*Striker* et al. 1968).

In the present experiments, the fatty acid level was observed for two hours after intravenous CCl_4 administration and no rise whatever in the plasma FFA level was noted. On the contrary, in rats (*Szlamka* et al. in press) a late increase in the plasma FFA concentration occurred after the oral administration of CCl_4. On the basis of the present result it is supposed that the metabolite of CCl_4 exerts a toxic effect on adipose tissue and prevents by this means the increase in fatty acid release. The question arose how the late rise of the fatty acid level came about in our rat experiments. The phenomenon can be explained in all probability by the fact that the FFA uptake of fatty acid consuming organs (liver, heart, muscle tissue) decreases in CCl_4 poisoning.

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INTERACTION OF cAMP AND ADRENORECEPTORS ON REGULATION OF RENAL FUNCTION AND RENIN SECRETION

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The effect of d-cAMP, infused in a dose of 1.0 mg/min into the renal artery, has been studied on renal haemodynamics and on renin secretion on normotensive, isonatraemic, anaesthetized dogs as well as in anaesthetized dogs pretreated with selective alpha or beta receptor blockers. Renin secretion was stimulated by c-AMP and this action was brought about by a direct effect on JGA rather than by a haemodynamic route or by influencing the intrarenal adrenoreceptors.

The intracellular level of cAMP, a mediator of several hormones, depends on the activity of adenyl cyclase and phosphodiesterase. Adenyl cyclase, an enzyme bound to the cell membrane, is activated as a result of action of the particular hormone and the role of this activated adenyl cyclase is to catalyse the conversion of ATP to cAMP, by producing one pyrophosphate molecule (*Sutherland* et al. 1962). cAMP is then quickly catabolyzed to 5'-AMP cytoplasmatic phosphodiesterase (*Sutherland* and *Rall* 1958). The dibutyric salt of cAMP (N_6-O_2-dibutyryl cAMP, d-cAMP) passes the cell membrane more readily than cAMP itself and may be converted intracellularly to cAMP. Furthermore, d-cAMP is comparatively resistant to phosphodiesterase (*Posternak* et al. 1962; *Falbriard* et al. 1967).

It has been shown (*Gaál* and *Forgács* 1974) that cAMP infused into the renal artery caused a significant diminution of renin secretion. The effect was ascribed to haemodynamic changes or an eventual splitting of cAMP.

It has been observed (*Butcher* et al. 1968; *Melson* et al. 1970) that the specific changes induced by catecholamines were accompanied by simultaneous alterations of the cAMP level in various tissues.

In order to decide whether exogenous cAMP affects renin secretion (RS) directly or *via* intrarenal adrenergic receptors, RS as well as renal haemodynamic parameters were investigated following the application of d-cAMP in normotensive, isonatraemic, anaesthetized dogs and in animals after selective blockade of α and/or β receptors.

Methods

Thirty dogs of both sexes, weighing 11—17 kg, were used. *Via* median laparotomy a catheter was introduced into the left ureter under pentobarbital anaesthesia (30 mg/kg b.w.). A siliconized catheter was placed into the left renal vein and connected to the left jugular vein, to estimate renal blood flow (RBF) directly A No. 19 Injection cannula was then inserted into the renal artery about 0.5 cm from the kidney, to permit drug application. While inserting the needle, special care was taken to spare the hilar nerves.

Blood pressure was measured by a manometer connected to the femoral artery. At the end of the surgical procedure, the animals were given an infusion of physiological saline, 2 ml/kg i.v.

Plasma renin activity (PRA) of the blood samples withdrawn from the renal artery and the left renal vein was measured by the rat bioassay described by *Kaneko* et al. (1967). PRA was expressed as ng angiotensin equivalent/ml plasma/24 hr. Renin secretion was calculated from the arteriovenous PRA difference and from the direct RPF; it was expressed as μg/min ($PRA_{v-a} \cdot RPF_{dir}$).

Renal blood flow (RBF_{dir}) of the left kidney and renal vascular resistance (R_{ren}) were calculated per 100 g and R unit/kg kidney weight respectively.

Haematocrit was estimated with the aid of a Janetzki-centrifuge, plasma Na level with flame-photometry.

After a 30 min control the animals were divided in four experimental groups according to the subsequent drug treatment.

Group I: 1.0 mg/min d-cAMP (Calbiochem);

Group II: 10 μg/kg/min phenoxybenzamine (POB) with a subsequent infusion of 1.0 mg/min d-cAMP;

Group III: 10 μg/kg/min propranolol, with a subsequent infusion of 1.0 mg/min d-cAMP;

Group IV: 10 μg/min POB plus 10 μg/kg/min propranolol, with a subsequent infusion of 1.0 mg/kg d-cAMP.

In each case, the drugs were infused into the left renal artery in 20 min.

For statistical analysis, Student's one-tailed *t*-test was used. A probability level of 0.05 was accepted as a significant difference.

Results

Results are summarized in Table I.

Group I (n=8). The effect of d-cAMP

Following d-cAMP treatment, RBF increased by 12% during the first 10 min. It showed a further increase in the second 10 min, while the renal vascular resistance decreased by 26%. Renin activity of the arterial blood did not change during the first 10 min, while it increased significantly by 46% in the second 10-min period. Both renin activity of renal venous blood and renin secretion increased significantly in both 10-min intervals. The arteriovenous difference was also increased, a statistical difference, however, could be found only in the first 10 min, since in the second 10 min arterial renin activity showed a higher increase (46%) than venous activity (34%).

Group II (n=8). The effect of d-cAMP in POB-pretreated animals

Phenoxybenzamine, an alpha blocking drug infused into the renal artery, caused a significant (19%) decrease in systemic blood pressure. RBF did not change, neither

was the decrease in renal vascular resistance significant. Venous renin activity, arteriovenous renin difference and renin secretion decreased significantly (49, 81 and 81%, respectively), while arterial renin activity did not change. A negative arteriovenous difference was found in two cases.

After POB administration, the effect of d-cAMP was studied. Neither the haemodynamic parameters of the kidney, nor arterial renin activity changed after d-cAMP treatment. There was, on the other hand, an increase in both the first and second 10-min period in renin activity of renal venous blood (76 and 266%, respectively), in the arteriovenous renin difference (543 and 856%, respectively) and in renin secretion (531 and 808%, respectively).

Group III (n=8). The effect of d-cAMP in propranolol-pretreated animals

Haemodynamic parameters of the kidney did not change after propranolol administration and renin activity showed only a slight, non-significant decrease. Infusion of d-cAMP in propranolol-pretreated animals failed to influence renal haemodynamics or peripheral arterial renin activity. Venous renin activity, arteriovenous renin difference and renin secretion were increased significantly in the first 10 min of treatment and by the end of infusion they were 206, 300 and 395% higher than the respective propranolol values.

Group IV (n=8). The effect of d-cAMP in POB plus propranolol-pretreated animals

After a simultaneous blockade of alpha and beta receptors, renin secretion showed the same decline as after POB infusion. Administration of d-cAMP to these animals resulted in an increase 3-fold in renal venous renin activity, 14-fold in veno-arterial renin difference and 13-fold in renin secretion. Arterial renin activity had a decreasing trend; this was, however, non-significant. The haemodynamic parameters (blood pressure, RBF, renal vascular resistance) did not change after d-cAMP treatment.

Discussion

Infusion of d-cAMP into the renal artery, at a rate of 1 mg/min, resulted in a significant increase in renin secretion and in direct RBF, while systemic blood pressure showed a statistically significant but biologically irrelevant decrease.

GFR and RPF were formed to increase after a 0.5 mg/kg/min infusion of d-cAMP (*Abe* et al. 1968). Similarly, renin secretion increased in rats (*Hauger-Klevene* 1970) as well as in dogs (*Yamamoto* et al. 1973). These authors observed a simultaneous increase in RBF, urine volume, excreted Na amount and free water reabsorption after d-cAMP treatment; cAMP itself, on the other hand, did not significantly affect renin release (*Tagawa* and *Vander* 1970). *Winer* et al. (1969) tested the effect

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Table I

		Group I			Group II			
n:		Control	d-cAMP 10′	d-cAMP 20′	Control	POB 20′	d-cAMP 10′	d-cAMP 20′
		8	8	8	8	8	8	8
PRA_a, ng/ml	$\bar{x}$	67	73	98*	44	34	34	49
	S.E.	13	12	20	5	7	3	2
PRA_v, ng/ml	$\bar{x}$	107	126*	144*	81	41*	72□	109*□
	S.E.	23	19	26	13	6	11	15
PRA_{v-a}, ng/ml	$\bar{x}$	40	53*	46	37	7*	38□	60*□
	S.E.	10	7	6	9	3	9	13
$RPF \cdot PRA_{v-a}$, μg/min	$\bar{x}$	10.900	15.640*	16.430	11.935	2.305*	12.224□	18.562*□
	S.E.	1.760	1.523	1.470	2.994	0.925	3.450	4.418
BP, mm Hg	$\bar{x}$	128	116	111*	117	95*	93*	88*
	S.E.	6	3	6	8	10	10	8
RBF_{dir}, ml/min	$\bar{x}$	531	596*	633*	552	531	563	569
	S.E.	50	51	56	30	53	71	58
R_{ren}	$\bar{x}$	1.52	1.23	1.12*	1.32	1.06	1.05	0.96*
	S.E.	0.22	0.13	0.15	0.11	0.07	0.12	0.07
Se [Na+] mEq/l	$\bar{x}$	149	148	148	145	145	143	144
	S.E.	2	1	1	1	1	1	1

* $p < 0.05$ v. control period

□ $p < 0.05$ vs. POB or propranolol period

Table I (cont.)

		Group III				Group IV			
n:		Control	PROP 20′	d-cAMP 10′	d-cAMP 20′	Control	POB+PROP 20′	d-cAMP 10′	d-cAMP 20′
		8	8	8	8	8	8	8	8
PRA_a,	$\bar{x}$	41	33	37	53	43	32	69	69
ng/ml	S.E.	2	6	1	7	5	2	13	17
PRA_v,	$\bar{x}$	75	49	98*□	101*□	64	35*	99□	112*□
ng/ml	S.E.	11	6	11	11	7	6	19	22
PRA_{v-a},	$\bar{x}$	34	16	61*□	48*□	21	3*	30□	43*□
ng/ml	S.E.	10	4	10	10	5	4	7	10
$RPF \cdot PRA_{v-a}$,	$\bar{x}$	11.845	4.185	19.883*□	16.492*□	9.054	1.316*	12.337□	17.312*□
μg/min	S.E.	3.302	0.545	3.604	3.723	2.339	1.270	2.915	3.107
BP,	$\bar{x}$	130	111	103*	103*	126	108*	108*	98*
mm Hg	S.E.	4	8	6	6	7	8	8	8
RBF_{dir},	$\bar{x}$	620	568	616	616	748	693	716	741
ml/min	S.E.	71	123	105	111	26	49	22	22
R_{ren}	$\bar{x}$	1.35	1.38	1.23	1.20	1.06	1.07	0.89	0.78*
	S.E.	0.15	0.18	0.13	0.18	0.06	0.10	0.04	0.06
Se [Na+],	$\bar{x}$	147	147	147	146	142	143	144	143
mEq/l	S.E.	2	1	1	1	1	2	2	1

* $p < 0.05$ vs. control period
□ $p < 0.05$ vs. POB or propranol period

of some adenosine derivatives *in vivo* (adenosine, 5'-AMP, ATP, cAMP) in dogs and only cAMP was found to increase renin secretion. Increased renin release was observed after cAMP by *Michelakis* et al. (1969), measured in a kidney-cell suspension system *in vitro*. Renin release *in vitro* increased both after cAMP and d-cAMP, but significantly only after d-cAMP (*Yamamoto* et al. 1973). In our previous experiments *in vitro* (*Gaál* et al. 1975) increased renin release could be observed from the renal cortical slices of dogs after cAMP treatment, while its infusion into the renal artery (*Gaál* and *Forgács* 1974) significantly increased RBF and decreased renin secretion. The facilitatory action of cAMP on renin secretion is a direct cellular effect (*Winer* et al. 1972) and does not depend on the changes of RBF, heart rate, sodium excretion or systemic blood pressure. This direct effect was corroborated by data *in vitro*.

Numerous authors reported on the beta adrenergic mediation of the effects by cAMP. Drugs, which stimulate beta receptors are known to stimulate adenyl cyclase and *via* this route they result in an increased level of tissue cAMP (*Robison* et al. 1967). Accordingly, isoproterenol, a beta mimetic drug, was described to increase renal renin release (*Ueda* et al. 1970; *Reid* et al. 1972; *Vandongen* et al. 1973; *Winer* et al. 1972). *Wiener* et al. (1972) supposed two pathways for beta receptor agonists to induce renin release: either by inhibition of phosphodiesterase, the degrading enzyme of cAMP (by ethacrinic acid or theophylline), or by facilitating the synthetising enzyme (adenyl cyclase) by isoproterenol, norepinephrine, hydralazine, etc. The facilitatory action of cAMP on renin secretion was blocked by alphalytic agents such as phentolamine and also by betalytic agents such as propranolol. *Meurer* et al. (1972) assumed that the facilitation of renin release was brought about by adenyl cyclase *via* specific receptors different from the known $beta_1$ or $beta_2$ receptors.

Michelakis et al. (1969) have shown that renin release from a kidney-cell suspension was increased following epinephrine and norepinephrine treatment as well as under the effect of cAMP. They concluded that cAMP would be the intracellular mediator of catecholamines.

Winer's hypothesis (*Winer* et al. 1972), according to which beta mimetics facilitate renin secretion by stimulating adenyl cyclase and by inhibiting phosphodiesterase, was corroborated by the observation that theophylline, a drug known to inhibit phosphodiesterase, increased renin secretion both *in vitro* (*Reid* et al. 1972) and *in vivo* (*Gaál* et al. 1975).

This enzyme theory was further supported by the differential effects of the increased level of various ions on renin secretion. The presence of some ions is necessary for phosphodiesterase to have a biological activity, since the enzyme is active only as a metal-complex (Mg^{2+}, Mn, Co, Ni, etc.). *Cheung* (1967) demonstrated that phosphodiesterase was inactivated in brain tissue in the presence of ATP or other nucleoside-triphosphates, because these triphosphate analogues had bound Mg^{2+} ions. *Székács* et al. (1969) observed a decreased sensitivity to angiotensin

in rats maintained on a Mg^{2+}-rich diet. According to some authors the increased adenyl cyclase activity and the concomitant elevation in the cAMP level might result in a facilitated accumulation of free Ca^{2+} in the microsomal fraction (*Entman* et al. 1969). On the other hand, increased renin release was described by *Yamamoto* et al. (1974) as a consequence of Ca^{2+} ions.

In the present experiments, infusion of POB and propranolol, POB caused, as in our earlier experiments (*Forgács* et al. 1974), a significant decrease in systemic blood pressure and renin secretion while propranolol did not change any parameter. Application of d-cAMP after selective alpha blockade (POB infusion) or selective beta blockade (propranolol infusion) significantly increased renin secretion without, however, affecting the haemodynamic parameters. This supports the idea that the facilitatory effect of d-cAMP on renin secretion is not necessarily bound to alpha or beta receptors. One must, however, be aware of the fact that d-cAMP might have increased renin secretion by a direct stimulation of the receptor which remained intact after drug application: by stimulation of the intact beta receptors after alpha-blockade and *vice versa*. To exclude this hypothesis, d-cAMP was applied after the simultaneous blockade of beta kinds of receptor. Renin secretion was increased in this case too but—like in the animals with selective blockade—the haemodynamic parameters remained unaffected.

These data support our hypothesis that d-cAMP increased renin secretion by influencing the JGA directly, thus the effect might be independent from the renal haemodynamic parameters and from intrarenal adrenoreceptors.

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BRAIN CATECHOLAMINE METABOLISM AND AVOIDANCE CONDITIONING IN RATS

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Male albino rats were trained in a shuttle-box by presentation of 50 trials and were grouped according to the performance level. The disappearance of labelled catecholamine pool was studied after intraventricular injection of tritiated noradrenaline in the neocortex, brain stem, hypothalamus and hippocampus. Rats with a high performance level showed a greater disappearance rate of the labelled catecholamine pool than low or medium performers.

In another experimental series the animals were grouped on the basis of their performance level which was tested 2 days before the intraventricular injection of the tracer. There was no significant difference in disappearance rates between the good and poor performances.

It is assumed that integration of the goal-directed motor pattern is mediated through catecholaminergic (noradrenergic) neuronal mechanisms.

A widespread activation of the central noradrenergic (NA) neuronal system and a less marked activation of the dopaminergic neurones during acquisition of conditioned response could be observed by *Fuxe* and *Hanson* (1967). Their histochemical fluorescence study suggested that the NA nerve terminals to the telencephalon play an important role as an arousal system in the avoidance behavioural response. In accordance with this assumption there are observations that a functional blockade of catecholaminergic transmission is followed by an impairment of avoidance behaviour (*Seiden* and *Carlsson* 1963; *Seiden* and *Hanson* 1964; *Corrodi* and *Hanson* 1966; *Hanson* 1967). Administration of α-methyltyrosine resulted in a depression of conditioned avoidance response (*Spector* et al. 1965; *Moore* 1966), but this depressive action may have been due to the toxicity of methyltyrosine (*Weissman* and *Koe* 1965).

It is known from earlier studies that NA turnover is significantly increased throughout the brain and the spinal cord after administration of paw shocks (*Thierry* et al. 1968; *Endrőczi* et al. 1975). These observations suggested an increased synthesis coupled with release in the peripheral nerves of the sympathetic system (*Alousi* and *Weiner* 1966; *Sedwall* et al. 1969).

In the present investigations we have studied the correlation of brain catecholamine metabolism with the performance of rats in two-way active avoidance conditioning with fixed trial contingent.

Methods

Eighty male R-Amsterdam rats of 150—200 g were used in the study. The intraventricular injection of 2 μCi ^{3}H-noradrenaline (^{3}H-noradrenaline-7-L, 10.6 Ci/mM, Amersham) was given in 10 μl physiological saline containing 0.1% ascorbic acid and buffered to pH 7.0. The rats were sacrificed by decapitation 16 hr later and the brain was removed for dissecting samples from the neocortex, mesencephalon, hypothalamus and hippocampus. After weighing the tissue on a torsion balance it was homogenized in 0.5 ml of 0.6 N perchloric acid at 0 °C for 15 minutes. The homogenate was centrifuged at 5,000 r.p.m. for 15 min and the radioactivity of 200 μl supernatant was measured after addition of 2 ml ethanol and 10 ml liquid-scintillation fluid with a Nuclear Chicago Mark II apparatus by the use of external standard and quenching correction.

Two-way active avoidance conditioning was performed in a shuttle-box. A total of 50 trials was presented in 30 sec intervals. As conditioned signal a buzzer tone was presented for 5 sec and it was followed by administration of electrical shocks (approximately 1.5 mA/shock lasting for 1 sec) to the paw during a 15 sec period or until leaving for the safe compartment within this time. Before the experiment the rats were habituated to the experimental box for 1 minute.

The intraventricular injection of ^{3}H-NA was performed 16 hr prior to the behavioural study and the animals were sacrificed immediately after presentations of 50 trials. According to the performance levels the rats were grouped into poor learners (< 10%), medium learners (< 50%) and good learners (> 50%). The maximum performance level was near to 80%.

In the experiments when the rats were preselected according to their performance only two groups were used: poor learners (< 10%) and good learners (> 50%).

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

Results

Although there was no difference in the total duration of shocks given to each group, the performance levels showed a wide variation during the fixed shock contingent training period (Table I).

In comparison to the poor and medium learners, the good learners showed a significant decrease of the labelled catecholamine pool in the hippocampus, hypothalamus and to a lesser extent in the brain stem. A slight increase of the labelled pool was found in the neocortex of medium performing rats. A more marked decrease of the labelled catecholamine pool in the hippocampus and hypothalamus than in the brain stem can be ascribed to the high density of catecholamine terminals in the former structures which respond to excitatory states more readily than do the catecholamine stores in the cell body located at the brain stem level (Fig. 1). A lack of change in the labelled catecholamine pool in the neocortex cannot be interpreted in the light of the present findings; for the phenomenon the different turnover rates of the cortical and subcortical structures may be responsible.

In another experimental series the animals were preselected according to their performance level, ^{3}H-NA tracer was administered 2 days later and the animals were sacrificed 16 hr following the administration. There was no significant difference in labelled NA pool between the rats showing poor and good performances (Fig. 2).

Table I

Total duration of shocks given to each group with different performance levels in a two-way avoidance conditioning

Mean pad-shock time (sec)

	PL	ML	GL
Exp. I	70	72	58
Exp. II	65		53

PL: poor; ML: medium; GL: good learners. Exp. I and Exp. II refer to experiments demonstrated in Fig. 1 and Fig. 2

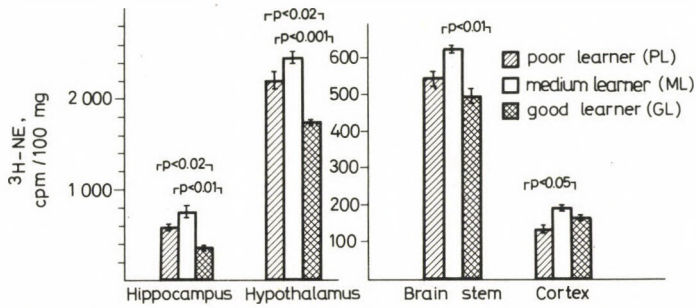

Fig. 1. Labelled catecholamine pool in rat with different performance levels in a two-way avoidance conditioning with fixed trial contingent

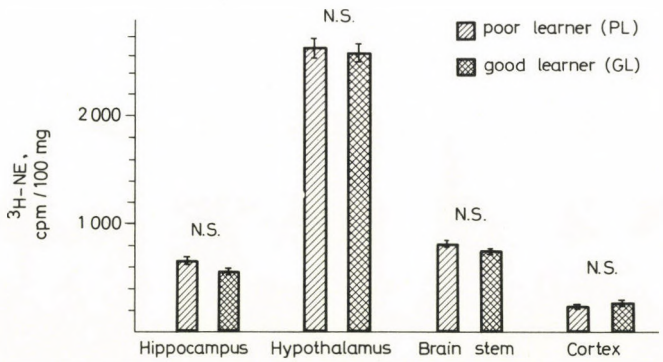

Fig. 2. Labelled catecholamine pool of brain regions of rats with poor and good performance levels. There was no significant difference between the groups when catecholamine turnover was tested 2 days after conditioning

Discussion

Stress-induced changes in brain catecholamine turnover have been observed by many investigators (*Barchas* and *Freedman* 1963; *Maynert* and *Levy* 1964; *Moore* and *Lariviere* 1964; *Gordon* et al. 1966, among others). The labelled NA given into the ventricle mixes with endogenous catecholamines and can be used as a tracer to monitor synthesis, release and uptake (*Milhaud* and *Glowinski* 1962; *Goldstein* and *Gerber* 1963; *Glowinski* and *Axelrod* 1966; *Glowinski* and *Iversen* 1966; *Carr* and *Moore* 1968). At least two compartments have been postulated for catecholamine metabolism: one with a rapid and the other with a slower turnover rate. We have studied the influence of avoidance learning on catecholamine turnover in the second or slow metabolic phase which proved to be sensitive for either noxious stimuli and pituitary hormones (*Endrőczi* et al. 1975).

The present findings indicate the involvement of catecholaminergic neurones in the integration of goal-directed motor response. The shock-induced changes do not explain the differences in catecholamine turnover rates observed in rats with different performance levels. The observations are in accordance with the conclusion of *Fuxe* and *Hanson* (1967) who found a decrease of the catecholamine fluorescence reaction of the telencephalon monoaminergic neurones in rats with avoidance conditioning but not after shock presentations.

There are observations indicating the participation of noradrenergic transmission in "reward" self-stimulation (*Stein* 1969; *Stein* and *Weise* 1970) but not in punishment. In the light of the recent findings it seems likely that a repetition of the motor patterns rather than a reward is responsible for the release of catecholamines during self-stimulation. This view is in agreement with the findings of *Gordon* et al. (1966) that motor activity is coupled with an increased NA turnover in the brain stem.

The role of central NA neurones in the organization of behavioural activity is less known than that of the dopaminergic neuronal mechanisms, however, the exploratory activity of rats seems to depend on noradrenergic transmission (*Fuxe* and *Ungerstedt* 1970). Further studies are required to clarify the involvement of the NA neuronal system in the integration of goal-directed motor patterns and its role in controlling the facilitatory and inhibitory processes at the brain stem–limbic circuit level.

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Pathophysiologia

EFFECTS OF HAEMODILUTION IN EXPERIMENTAL CARDIOGENIC SHOCK

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The effect of isovolaemic haemodilution with dextran has been studied in dogs in cardiogenic shock.

Cardiac output in cardiogenic shock was increased by haemodilution. This increase, however, did not compensate the decrease of oxygen transport, due to the lowered oxygen binding capacity of blood.

Lowering of haematocrit below 30% appears to be especially dangerous in cardiogenic shock.

In recent years several experimental and clinical studies have emphasized the importance of the rheological properties of the blood in various pathological conditions including myocardial infarction. *Bloch* (1955), *Ditzel* et al. (1968), *Langsjoen* (1966) and *Jan* et al. (1975) showed that whole blood viscosity, haematocrit, plasma fibrinogen and α_2-globulin levels rise in the initial stage of acute myocardial infarction leading to red cell aggregation and microcirculatory impairment. *Langsjoen* reported favourable results of dextran infusions in infarct patients (*Langsjoen* 1973; *Langsjoen* et al. 1963). The infusion, however, could expand plasma volume and placed an excessive load on the damaged heart. This has raised the possibility of using isovolaemic haemodilution in this condition.

Haemodilution is routinely applied in open heart surgery and coronary revascularization procedures, and the question arose of the danger of haemodilution in the presence of damaged coronary arteries.

Isovolaemic haemodilution leads to a decrease of peripheral resistance and an elevation of cardiac output in dogs and systemic oxygen transport may increase in spite of a decreased oxygen-binding capacity (*Sunder-Plassmann* et al. 1972). It has been shown that haemodilution does not aggravate ECG changes after tempor-

to what extent a damaged heart can compensate the low oxygen-binding capacity by an increase of cardiac output to provide an adequate peripheral oxygen supply. In the experiments to be described, this problem has been studied.

Methods

A total of 17 dogs was anaesthetized with 30 mg/kg intravenous pentobarbital. They were ventilated with air using a Harvard respirator. The thorax was opened in the left fourth intercostal space and electromagnetic flow transducers were placed around the pulmonary artery and the circumflex branch of the left coronary artery. The transducers were connected to a Carolina Medical Electronics flowmeter. A polyethylene catheter was introduced into the ascending aorta through the femoral artery for blood pressure measurement with a Statham P23Db transducer. Flow and pressure signals were recorded on a Beckman Dynograph recorder.

Oxygen content of arterial blood samples was determined by a modification of the polarographic method of *Laver* (*Laver* et al. 1965).

Following control measurements, the ventral interventricular branch (cranial descending coronary artery) (*Miller* et al. 1964) was ligated approximately 4 cm from its origin. Proximal to this ligature a polyethylene cannula was placed into the artery and advanced to the origin of the circumflex branch. This branch was then embolized by methyl-methacrylate beads 25 μ in average diameter and less than 50 μ in maximum diameter. Embolization was carried out in several steps consisting of 0.1 ml injections of the beads suspended in saline at a concentration of 5 mg/ml till cardiac output decreased below 70% of its value before ligation of the descending branch and mean arterial blood pressure decreased below 70 mm Hg. If blood pressure did not decrease below 70 mm Hg embolization was continued until cardiac output decreased below 60% of its control value.

Isovolaemic haemodilution was induced by bleeding and simultaneous infusion of an equal volume of a 6% dextran solution of 60,000 average molecular weight. Exchange volumes in a single dilution step were 100 or 200 ml depending on the animal's weight. Five minutes after each dilution step a 1.5 ml arterial blood sample was obtained for haematocrit and blood oxygen content determination. Flow and pressure values were also determined at the same time.

Results are presented in the following units: mean arterial blood pressure in mm Hg, cardiac output in ml/min/kg, systemic conductance in ml/min/mm Hg/kg, circumflex branch flow in ml/min/kg, systemic oxygen transport (cardiac output multiplied by arterial oxygen content) in ml/min/kg, circumflex branch oxygen transport in ml/min/kg, external work of the heart (mean arterial blood pressure multiplied by cardiac output) in mm Hg·l/kg.

In the Tables mean and median values are shown and 20th and 80th percentiles (*Herrera* 1958) are given for the estimation of scattering. Statistical analysis was performed by the signed rank test (*Wilcoxon* et al. 1963).

Results

Mean arterial blood pressure (Table I) decreased in cardiogenic shock. Following moderate haemodilution to a haematocrit range of 35—31 it averaged 85 mm Hg, which did not significantly differ from the initial shock values (i.e. before haemodilution, haematocrit values above 40). Moderate haemodilution therefore did not influence arterial blood pressure in cardiogenic shock. Further haemodilution, however, was accompanied by a decrease of blood pressure; it was significantly lower in the 30—26 and 25—21 haematocrit ranges than in the 35—31 range.

Cardiac output (Table II) was decreased due to the elicitation of cardiogenic shock. It increased after haemodilution but did not reach the control value. The somewhat low level of control cardiac output was due to the fact that it was determined after thoracotomy which in itself decreases cardiac output.

Table I

Effect of haemodilution on mean arterial blood pressure in cardiogenic shock (mm Hg)

	Control	Cardiogenic shock				
		Hematocrit				
		> 40%	40—36%	35—31%	30—26%	25—21%
Mean	120.0	93.0*	86.0*	85.0*	80.0***	67.0***
Median	120.0	94.5	90.0	82.0	80.0	69.0
20th percentile	95.6	73.6	66.0	72.0	59.6	50.4
80th percentile	148.0	103.2	98.4	96.0	93.6	80.6

* Compared to control, $p < 0.01$
** Compared to 35—31% haematocrit range, $p < 0.05$
*** Compared to haematocrit ranges 35—31 and 30—26%, $p < 0.05$
Compared to haematocrit > 40%, $p < 0.05$

Table II

Effect of haemodilution on cardiac output in cardiogenic shock (ml/min/kg)

	Control	Cardiogenic shock				
		Hematocrit				
		> 40%	40—36%	35—31%	30—26%	25—21%
Mean	98.2	60.2*	74.9**	76.1***	81.1***	81.9**
Median	84.9	58.7	64.4	64.7	73.9	77.6
20th percentile	73.1	40.3	42.2	51.5	50.1	57.3
80th percentile	137.0	87.1	113.0	107.3	113.0	113.0

* Compared to control, $p < 0.01$
** Compared to haematocrit > 40%, $p < 0.05$
*** Compared to haematocrit > 40%, $p < 0.01$

External work of the heart (Table III) decreased in shock. Haemodilution increased it but the control values were not reached. The value was highest in the 35—31 haematocrit range, then a decrease occurred. In the 25—21 range it was close to the initial shock value at haematocrit above 40.

Systemic conductance (Table IV) decreased moderately in shock and increased progressively during haemodilution, to values significantly higher than the control with a haematocrit less than 35.

Table III

Effect of haemodilution on external cardic work in cardiogenic shock (mm Hg · L/kg)

	Control	Cardiogenic shock				
		Hematocrit				
		> 40%	40—36%	35—31%	30—26%	25—21%
Mean	12.26	5.61*	5.82*	7.18***	6.94**	5.58*
Median	10.62	5.76	4.69	5.06	4.77	4.78
20th percentile	8.52	2.93	2.52	3.71	3.14	3.44
80th percentile	16.93	10.67	10.37	9.31	9.97	8.21

* Compared to control, $p < 0.01$
** Compared to haematocrit > 40%, $p < 0.05$
*** Compared to haematocrit > 40%, $p < 0.01$

Table IV

Effect of haemodilution on systemic conductance in cardiogenic shock (ml/min/mm Hg)

	Control	Cardiogenic shock				
		Hematocrit				
		> 40%	40—36%	35—31%	30—26%	25—21%
Mean	13.41	11.21*	14.76	14.55**	16.50***	20.01****
Median	11.19	11.94	15.20	10.33	15.82	21.72
20th percentile	9.06	6.16	7.69	8.50	10.18	13.65
80th percentile	18.76	15.16	22.70	23.48	22.96	26.39

* Compared to control, $p < 0.05$
** Compared to control, $p < 0.01$
*** Compared to 40—36% haematocrit range, $p < 0.01$
**** Compared to 35—31% haematocrit range, $p < 0.01$

Blood flow in the circumflex branch of the left coronary artery (Table V) changed little in shock and subsequent haemodilution down to a haematocrit of approximately 31. At haematocrits of 30 to 26, coronary flow was higher than before shock. Conductance of the circumflex branch (Table VI) increased on haemodilution; in the 35—31 range it was significantly higher than before shock and a further increase occurred at haematocrits of 30 to 26.

Table V

Effect of haemodilution on blood flow in the circumflex branch in cardiogenic shock (ml/min/kg)

	Control	Cardiogenic shock				
		Hematocrit				
		> 40%	40—36%	35—31%	30—26%	25—21%
Mean	1.78	1.72	2.34	2.08	2.39*	2.05
Median	1.26	1.55	1.80	1.34	1.63	1.72
20th percentile	0.92	0.78	1.03	0.83	1.16	1,02
80th percentile	3.35	2.43	4.67	2.63	3.14	2.53

* Compared to control, $p < 0.05$

Table VI

Effect of haemodilution on conductance of the circumflex branch in cardiogenic shock (ml/min/Hg mm)

	Control	Cardiogenic shock				
		Hematocrit				
		> 40%	40—36%	35—31%	30—26%	25—21%
Mean	0.251	0.296	0.476	0.406*	0.490**	0.508
Median	0.149	0.211	0.314	0.234	0.318	0.333
20th percentile	0.100	0.145	0.157	0.132	0.205	0.200
80th percentile	0.277	0.386	0.839	0.451	0.688	0.654

* Compared to control, $p < 0.01$
** Compared to 35—31% haematocrit range, $p < 0.01$

Oxygen transport of the circumflex branch of the left coronary artery (Table VII) was essentially unchanged at a dilution to the 40—36 range. Further dilution caused a progressive diminution of circumflex oxygen transport and at haematocrits of 25 to 21 it was significantly less than the control value.

Systemic oxygen transport (Table VIII) decreased in shock. Haemodilution down to 35—31 did not cause any substantial change but at haematocrits below 30 a further significant decrease occurred.

Table VII

Effect of haemodilution on circumflex branch oxygen transport in cardiogenic shock (ml/min/kg)

	Control	Cardiogenic shock				
		Haematocrit				
		> 40%	40—36%	35—31%	30—26%	25—21%
Mean	0.327	0.292	0.343	0.276	0.252	0.171*
Median	0.217	0.253	0.254	0.176	0.168	0.131
20th percentile	0.141	0.114	0.140	0.139	0.131	0.090
80th percentile	0.625	0.398	0.683	0.345	0.344	0.277

* Compared to control, $p < 0.05$

Table VIII

Effect of haemodilution on systemic oxygen transport in cardiogenic shock (ml/min/kg)

	Control	Cardiogenic shock				
		Haematocrit				
		> 40%	40—36%	35—31%	30—26%	25—21%
Mean	16.70	10.24*	11.01*	10.22*	8.92**	7.19**
Median	14.50	9.16	8.90	9.64	7.93	7.35
20th percentile	11.50	6.17	6.19	6.78	6.84	5.17
80th percentile	21.70	15.02	16.64	12.54	10.38	9.59

* Compared to control, $p < 0.01$
** Compared to 35—31% haematocrit range, $p < 0.01$

Discussion

The application of haemodilution in myocardial infarction and during coronary revascularization operations still involves controversies. According to *Johnson* et al. (1969) if the haematocrit falls below 32 during revascularization procedures there is an increased frequency of heart arrest and it is difficult to make the heart beat again. *Hallowell* et al. (1972) on the other hand performed more than 500 successful coronary revascularization operations at haematocrit levels of 20 to 15.

In myocardial infarction only *Langsjoen* and his group reported favourable effects of dextran infusion. Mortality in their treated group was 13% and in the control group 32% (*Langsjoen* et al. 1968). This study is, however, open to criticism as the rules of double blind investigation were not strictly observed.

In appropriately randomized groups *Borchgrevink* and *Enger* (1966) found no difference in the mortality of dextran-infused and control infarct patients. *Ditzel* (1972) observed a rise in serum GOT activity in dextran-infused infarct patients on the 4th to 7th days of treatment when compared to patients treated with an equal volume of 5% glucose. This, however, could be due to the wash-out phenomenon and was not necessarily associated with a more severe circulatory state (see discussion by *Lewis* 1972).

As regards the results of animal experiments several problems remain open. The basic compensating mechanism in isovolaemic haemodilution is an increase of flow (*Messmer* et al. 1969; *Sunder-Plassmann* et al. 1971a, b). According to the calculations of *Hint* (1968) systemic oxygen transport could increase in acute haemodilution even though the oxygen binding capacity of blood decreases. Studies by *Messmer* (1973) and *Sunder-Plassmann* et al. (1971b) based on 234 measurements supported this hypothesis; they showed that the increase of cardiac output more than compensates the decrease of oxygen binding capacity and systemic oxygen transport reaches a maximum at a haematocrit of about 30% if dextran is used for dilution. There are, however, also results to the contrary. According to the calculations of *Crowell* and *Smith* (1967), the optimum haematocrit for oxygen transport is around 40. If 3.5% gelatin (Haemaccel^{R}) is used for dilution systemic oxygen transport decreases owing to the increase in cardiac output being more moderate. The compensating mechanisms for the decrease in oxygen binding capacity apparently depend on the kind of colloid used for dilution. This is probably due to their different effects on blood viscosity and on plasma volume. Clinical dextran solutions are hyperoncotic and mobilize fluid from the interstitial to the intravascular space.

In our experiments the moderate increase of cardiac output in cardiogenic shock could only compensate the lowering of O_2 binding capacity until a dilution range of 35—31% haematocrit had been reached. The markedly decreased systemic oxygen transport (compared to the control value), however, did not rise, and decreased further with a haematocrit of 30 or less. In cardiogenic shock the phenomenon observed by *Sunder-Plassmann* et al. (1972), that systemic O_2 transport is at its maximum at a haematocrit of 30, does not occur.

The blood and oxygen supply to the relatively intact part of the myocardium is extremely important in cardiogenic shock because the function of the infarcted muscle has to be taken over by the intact parts of the heart and the latter also supply blood to the occluded area *via* collaterals. In our experiments the left heart was almost entirely dependent on flow in the circumflex branch of the left coronary artery as the cranial descending coronary artery had been ligated. The circumflex

vascular bed was, however, not entirely intact because part of the capillaries had been occluded by microspheres. Circumflex flow increased only slightly on haemodilution showing that the vascular bed had no more reserve capacity. Circumflex O_2 transport remained essentially unchanged and decreased significantly in the 25 to 21 haematocrit range. The remaining left heart muscle supplied by the circumflex branch had to maintain a higher cardiac output after haemodilution when compared to the initial shock value. In spite of the increase of vascular conductance, the external work of the heart increased and therefore the oxygen supply of the heart deteriorated comparatively.

In conclusion, the results failed to support the view that haemodilution would improve the oxygen supply of the heart and peripheral tissues in cardiogenic shock although even the severely damaged heart could somewhat increase its output. Haematocrit levels under 30% seem especially dangerous with both systemic and coronary oxygen transports decreasing. However, before a final evaluation of this form of therapy, survival studies are needed.

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AN EXPERIMENTAL MODEL OF TRYPTIC HAEMORRHAGIC ENTEROPATHY OF THE DOG

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To study the enteropathy characteristic of the shock state of the dog a simple experimental model has been developed. The cranial mesenteric artery was perfused with 2 μg/kg/min norepinephrine for an hour *via* a diagnostic cardiac catheter inserted under fluoroscopic control. This caused a "pharmacological occlusion" of the artery by local alpha receptor stimulation, and tryptic haemorrhagic enteritis was produced without hypovolaemia or assay operative procedure.

In certain mammals—especially in dogs and rats—the irreversible shock state is followed by a typical lesion of the gastrointestinal tract, a haemorrhagic mucosal effusion from the pylorus to the anus. First the epithelium and then the whole mucosa becomes necrotic and finally it is partially shed. Accompanying the condition is a watery diarrhoea which soon becomes bloody. The characteristic phenomenon was termed by *Lillehei* "the common denominator" of the shock-syndrome of the dog (*Lillehei* et al. 1962). The precipitating factor of the shock is indifferent from this point of view. Sometimes the lesion can be observed in humans as well and there is a certain morphological and aetiological similarity between the above mentioned experimental enteropathy and the clinical cases of necrotizing enterocolitis observed after major abdominal and cardiac surgery (*Drucker* et al. 1964).

The pathomechanism of the lesion was partly clarified by a Montreal team (*Bounous* et al. 1963, 1964a; *Gurd* 1965). The role of trypsin in the process was proved by the local protective effect of the trypsin-antagonist Trasylol[R] (*Bounous* et al. 1964b) and by preventive ligation of pancreatic ducts (*Bounous* et al. 1965). The latter prevented not only the mucosal necrosis but caused an increased shock-tolerance in preoperated dogs; so it was justified to call the lesion a tryptic haemorrhagic enteropathy. However, according to *Black-Schaffer* and associates the haemorhagic enteropathy is the ultimate outcome of osmotic shedding of the enteric epithelium, brought about by impairment of ionic pumping, i.e. active transport of potassium ion diminishes, while passive movement into the cells of hydrated Na continues. The result is an osmotic explosion (disruption) of the mucosal cells (*Black-Schaffer* et al. 1967).

For the study of haemorrhagic enteropathy we have developed a quick, simple and effective model, based on "pharmacological occlusion" of the cranial mesenteric artery of the dog.

Methods and results

Insertion of catheter. In dogs anaesthetized with 30 mg/kg of pentobarbital intravenously a cardiac catheter was inserted into the right femoral artery by the Seldinger method or by cut-down. In this position the leader was removed, the catheter was filled with heparinous saline and closed. Under fluoroscopic control the tip of the catheter was introduced into the cranial mesenteric artery. The correct position of the catheter in a dog lying on its right side is shown in Fig. 1. The manipulation process is not difficult as, in the dog, like in humans, there are only two vessels originating from the beginning of the abdominal aorta close to each other and running in the sagittal plane: cranially the coeliac artery, and caudally the cranial (i.e. superior) mesenteric artery, corresponding to the superior mesenteric artery in humans. When the catheter has reached its target, its tip is bent in a small angle; if it is in the coeliac artery, the tip forms a right angle (Fig. 1). In the latter case the catheter has to be pulled back a little and its tip will soon enter into the correct vessel. The main point is to manipulate the tip of the catheter at the level of the liver-shadow and on the anterior surface of the aorta in the sagittal plane. During the procedure which takes not more than 1—2 minutes the catheter has to be rotated and moved gently.

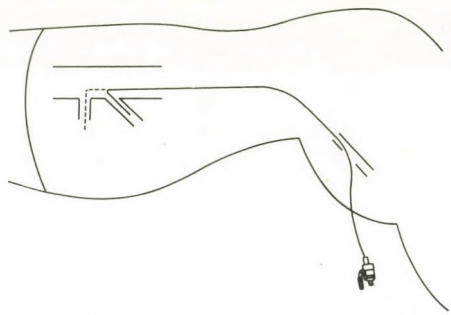

Fig. 1. Position of cardiac catheter in cranial mesenteric artery, inserted *via* the right femoral artery in a dog lying on its right side (for details, see text)

Infusion. After the required position has been reached the catheter is connected to an infusion pump and the vessel is perfused with 2 μg/kg/min norepinephrine for one hour.

The lesion. This quantity of the drug is enough to produce gross (Fig. 2) and microscopic (Figs 3, 4) enteropathy in most cases; the lesion is identical with the tryptic haemorrhagic enteritis of the dog.

Characteristic difference. The only, and rather characteristic, difference is that the first sign of haemorrhagic mucosal suffusion starts with a sharp border in the duodenum approximately 15 cm from the pylorus, corresponding to the blood supply of this part of the gastrointestinal tract.

Discussion

It has been shown that shock can be produced by a large quantity of catecholamines (*Lillehei* et al. 1964; *Gilbert* and *Hohl* 1964). The 2 μg/kg/min dose of norepinephrine applied by us is far less than the amount necessary for inducing

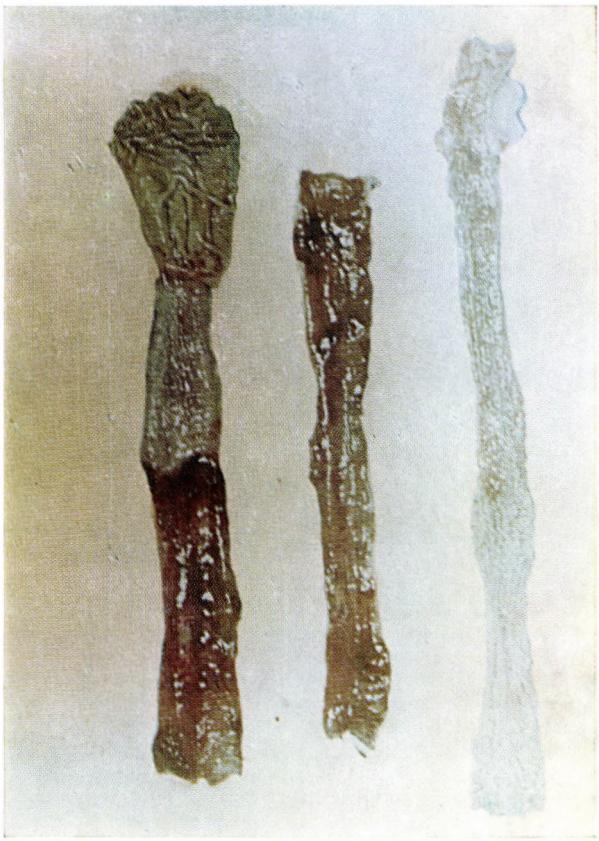

Fig 2. Typical tryptic haemorrhagic enteropathy produced by perfus ioninto the crauial mesenteric artery of 2 μg/kg/min norepinephrine for one hour. The gross suffusion in the duodenum starts, with a sharp border apqroximately 15 cm from the pylorus. It is marked iu the small bowel but there is only a mild lesion in the oral half of the colon

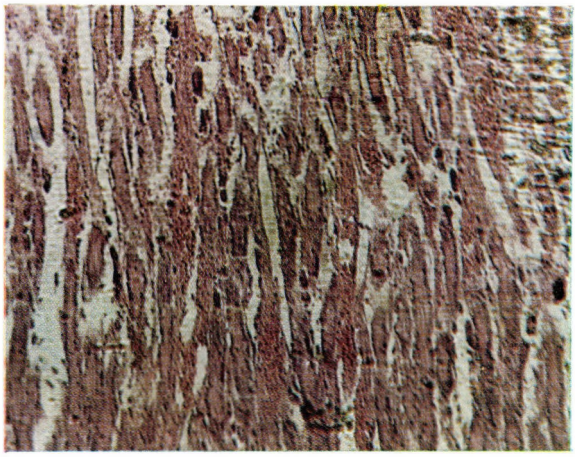

Fig 3. Grave stasis and early disintegration of duodenal mucosa. Haematoxylin-eosiu, approx, x 85

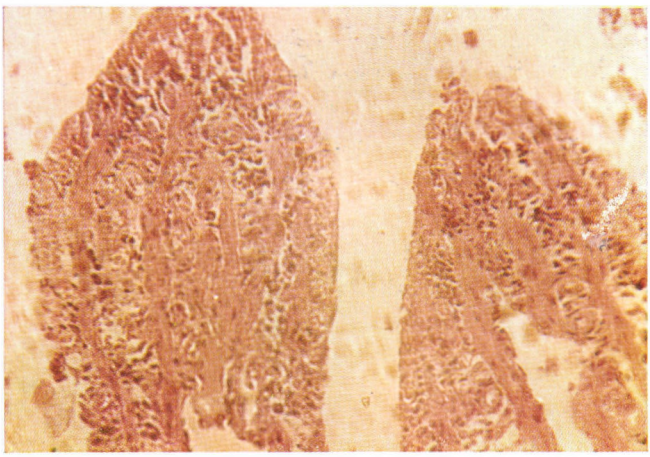

Fig 4. A characteristic sign of the early stage of enteropathy under high power: on the tips of mucosal villi the epithelium has been shed, the villi are denuded with no nublear staining. Haematoxylin-eosin, approx. 210

shock. In preliminary experiments all animals survived if the perfusion did not exceed two hours in duration or a quantity of 4 μg/kg/min of norepinephrine. The 2 μg/kg/min dose used in our experiments was in most cases sufficient to produce a typical enteropathy without shock.

In some dogs, however, no enteropathy had developed in spite of the same experimental procedure. With the use of an electromagnetic flowmeter (type Carolina Medical Electronics 322) it was shown that development of the lesion was correlated with the decrease of flow. During the perfusion, blood flow in the cranial mesenteric artery stops in most cases and this leads to the mucosal suffusion. If the flow does not stop completely, no mucosal haemorrhage is seen. This observation can be explained by the different sensitivity to catecholamine of individual dogs.

In a dose of 2 μg/kg/min, norepinephrine has practically only local effects. The drug-induced vasoconstriction is confined to a restricted part of the gastrointestinal tract, the one supplied by the cranial mesenteric artery, i.e. from the distal two thirds of the duodenum to the aboral half of the large bowel (*Miller* et al. 1964). The experimental enteropathy itself is perfectly identical with the lesion of bowel mucosa produced by any kind of shock.

Direct perfusion of the cranial mesenteric artery with norepinephrine has two main advantages, viz. the haemorrhagic enteropathy can be produced without hypovolaemia and shock in a short period; and there is no need of a surgical intervention (*Williams* et al. 1968).

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Pharmacologia

PHARMACOLOGICAL INVESTIGATION ON THE NEUROHUMORAL TRANSMISSION OF THE VASOMOTOR REGULATION

By

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Renal efferent sympathetic activity and its changes due to stimulation of the central stump of the vagal, sciatic and ulnar nerves were investigated. In addition, the effect on basal activity and sympathetic reflexes of drugs with well defined site of action was studied (diazepam, tofizopam, phentolamine, dihydroergotamine, chlorpromazine, reserpine, clonidine, atropine, methysergide and phenindamine).

The sympathetic efferent activity and the changes in sympathetic reflexes allowed conclusions to be drawn as to the functional state of the vasomotor centre.

Neither methysergide nor phenindamine inhibited efferent sympathetic activity or influenced sympathetic reflexes. These findings exclude the possibility of serotonin or histamine being the transmitter substance in the vasomotor neurone. Experiments with atropine revealed that the muscarinic action of acetylcholine does not figure in the sympathetic inhibitory or excitatory reflex processes.

Of the drugs investigated only diazepam and clonidine inhibited efferent sympathetic activity. Clonidine was more selective and acted in much lower doses (20 μg/kg) than diazepam (0.5—1 mg/kg).

The alpha blocking agents inhibited the viscero-sympathetic inhibitory reflex arch more intensely than the somato-sympathetic inhibitory one. The inhibitory transmitter is presumably noradrenaline.

The sympathetic excitatory reflexes were decreased by diazepam and tofizopam and increased by clonidine and phentolamine. The other substances were ineffective. As to the transmitter substance figuring in the sympathetic excitatory reflexes no unequivocal answer could be obtained in the present experiments.

The executive role in the maintenance of vasomotor tone is played by the sympathetic nervous system. This has been confirmed not only by electrical stimulation of the peripheral sympathetic nerves but also by neurophysiological tests. A continuous tonic activity can be observed on the efferent sympathetic fibres running to the heart (*Bronk* et al. 1936; *Fischer* et al. 1955; *Downing* and *Siegel* 1963; *Tuttle* 1963, 1965), blood vessels (*Folkow* 1955, 1960), splanchnic region (*Gernandt* et al. 1946; *Dontas* 1955; *Tang* et al. 1957; *Peiper* and *Hauck* 1961), limbs (*Celander* and *Folkow* 1953; *Folkow* 1956), and kidneys (*Engelhorn* 1957; *Sell* et al. 1958; *Weidinger* et al. 1961; *Kehrel* et al. 1962; *Weidinger* and *Leschorn* 1964; *Weidinger* and *Huber* 1964). The part played by these fibres in blood pressure regulation is also evidenced by observations according to which the activity of these fibres is modulated synchronously with the changes in blood pressure. A blood pressure elevation and increased neuronal activity are evoked by asphyxia, by excitation

of the chemoreceptors and by stimulation of peripheral somatic afferents. A fall in blood pressure and diminished neuronal activity are caused by excitation of the autonomic (sinus and vagal nerves) and of the somatic afferents (sciatic nerve).

On the basis of the above observations, the action potentials led off from sympathetic efferents allow to investigate the activity of vasomotor neurones (*Baust* et al. 1962).

The vasomotor neurones (*Pórszász* et al. 1962, 1965) receive both stimulatory and inhibitory impulses from the periphery and from the higher hypothalamic centres. Little is known, however, about the humoral factors participating in these connections, i. e. about the nature of the chemical substances responsible for neurochemical transmission.

No systematic investigations have been performed in this field. Studies by means of classical methods of the putative chemical substances involved in stimulus transmission are not feasible at the present methodological possibilities. It would therefore be necessary to demonstrate in the synapses of the vasomotor neurones a substance which will be liberated at the neuronal transmission and by the use of the substance one should be able to elicit an excitation or inhibition of the vasomotor neurones. The situation is complicated by the fact that the vasomotor neurones receive simultaneously both excitatory and inhibitory impulses. At present, knowledge of the substance playing a role in neuronal transmission must be based on indirect evidence. For this purpose, drugs of well-defined site of action are the most suitable tools.

On the basis of the above considerations, taking into account the pertaining data in the literature, we started investigations as early as 1965 to elucidate the nature of the presumably humoral factors which figure in the excitatory and inhibitory connections of the vasomotor neurones (*Pórszász* 1966; *Pórszász* and *P.-Gibiszer* 1967).

Methods

The experiments were performed on 150 cats anaesthetized with chloralose (35 mg/kg) plus urethane (300 mg/kg) by the intraperitoneal route. We investigated the vasodepressor reflex elicited by stimulation of the central stump of the posterior tibial nerve (SDR = sciatic depressor reflex), the depressor responses elicited by stimulation of the central stump of the vagal nerve (VDR = vagodepressor reflex), as well as the sympathetic activity preceding and accompanying the above reflex responses. In some cases the pressor responses to stimulation of the central stump of the posterior tibial nerve and of the ulnar nerve were also investigated together with the sympathetic activity preceding or accompanying these pressor responses. The same procedure was employed when investigating the carotid pressor reflexes.

The postganglionic sympathetic activity of the kidney was amplified by means of an RC amplifier (lower cut at 0.8 to 50 Hz, upper cut at 1 to 2 KHz) and continuously recorded in its integrated form on one channel of a Beckman Dynograph, simultaneously with registration of blood pressure and respiration. To integrate action potentials, we used a device functioning with active elements and having a time constant of 0.2 sec. The sympathetic nerve was always kept in paraffin oil, and the animal was warmed from a distance of 80 cm by means of an infrared lamp. Under these conditions

the activity of the nerve remained satisfactory for several hours. In some cases action potentials were led off from the splanchnic as well as from the hypogastric nerve; similar changes were obtained also on these nerves. Evaluation of the integrated neuronograms was made by means of the basal activity before stimulation always taking for basis an identical time unit, generally 15 seconds. Artefacts due to stimulation with the square-wave impulse generator were reduced to a minimum by means of a home-made high-frequency isolation transformer (*Pórszász* and *Szabó* 1959). To avoid movement artefacts the animals were immobilized with gallamine (2 to 3 mg/kg, intravenously) and kept under artificial respiration.

The drugs used were: diazepam (Seduxen^R, Richter, Budapest), tofizopam (Grandaxin^R, EGYT-341, EGYT Pharmacochemical Works, Budapest), phentolamine (Regitin^R, CIBA), dihydroergotamine (Sandoz), chlorpromazine (Hibernal^R, EGYT Pharmacochemical Works, Budapest), reserpine (Rausedyl^R, Richter, Budapest), clonidine (St-155, Catapresan^R, Boehringer), phenindamine (Pernovin^R, Chinoin, Budapest), methysergide (Deseril^R, Sandoz), cholinephenylether.

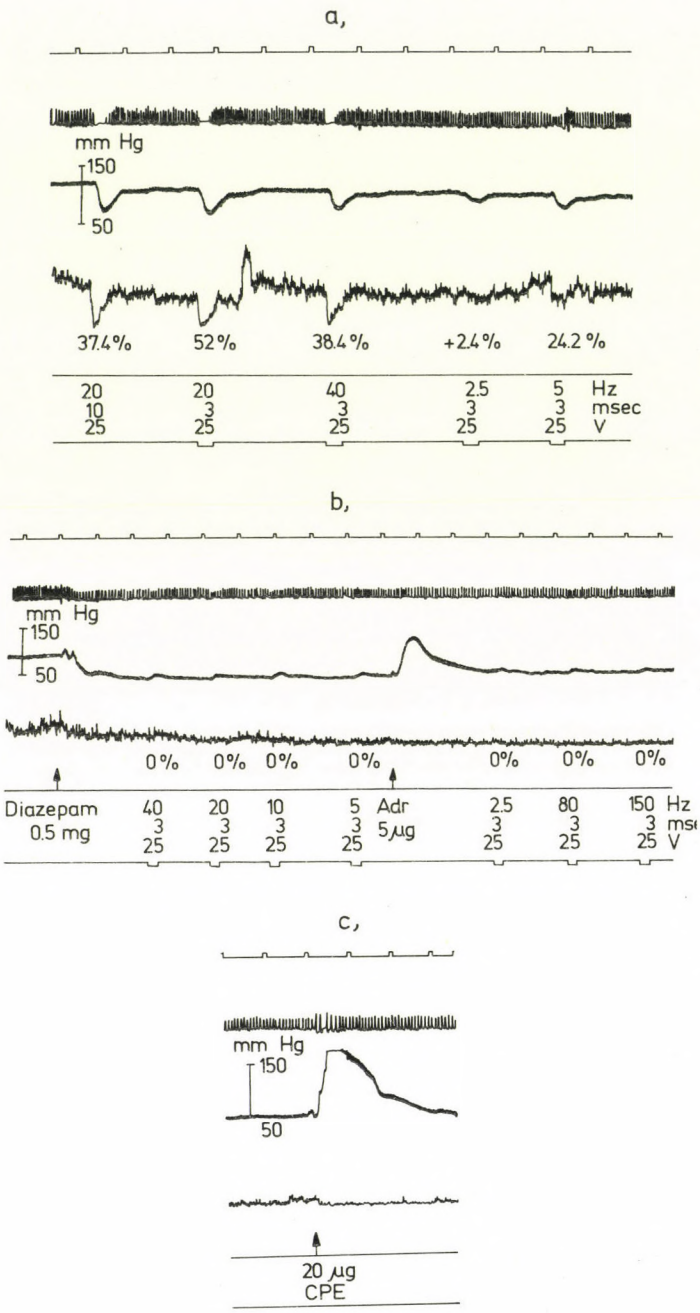
Results

1. Alterations of the vagodepressor reflex (VDR) and of the accompanying sympathetic reflex responses

a) Diazepam. A typical record taken from one of the six cats investigated is shown in Fig. 1. Part a) of Fig. 1 shows the effect on blood pressure and integrated sympathetic activity of the stimulation of the central vagal stump with 20, 40, 2.5, 5 Hz, 3 msec and 5 V. Part b) shows that 0.5 mg/kg diazepam reduced blood pressure by 40 mm Hg, and the sympathetic activity decreased gradually. Stimulation of the vagal nerve (40, 20, 10 and 5 Hz) elevated blood pressure by 9 to 10 mm Hg without affecting the sympathetic activity. This dose of diazepam did not inhibit the sympathetic ganglionic structures as proved by the finding that the intravenous injection of 20 μg/kg of cholinephenylether considerably increased the blood pressure. Diazepam was investigated in doses of 0.5—1—2 and 3 mg/kg given intravenously. Fig. 2 shows the effect of 0.5 mg/kg of diazepam. In this Figure and in the following ones, the abscissa shows the logarithm of stimulus frequency and the ordinate the percentage inhibition of sympathetic activity. It is clearly seen that diazepam fully inhibited the reflex inhibition of sympathetic activity; actually, an activity-increasing tendency appeared at higher frequencies.

Thirty minutes after administration of 3 mg/kg of diazepam lower stimulation frequencies did not inhibit but increased the sympathetic activity. After one hour, stimulation with 2.5—5—10—20 Hz of the central vagal stump caused a considerable augmentation of sympathetic activity, and also a blood pressure elevation (Figs 3 and 4).

b) Tofizopam, a drug related chemically to diazepam, in intravenous doses of 10—20 mg/kg caused a lasting fall in blood pressure, but did not affect the efferent sympathetic activity. The sympathetic reflexes were, however, altered after smaller (1—2—3 mg/kg) doses of the drug.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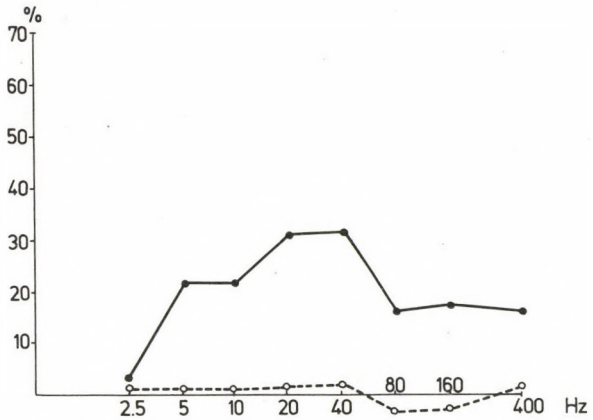


Fig. 2. Inhibitory effect of diazepam on the viscero-sympathetic inhibitory reflex. Abscissa: stimulation frequency applied on the central vagal trunk: impulse width 3 msec, amplitude 5 V. Ordinate: inhibition in per cent. Full line shows the effects of stimulation before drug administration, broken line those after 0.5 mg/kg diazepam i.v.

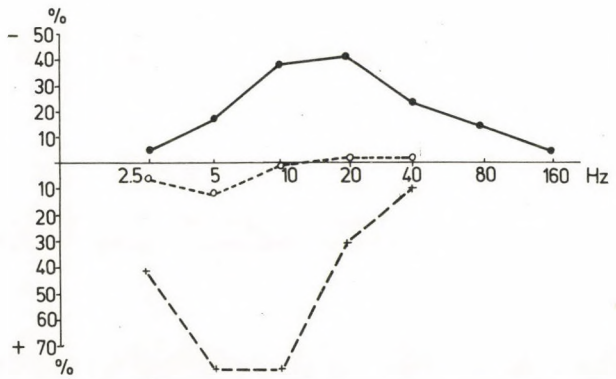


Fig. 3. Inhibitory effect of diazepam on the viscero-sympathetic inhibitory reflex. Abbreviations as in Fig. 18. −%: inhibition in per cent; +%: augmentation in per cent. Full line: before drug administration; •—•—•: 30 min after 3 mg/kg diazepam; +—+—+: one hour after 3 mg/kg diazepam

←

Fig. 1. Modifying effect of diazepam on the sympathetic-inhibitory reflex. Anaesthesia: 35 mg/kg chloralose and 300 mg/kg urethane intraperitoneally. From top to bottom: time in min, respiration, arterial blood pressure, integrated sympathetic activity. The number under the latter means the percentage decrease of sympathetic activity. Below: stimulus mark. A high frequency isolation transformer was used. a) Changes before diazepam; b) changes after diazepam; c) the effect of cholinephenylether. CPE: cholinephenylether; Adr.: adrenaline

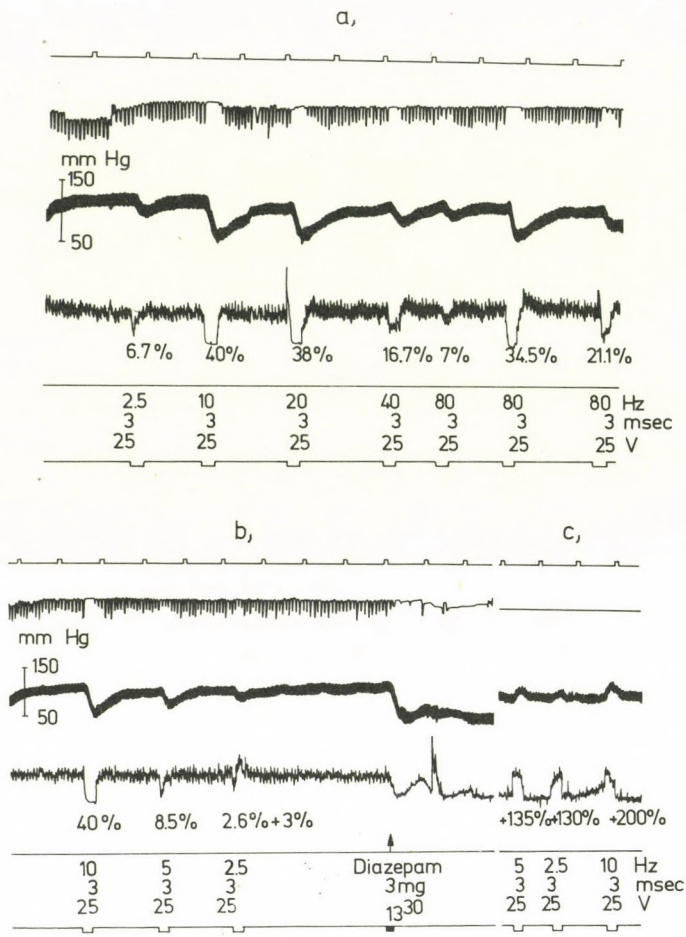


Fig. 4. Inhibitory effect of diazepam on the viscero-sympathetic inhibitory and on the vagodepressor reflex. Marks from top to bottom as in Fig. 1. Part a): sympathetic reflex responses before the drug; part b): and part c): after 3 mg/kg diazepam. Stimulation of the central trunk of the left vagal nerve. High frequency isolation transformer was used. It is seen that vasodepression turned into vasopression

In the present series of experiments the central vagal stump was stimulated at an impulse duration of 5 msec, and with 3 V at various frequencies. Within 30 minutes after 1 mg/kg tofizopam both the sympathetic reflex responses and the blood pressure responses elicited by vagal stimulation were considerably enhanced. The reflex responses were reversed from inhibitory to excitatory 60 min after the injection of tofizopam. At the same time the reflex responses in blood pressure are biphasic. The heart rate was unaffected (Fig. 5).

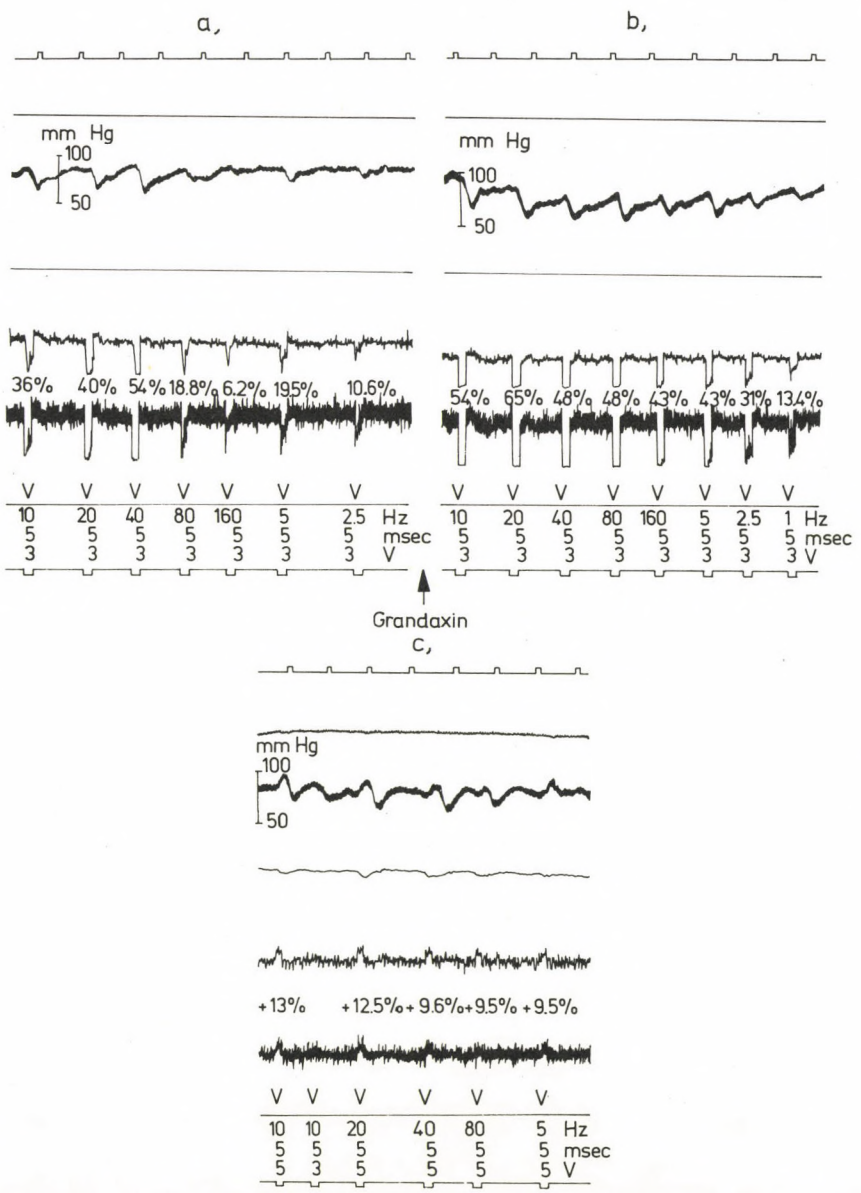


Fig. 5. Effect of tofizopam on vagoinhibitory reflex. Parallel registration of nictitating membrane contraction, blood pressure, heart rate, and efferent sympathetic activity of renal plexus by means of two different time constant integrations (0.4 and 0.2 sec) from top to bottom, respectively. Stimulation parameters msec; V) and the extent of evoked sympathetic reflexes are expressed in per cent of basic activity. Top line: time in minutes. Bottom line: stimulation time (10 sec). a) Reflex response of untreated animal; b) 30 min after 1 mg/kg tofizopam; c) 60 min after 1 mg/kg tofizopam

Tofizopam (1 mg/kg intravenously) caused an enhancement of the inhibition of sympathetic activity elicited by vagal stimulation in the first 30 min, and the inhibition changed into excitation 60 min after administration. 2 mg/kg of the drug immediately diminished the inhibitory viscero-sympathetic reflex and 60 min later this turned into an excitatory one. These results are shown in Fig. 6.

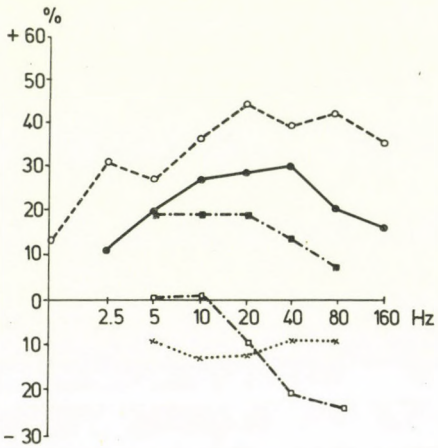


Fig. 6. Alteration of inhibitory response evoked by central vagal stump stimulation after 1 mg/kg and 2 mg/kg tofizopam (average values of 5 experiments). ●—●: Extent of inhibition, 30 min after 1 mg/kg tofizopam; x—x: extent of inhibition, 60 min after 1 mg/kg tofizopam; ■—■: inhibition immediately after 2 mg/kg tofizopam; □—□: excitation, 60 min after 2 mg/kg tofizopam; ———: control inhibitory reflex response to vagal stimulation

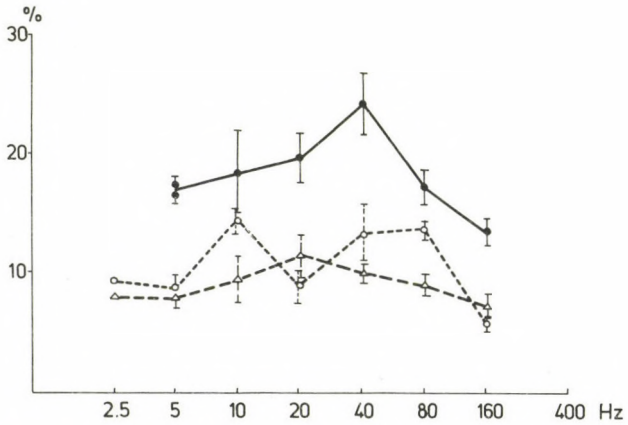


Fig. 7. Effect of chlorpromazine on the viscero-sympathetic inhibitory reflex. Average of 5 experiments. Abscissa: stimulus frequency. Ordinate: inhibition in per cent. ———: Control value; — — —: 30 min after chlorpromazine; —— ——: one hour after chlorpromazine. Stimulation of central trunk of left vagal nerve ± standard error

c) Chlorpromazine given in doses from 2 to 5 mg/kg intravenously evokes a lasting decrease of blood pressure and a parallel increase in renal sympathetic activity. These findings indicate that the vasodepression is not due to a diminished activity of the vasomotor neurones (VMN); in all certainty, the adrenolytic and spasmolytic effects of the drug are responsible for the action. Sympathetic activity begins to decrease after 10 to 15 min, a fact indicative of the slow onset of the drug effect.

Chlorpromazine given in doses from 2 to 5 mg/kg inhibited in four out of five animals the inhibition of sympathetic activity preceding the vasodepressor reflex (Fig. 7). In one cat, the reflex inhibition of sympathetic activity was even enhanced.

d) Phentolamine (1 to 2 mg/kg) evokes a lasting diminution of blood pressure, with a simultaneous increase in sympathetic activity. Thus, the vasodepression is of peripheral origin. Later, sympathetic activity reaches the initial level but does not fall below it. The reflex inhibition of sympathetic activity is diminished by phentolamine (Fig. 8). It revealed the inhibition in four out of the six investigated animals, and enhanced it in two animals. In one, the reflex inhibition turned into excitation upon the effect of stimulation with a voltage higher than the initial one.

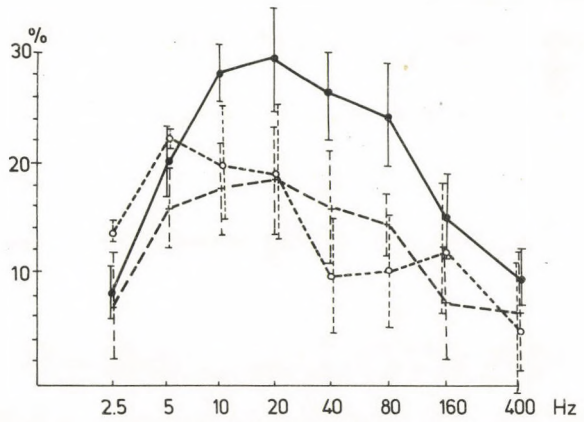


Fig. 8. Effect of phentolamine on the viscero-sympathetic inhibitory reflex. Average of 6 experiments. Marks as in Fig. 7. Stimulation of central trunk of left vagal nerve ± standard error

e) Dihydroergotamine (DHE). A dose of 1 to 2 mg/kg of DHE decreases blood pressure and causes an increase in sympathetic activity which later falls below the initial value. In four animals out of five the reflex inhibition of sympathetic activity was inhibited (in one cat the inhibition developed after a transient enhancement); in one animal the inhibitory effect was only increased.

f) Reserpine. The animals were pretreated with 3 mg/kg of reserpine 24 hours prior to the experiment. An investigation performed on such an animal is shown in Fig. 9 which clearly shows that, at a blood pressure level of 90 mm Hg, stimulation of the central vagal stump completely inhibited sympathetic activity during the stimulation without causing any fall in blood pressure. Thus there is a "dissociation"

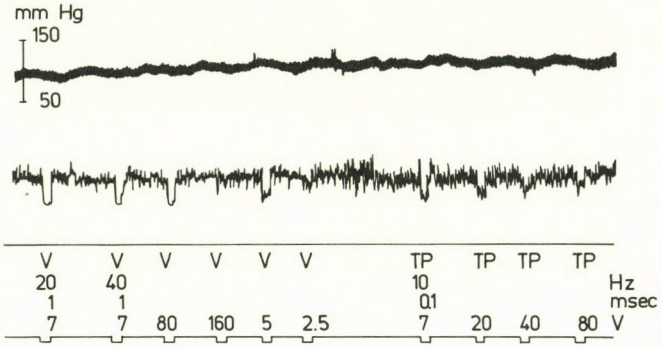


Fig. 9. Effect of reserpine on the viscero-sympathetic inhibitory reflex. Anaesthesia: 35 mg/kg chloralose + 300 mg/kg urethane intraperitoneally. Pretreatment with 3 mg/kg reserpine 24 hours before the experiment. V: central trunk of left vagal nerve; TP: central trunk of posterior tibial nerve. For details see the text

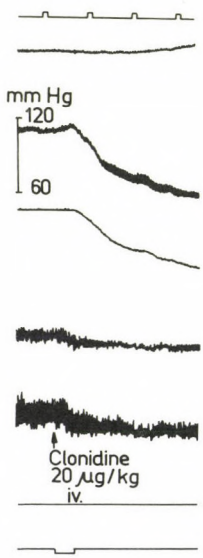


Fig. 10. Influence of 20 µg/kg clonidine on blood pressure and on efferent sympathetic activity of cat anaesthetized with a mixture of chloralose–urethane (45 mg/kg + 350 mg/kg, respectively). Parallel registration from top to bottom: time in min, nictitating membrane contractions, blood pressure, heart rate and efferent sympathetic activity of renal plexus by means of two different time constant integrations (0.4 and 0.2 sec)

between the changes in sympathetic activity and blood pressure. It has been shown earlier (*Such* et al. 1972) that, in some cases, there is no close parallelism between the changes of blood pressure and renal sympathetic activity. This is only natural, as peripheral resistance is controlled not only through renal, but also through other sympathetic efferent channels. It is also seen in Fig. 9 that stimulation of the central stump of the posterior tibial nerve inhibits sympathetic activity without any significant change in blood pressure.

Reserpine fails to influence the reflectorily induced sympathetic responses even if applied in acute experiments by the intravenous route (1 mg/kg).

g) Clonidine. 10—20 μg/kg of clonidine given intravenously causes a lasting fall in blood pressure synchronously with an inhibition of efferent sympathetic activity. This diminution caused by 10 μg/kg clonidine lasts about 8—10 min, but 20 μg/kg clonidine induces a depression in blood pressure of 34 ± 14.3 mm Hg lasting for 20 min and this fall is associated with a $24 \pm 8\%$ diminution of sympathetic activity. Furthermore, clonidine causes bradycardia and a lasting contraction of the nictitating membrane. The prompt modulating effect of clonidine on sympathetic activity suggests a central origin (Fig. 10).

The viscero-sympathetic inhibitory reflex is diminished by 20 μg/kg clonidine (Figs 11 and 12). In these experiments the sympathetic reflexes were different from animal to animal, but all their alterations were in direction identical with their

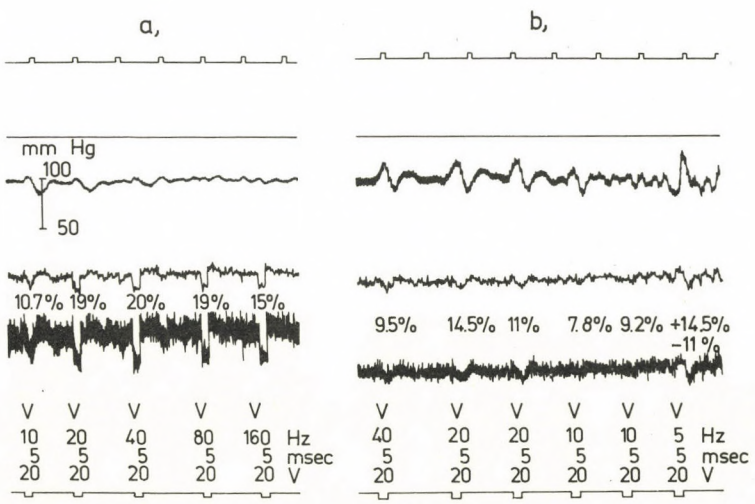


Fig. 11. Alteration of inhibitory reflex evoked by central vagal stump stimulation after 20 μg/kg clonidine intravenously. From top to bottom: time in min, nictitating membrane, blood pressure and efferent sympathetic activity of renal plexus by means of two different time constant integrations (0.4 and 0.2 sec), inhibition in per cent, stimulus mark. High frequency isolation transformer was used. a) Reflex response of untreated animal; b) 20 min after 20 μg/kg clonidine

common tendencies. Therefore, the results of these experiments have not been averaged. In all cases, clonidine diminishes the degree of reflex inhibition and turns it into a biphasic one. The phenomenon would be defined as a biphasic response, which appears during a single stimulation, first in the form of inhibition and after a few seconds as an excitation. The curves in Fig. 12 are characteristic; they show that the diminution of the reflex response is especially marked in the case of medium stimulating frequencies.

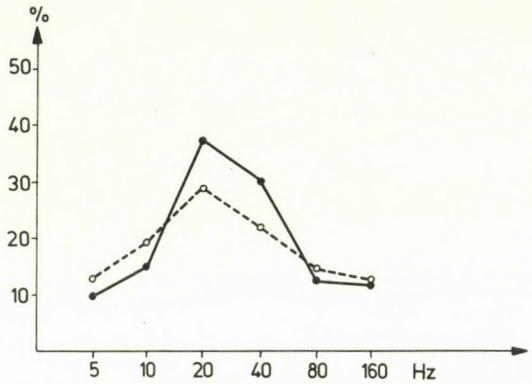


Fig. 12. Influence of clonidine on the viscero-sympathetic inhibitory reflex. ———: Reflex response of untreated animal; — — —: reflex response after 20 μg/kg clonidine

2. Alterations of the sciatic depressor reflex (SDR) and of the accompanying sympathetic reflex responses

a) Diazepam. The compound given intravenously in doses from 0.5 to 2 mg/kg inhibits basal sympathetic activity, and completely abolishes the reflectory inhibition of sympathetic activity evoked by stimulation of the central stump of the posterior tibial nerve. These results were identical in all the four animals investigated.

b) Tofizopam. This substance given in doses of 1 mg/kg intravenously, within 30 min after the injection decreases the intensity of the somato-sympathetic inhibitory reflex evoked by stimulation of the central stump of the sciatic nerve. After 60 min the effect is even more enhanced, and with doses of 2 mg/kg this inhibition of the inhibitory somato-sympathetic reflex is more marked than with a 1 mg/kg dose. It is seen in Fig. 13 that the sensitivity of the somato-inhibitory reflex is maximal at 10 Hz frequency stimulation of the sciatic nerve. Essentially the frequency-dependent responsiveness is not affected by tofizopam but at identical frequencies the maximum inhibition is depressed and this effect of the compound is more marked 60 min following the injection.

The diminishing effect of tofizopam on the reflex response grows with increasing doses of the compound from 1 mg/kg to 3 mg/kg. With the latter dose, reflex inhibition of sympathetic activity is completely abolished. In all experiments performed on 9 cats, the somato-inhibitory reflex diminished in dependence of the applied dose of the drug.

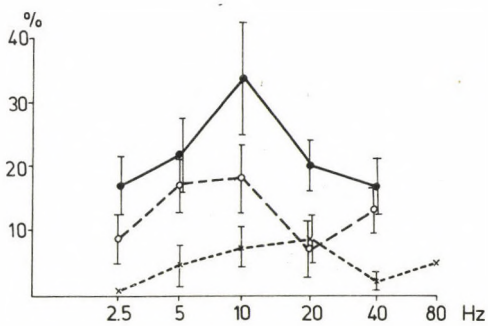


Fig. 13. Effect of tofizopam on the somato-sympathetic inhibitory reflex elicited by stimulation of the central stump of sciatic nerve (impulse duration, 0.1 msec; impulse amplitude, 2 V). The rate of inhibition is expressed in per cents of the basic sympathetic activity. ———: Reflex response of untreated animal; o—o: reflex response 30 min after 2 mg/kg tofizopam; x—x: reflex response 60 min after 2 mg/kg tofizopam

c) Chlorpromazine. Five animals were treated with 2 to 5 mg/kg of chlorpromazine. It increased the reflex sympathetic inhibition in 4 cats and inhibited it in one cat. Fig. 14 shows the results obtained in 4 animals. Inhibition of the sympathetic reflex inhibition increased gradually; the effect was manifest at 10 min, and considerably more intense at 30 min; it persisted for more than one hour.

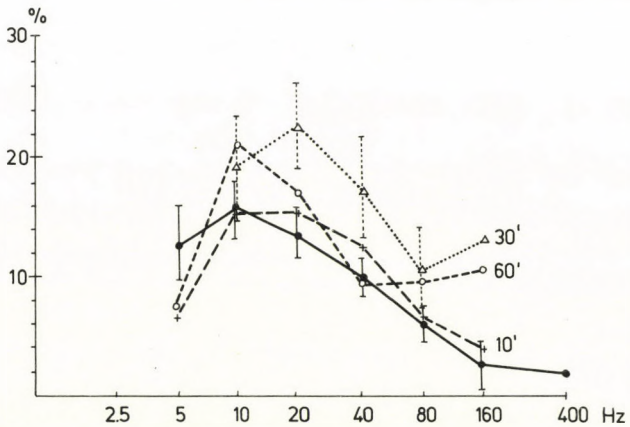
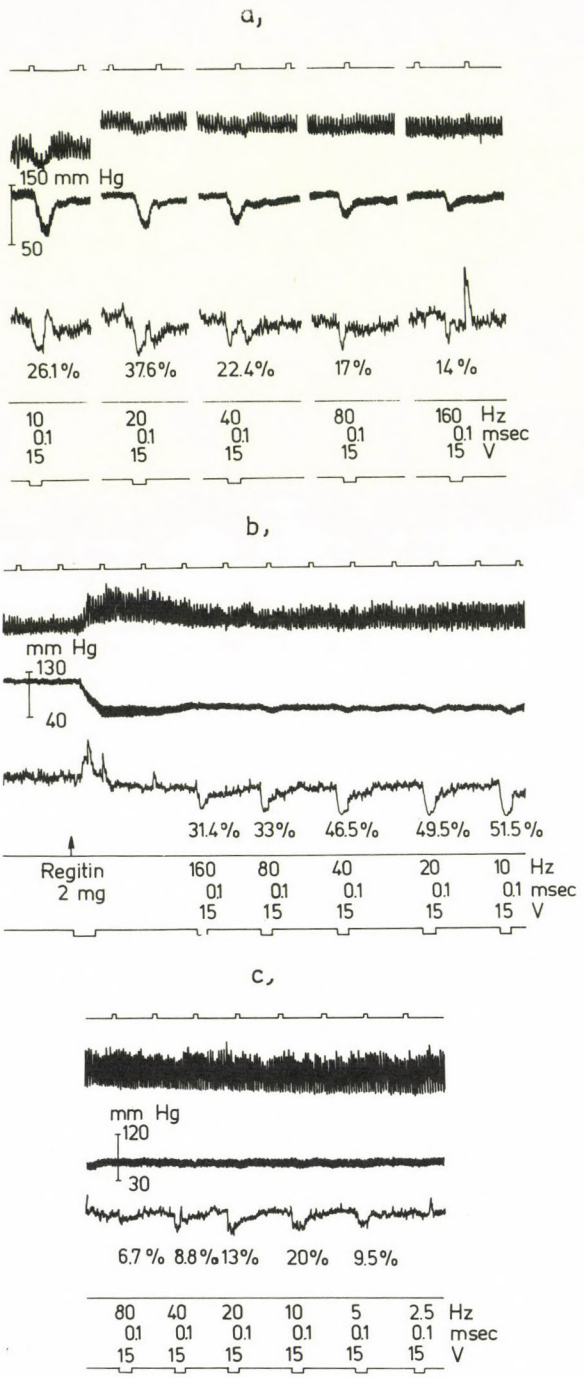


Fig. 14. Effect of chlorpromazine on the somato-sympathetic inhibitory reflex. Average of 4 experiments. Full line: initial control value. Other lines, as indicated: mean values of experiments performed 10, 30 and 60 min after drug administration. Stimulation of central stump of posterior tibial nerve ± standard error


a,
150 mm Hg
50
26.1 %
37.6 %
22.4 %
17 %
14 %
10 20 40 80 160 Hz
0.1 0.1 0.1 0.1 0.1 msec
15 15 15 15 15 V
b,
mm Hg
130
40
31.4 % 33 % 46.5 % 49.5 % 51.5 %
Regitin
2 mg
160 80 40 20 10 Hz
0.1 0.1 0.1 0.1 0.1 msec
15 15 15 15 15 V
c,
mm Hg
120
30
6.7 % 8.8 % 13 % 20 % 9.5 %
80 40 20 10 5 2.5 Hz
0.1 0.1 0.1 0.1 0.1 0.1 msec
15 15 15 15 15 15 V

d) Phentolamine. A typical record is shown in Fig. 15. Part a) demonstrates the inhibition of sympathetic activity due to stimulation of the central stump of the tibial nerve before drug administration. Part b) shows that immediately after the injection of 2 mg/kg of phentolamine the reflectory sympathetic inhibitions are enhanced. In Part c) the sympathetic inhibitions caused by stimulation of the posterior tibial nerve are markedly decreased, and are weaker than before phentolamine. The results of experiments of similar character performed on 4 animals are summarized in Fig. 16; the reflex inhibitions of sympathetic activity considerably increased after 10 min, but remained below the control level after 30 and 60 min, especially on stimulation at 20 Hz. Neither lower nor higher frequencies caused any significant decrease in the reflex inhibition of sympathetic activity.

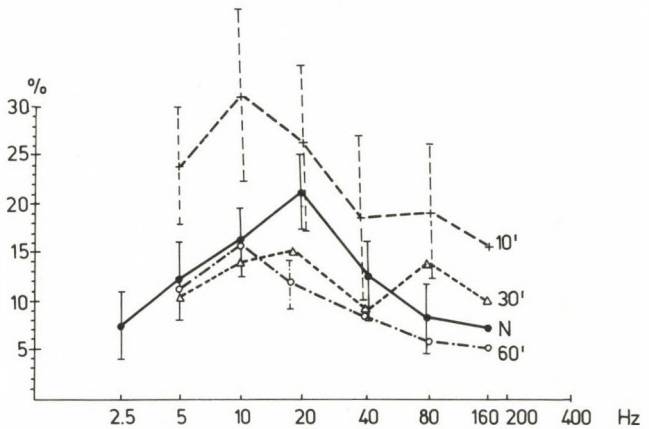


Fig. 16. Effect of phentolamine on the somato-sympathetic inhibitory reflex. Average of 4 experiments. Abscissa: stimulation frequency in Hz (log. scale); ordinate: inhibition in per cent of sympathetic activity due to stimulation of afferent tibial nerve. N: normal control value 10, 30 and 60 min after phentolamine administration. For details, see text

←

Fig. 15. Effect of phentolamine on the depressor reactions and sympathetic inhibitory responses evoked by stimulation of the posterior tibial nerve. Anaesthesia: 35 mg/kg chloralose + 300 mg/kg urethane intraperitoneally. From top to bottom: time in min, respiration, blood pressure, integrated sympathetic activity (inhibition in per cent). a) Responses before phentolamine; b) immediately after phentolamine; c) 30 min after phentolamine. High frequency isolation transformer was used

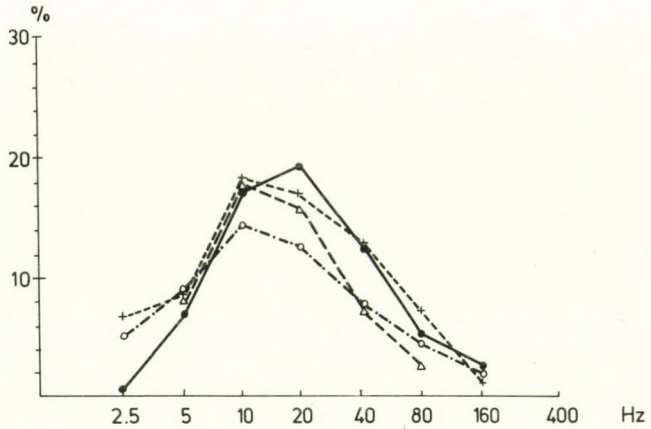


Fig. 17. Effect of dihydroergotamine on the somato-sympathetic inhibitory reflex. ———: Control value; +—+: 10 min; Δ—Δ: 30 min; ●—●—●: 60 min after dihydroergotamine. Abscissa: stimulation frequency in Hz, ordinate: inhibition of sympathetic activity in per cent

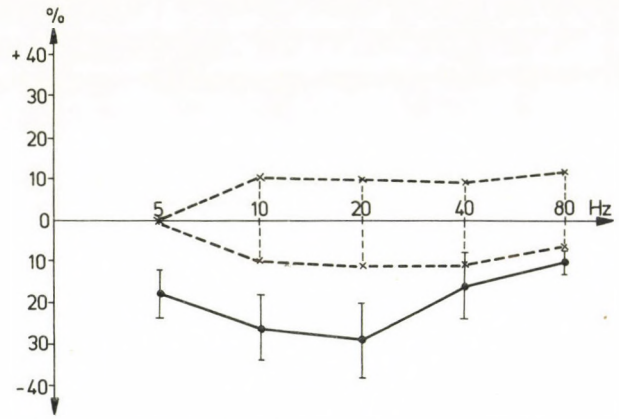


Fig. 18. Inhibition of somato-sympathetic inhibitory reflex by clonidine. ———: Reflex response of untreated animal; — — —: reflex response after 20 μg/kg clonidine (biphasic reflex) (per cent ±S.E.M.) The extent of inhibition and excitation of the stimulations are shown by vertical broken junction lines

e) Dihydroergotamine (DHE). Results obtained in 6 animals are shown in Fig. 17. DHE did not influence the reflectory sympathetic inhibition. Alterations were not evaluable statistically. The results resembled those obtained in animals treated with phentolamine. In 2 out of the 6 cats only an increase in reflectory sympathetic inhibition was observed, in one animal the inhibition developed after an enhancement, while in the remaining three cats a gradual decrease of the reflex inhibition was seen.

f) Clonidine. Results of 6 experiments showed that 20 μg/kg clonidine inhibits the development of inhibitory reflex elicited from the sciatic nerve. In 3 out of the 6 cats the reflex could not been elicited after clonidine treatment. In another 3 cats the inhibitory reflex turned into a biphasic one (Fig. 18).

g) Atropine, methysergide and phenindamine. One mg/kg each of the compounds failed to affect the sympathetic reflex.

3. Alteration of sympathetic activity accompanying the pressor reflexes

a) Diazepam decreased, in all cases, the sympathetic activation evoked by somatic afferentation.

b) Tofizopam. The rise of blood pressure which appears during occlusion of the carotid artery(ies) and the slight increase of sympathetic activity can be inhibited to about 50% with 6 mg/kg of tofizopam; with 10 mg/kg of the compound the carotid reflex is blocked. Excitation of sympathetic activity evoked by stimulation of the central stump of the ulnar nerve was inhibited with 2 mg/kg of tofizopam in 10 min for more than 30 min.

c) Chlorpromazine. Fig. 19 shows the modification of the carotid pressor reflex under the effect of chlorpromazine. Compression for 20 sec of one or both carotid arteries hardly increased sympathetic activity before chlorpromazine administration, even though bilateral compression caused a considerable increase in blood pressure (part a of Fig. 19). After the intravenous administration of 2 mg/kg of chlorpromazine, sympathetic activity markedly increased under the effect of the interventions (part b). The same result was consequently obtained in 4 animals. Sympathetic activity accompanying the carotid pressor reflex was enhanced by phentolamine, too.

Sympathetic activation evoked by stimulation of the central stump of the median nerve was not depressed by 2 mg/kg of chlorpromazine given intravenously (see Fig. 19, part b); while phentolamine somewhat enhanced it (Fig. 20). The possibility of sympathetic activation always persisted, even after reserpine pretreatment.

d) Clonidine. The somato-excitatory reflex of the ulnar nerve showed an increased excitation after 20 μg/kg clonidine, but there was no increase at each stimulation frequency. The difference is obviously significant of stimulations with 10—20 Hz; significancy at 40—80 Hz is at a level of $p < 0.05$ (Fig. 21).

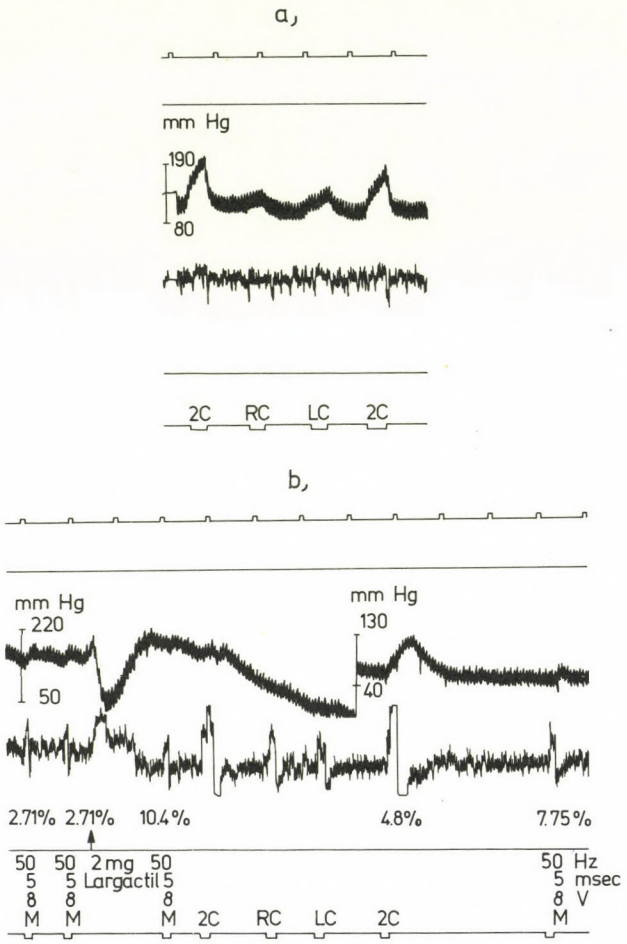


Fig. 19. Effect of chlorpromazine on carotid sinus reflex and on the consecutive sympathetic responses. Anaesthesia: 35 mg/kg chloralose + 300 mg/kg urethane. Marks: time in min, arterial blood pressure, integrated sympathetic activity. 2C: occlusion of both carotid arteries; RC: occlusion of right carotid; LC: occlusion of left carotid; M: stimulation of central stump of median nerve; Hz, msec, V. a) Blood pressure and sympathetic response before chlorpromazine; b) after 2 mg/kg chlorpromazine

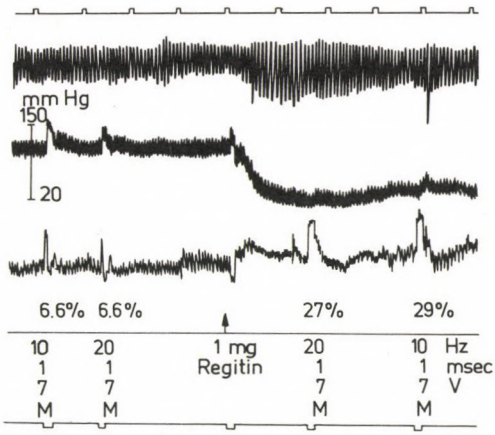


Fig. 20. Effect of phentolamine on somato-excitatory reflex. Anaesthesia: 35 mg/kg chloralose + 300 mg/kg urethane. Marks from top to bottom: time in min, respiration, blood pressure, integrated sympathetic activity. Numbers under sympathetic activity refer to increase in per cent. M: stimulation of central stump of median nerve. Isolation transformer was used

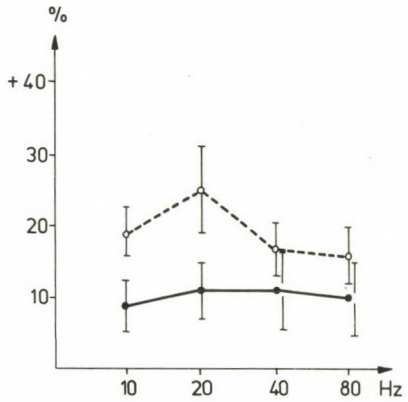


Fig. 21. Gradation by clonidine of somato-excitatory reflex (3 cats). ——: Untreated animal; — —: after 20 μg/kg clonidine. Abscissa: stimulation frequency in Hz (log. scale); ordinate: excitation in per cent of sympathetic activity due to stimulation of the afferent ulnar nerve

The results obtained with the drugs investigated are summarized in Table I.

Table I

Effect of various drugs on basal sympathetic activity and on its reflectory responses

Experiments performed on 72 cats

Compound	Efferent sympathetic activity	IDR	VDR	CAR	SPR
Diazepam 0.5—3 mg/kg	Decreased without transient enhancement	Inhibited	Inhibited or reversed	Somewhat decreased	Somewhat decreased
Tofisopam 1—3 mg/kg	Unaltered	Inhibited	Inhibited or reversed	Inhibited	Inhibited
Chlorpromazine, 2—5 mg/kg	Slowly decreased after transient enhancement	Enhanced	Inhibited after occasional transient enhancement	Enhanced	Unaltered, sometimes enhanced
Phentolamine, 1—2 mg/kg	Unaltered after transient enhancement	First enhanced, then inhibited	First enhanced, later inhibited or even reversed	Enhanced	Enhanced
Dihydroergotamine (DHE), 1—2 mg/kg	Inhibited after transient enhancement	Slightly inhibited after enhancement (no statistical change)	First enhanced, then inhibited	Not investigated	Not inhibited

Clonidine 20—40 μg/kg	Instantly decreased	Inhibited	First enhanced then inhibited	—	Enhanced
Reserpine, 3 mg/kg (24 hours prior to exp.)	Good	The inhibition persists	The inhibition persists	The enhancement persists	The enhancement persists
Guanethidine, 3 mg/kg (24 hours prior to exp.)	Good	The inhibition persists	The inhibition persists	The enhancement persists	The enhancement persists
Atropine, 1 mg/kg	Unaltered	Unaltered	Unaltered	Unaltered	Unaltered
Methysergide, 1 mg/kg	Unaltered	Unaltered	Unaltered	Unaltered	Unaltered
Phenindamine, 1 mg/kg	Unaltered	Unaltered	Unaltered	Unaltered	Unaltered

IDR : sympathetic inhibition eliciting depressor reflex from posterior tibial nerve
VDR: sympathetic inhibition eliciting vagodepressor reflex
CAR: sympathetic activation due to carotid occlusion
SPR : sympathetic activation due to excitation of somatic afferents

Discussion

In knowledge of the relationships and neurophysiological background of afferent stimuli reaching the bulbar sympathetic centres and the efferent responses to them one can obtain information on the transmitters figuring in the inhibitory and excitatory processes. For this purpose drugs with well-defined site of action may be of great help. The pharmacological significance of such experiments lies in the fact that the effect on the vasomotor centre of various compounds can be investigated, a feature useful in the search for centrally acting antihypertensive drugs.

Pórszász (1973) and *Pórszász* et al. (1972) published several data on the organization of excitatory and inhibitory connections in the bulbar sympathetic structure. It was shown that the electrophysiological analysis of the so-called vasomotor reflexes offered reliable information on the functioning of the bulbar vasomotor centre. With this aim, the conditions necessary to evoke sympathetic inhibitory and excitatory reflexes as well as the temporal and spatial summation of inhibition and excitation were studied. Analyzing the efferent responses essential differences were found between the neural pathways figuring in visceral and somatic afferentation, the visceral pathway containing much more synapses than the somatic one. A hypothesis was then put forward concerning the excitatory and inhibitory relations of the bulbar sympathetic neurone.

All the compounds used in the present experiments have a well-defined site of action. Many data are at disposal concerning the mode of action of ergot alkaloids (*Rothlin* 1925; *Wright* 1930), chlorpromazine (*Killam* and *Killam* 1958; *Bradley* 1958; *Domino* 1958), diazepam (*Marpurgo* 1968; *Sigg* and *Sigg* 1969), tofizopam (*Komlós* et al. 1968) and reserpine (*Iggo* and *Vogt* 1960).

The central effects of clonidine were first analysed by *Kobinger* and *Walland* (1967). In their experiments intracisternally applied clonidine inhibited the sympathetic cardiovascular centre. *Schmitt* et al. (1967, 1968), *Constantine* and *McShane* (1968), *Bently* and *Li* (1968) found that intravenously or intracisternally administered clonidine inhibited the action potential recorded on the splanchnic and cardiac nerves. The transection experiments of *Hukuhara* et al. (1968) and *Schmitt* and *Schmitt* (1969) verified that clonidine exerted its hypotensive action through the medulla oblongata. When applied centrally, clonidine inhibited efferent sympathetic activity, an effect antagonized by alpha blocking agents (*Schmitt* and *Schmitt* 1970; *Schmitt* et al. 1971). *Haeusler* (1973, 1974) investigated the activity of the efferent nerve and the increase of activity evoked by stimulation of the posterior hypothalamus. He found that clonidine, given intravenously, inhibited the increase in efferent activity. This inhibitory effect of clonidine would be counteracted by noradrenaline infusion and inhibited by piperoxane. Our results can be explained as follows.

The results obtained with methysergide and phenindamine excluded the possibility of serotonin or histamine being the transmitter substance in the vasomotor neurone.

The experiments with atropine excluded the possibility that the muscarinergic action of acetylcholine would play a part in the sympathetic-inhibitory or excitatory reflex processes. On the basis of the results of *Such* and *Mátrai* (1972) it may be assumed that it is the nicotine-like action of acetylcholine that activates the adrenergic system.

The effect of all the other substances investigated can be explained by an influence on noradrenaline metabolism.

Taylor and *Laverty*'s experiments on rats (1969) have raised the possibility that diazepam decreases noradrenaline turnover in the thalamus and midbrain. In our experiments diazepam diminished efferent sympathetic activity parallel with its hypotensive action, i.e. it depressed the functioning of the sympathetic structures. Diazepam decreases the vagodepressor and sciatic-depressor reflexes leaving the responsiveness of the vasomotor centre unaltered. All these effects can be explained by an inhibition of noradrenaline turnover.

Tofizopam, a drug related chemically to diazepam, can be assumed to influence sympathetic reflexes by the same mechanism as diazepam. Tofizopam does not decrease sympathetic activity; thus, its diminishing action on noradrenaline turnover is presumably weaker.

The histofluorescence investigations of *Fuxe* (1965) revealed a great number of catecholamine-containing cells and fibres in the n. tractus solitarius, n. motorius dorsalis vagi, the mesencephalic reticular formation and the n. gigantocellularis, at sites from which vasomotor reactions can be elicited. The transmitter role in the pathway system of the vagodepressor reflex is presumably played by catecholamines (*Pórszász* 1966; *Schmitt* et al. 1972).

Chlorpromazine, phentolamine and dihydroergotamine inhibit vagodepressor reflexes, an effect ascribed by *Pórszász* (1966) to an identical mechanism, i.e. the adrenolytic properties of the compounds.

It sometimes occurs that inhibition of the vagodepressor reflex does not develop immediately but is preceded by an initial augmentation of the reflex response. This phenomenon too may be due to an increased noradrenaline turnover. Such an increase in noradrenaline turnover was demonstrated by *Bigelow* et al. (1969) after the application of phenoxybenzamine. Chlorpromazine inhibits the re-uptake and increases the synthesis of noradrenaline (*Gey* and *Pletscher* 1961; *Carlsson* and *Lindquist* 1963; *Andén* 1964).

At the initial stage the augmenting effect of clonidine on inhibition of the vagodepressor reflex is also based on increased noradrenaline turnover (*Andén* et al. 1970). According to *Klupp* and *Knappen* (1970) clonidine inhibits the activity following stimulation of the saphenous nerve but the doses necessary for this action are higher than in the case of hypothalamic stimulation. Thus, the site of action of clonidine is the vasomotor centre.

The inhibition of the sympathetic reflex evoked by stimulation of the sciatic and posterior tibial nerves is augmented by chlorpromazine. Dihydroergotamine

has hardly any influence; phentolamine inhibits it after a transient increase while clonidine first inhibits then renders it biphasic and, finally, excitatory. In the blood pressure experiments of *Bergmann* and *Ramu* (1968) intraventricularly applied phentolamine inhibited and even reversed the vagodepressor reflex but left the depressor and pressor reflexes evoked by sciatic stimulation unaffected. When phentolamine was administered by the intravenous route, both the vagal and somatic depressor reactions were inhibited (*Bergmann* et al. 1967). In the light of our results the two inhibitory systems, differing in physiological characteristics, differ also quantitatively in their response to drugs, the inhibitory system of the somatic afferents being less sensitive than the vagal one.

Reserpine was applied in a dose of 3 mg/kg 24 hours before the experiments. This seems contradictory to the investigations of *Dahlström* and *Fuxe* (1965) in the rat, where the amine level returned to normal by the 24th hour. Since, however, the cat is more sensitive to reserpine than the rat we may accept the conclusion that reserpine does not influence sympathetic reflexes.

It was remarkable that, except diazepam and tofizopam, none of the substances investigated could abolish the sympathetic excitatory reflex response; they were even augmented. The most probable reason for the augmentation is the inhomogeneity of the reflex responses demonstrated in a previous paper (*Such* et al. 1972). Since the inhibitory connections can be blocked by the substances investigated it is easy to understand why the excitatory processes will increase.

The reflex elicited in untreated cats exhibits wide variations. This can be explained by the fact that the depth of anaesthesia depends on the general condition of the animal and also shows seasonal variations in spite of the dose of anaesthetic being identical. Thus, the individual responsiveness of the cats varies to a great extent. The sympathetic reflexes are frequency-related. Most sensitive is the range from 10 to 40 Hz, i.e. the drugs investigated mainly inhibit at these frequencies. The cause of this phenomenon was not investigated.

Summing up, alpha-blocking agents inhibit the viscero-sympathetic more effectively than the somato-sympathetic inhibitory reflex arch. The inhibitory transmitter is presumably noradrenaline. Presynaptic inhibitory effect of cholinergic transmitter on noradrenaline through α-receptor has been shown (*Vizi* 1968; *Paton* and *Vizi* 1969; *Vizi* 1974). The inhibition of noradrenaline release through presynaptic α-receptors has been shown in peripheral (*Starke* et al. 1974) and also in central noradrenaline nerve terminals (*Starke* and *Montel* 1973). Clonidine, exciting central presynaptic α-receptors, possibly mimicks the action of noradrenaline and this could be the reason why clonidine acts on viscero-sympathetic inhibitory pathway in a similar way as α-receptor blocking drugs. According to data in the literature clonidine directly excites alpha receptors. If this is so, the drug should facilitate the viscero-inhibitory reflex; actually, it inhibits it. The transmitter substance of the sympathetic-excitatory reflexes could not be elucidated by pharmacological antagonists under the

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TACHYPHYLAXIS TO THE HYPOTENSIVE EFFECT OF CLONIDINE AND THE POSSIBLE MECHANISM OF THIS ACTION

By

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In cats under chloralose–urethan anaesthesia, tachyphylaxis developed to the hypotensive effect of clonidine. Development of this tachyphylaxis was slow in the case of intravenous low doses (10—20 μg/kg) and rapid after high doses (50 μg/kg). No tachyphylaxis developed to contractions of the nictitating membrane. The phenomenon resembles the tachyphylaxis seen after the application of indirect sympathomimetics. A tachyphylaxis developed to the latter agents inhibited the hypotensive action of clonidine which, after such an intervention, invariably turned into a vasopressor effect. Clonidine exhibited the same behaviour after pretreatment with cocaine, bretylium or reserpine. Infusion of noradrenaline (10 μg/kg/min) counteracted the tachyphylaxis. Thus, clonidine is assumed to exert its hypotensive effect in an indirect way, i.e. by interference with the metabolism of noradrenaline.

Clonidine (Catapresan, ST-155) has been shown to possess a long-lasting hypotensive action (*Hoefke* nad *Kobinger* 1966; *Kobinger* 1967; *Kobinger* and *Walland* 1967) and that this action might be due to an effect on the central sympathetic structures (*Sattler* and *van Zwieten* 1967; *Schmitt* et al. 1967; *Schmitt* and *Schmitt* 1969; *Klupp* et al. 1970).

In previous experiments (*Pórszász* 1966) no drug was, however, found to exert its hypotensive action by a direct influence on the vasomotor centre. The central site of action of clonidine renders this drug suitable for elucidating the function of the vasomotor centre. In previous experiments it was observed that a second intravenous administration of clonidine decreased blood pressure to a much lesser extent than did the first one and that subsequent injections even increased it (*P.-Gibiszer* and *Pórszász* 1973).

The aim of present experiments was to study the mechanism of rapid tolerance (tachyphylaxis) developing on repeated administration of clonidine.

Methods

The experiments were performed on 35 cats under intraperitoneal chloralose + urethan anaesthesia (35 mg/kg and 300 mg/kg, respectively). Blood pressure was measured by means of a mercury manometer connected to the left femoral artery, respiration through a cannula inserted into the trachea and contraction of the nictitating membrane with an isotonic lever (1 : 12) on a Palmer kymograph in the usual way. The substances to be investigated were injected through a cannula inserted into the femoral vein.

The substances used were: clonidine (Boehringer, Ingelheim); dl-amphetamine sulphate (Merck); cocaine hydrochloride (Merck); tyramine hydrochloride (Merck); ephedrine hydrochloride (Merck); bretylium tosylate (B.W.); reserpine (Merck).

Results

Clonidine tachyphylaxis

Figure 1 shows a typical experiment concerning clonidine tachyphylaxis. Injection of 8 μg/kg of clonidine caused an initial elevation in blood pressure which was followed by a slow diminution with nearly complete return to the original level 18 min after injection of the drug. A new 8 μg dose given after the first injection caused a lasting elevation in blood pressure and augmented contractions of the nictitating membrane.

Subsequent administration of 16, 32 and 64 μg of clonidine intravenously elevated blood pressure and permanently increased the tone of the nictitating membrane until maximum contraction.

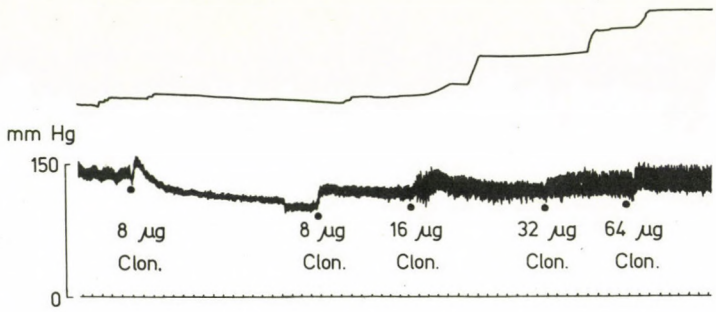

Fig. 1. Effect of repeated injections of clonidine on blood pressure and nictitating membrane. Cat, 2.5 kg. Intraperitoneal anaesthesia with 35 mg/kg of chloralose + 300 mg/kg of urethane. From top to bottom: nictitating membrane, blood pressure, time in minutes. Clon: clonidine

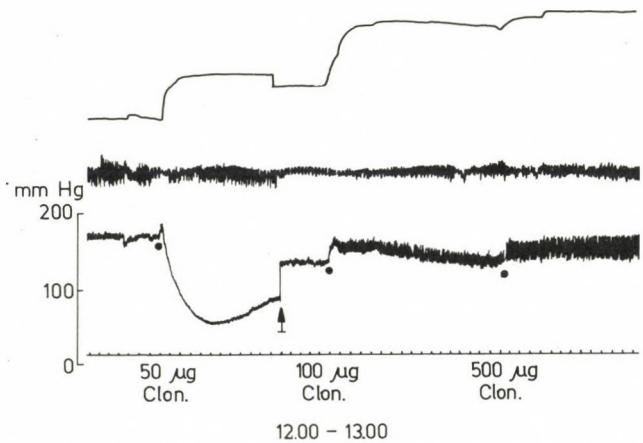

Fig. 2. Rapid tachyphylaxis elicited by a high dose of clonidine. Cat. 2.8 kg. Intraperitoneal anaesthesia with 35 mg/kg of chloralose + 300 mg/kg of urethane. From top to bottom: nictitating membrane, respiration, blood pressure, time in minutes. Clon: clonidine; ↑: break of recording for 60 min

Tachyphylaxis developed even more rapidly after a high first dose of clonidine. Such an experiment is shown in Fig. 2. Intravenous injection of 50 μg/kg of clonidine caused a rapid and lasting fall in blood pressure, from 180 to 50 mm Hg. The second injection of clonidine (100 μg/kg, intravenously) only elicited an elevation of blood pressure just as did the third dose (500 μg/kg, intravenously). The nictitating membrane was contracted to the maximum in all cases. The middle curve shows respiration; 500 μg of clonidine caused a reflex increase due to the bradycardia elicited.

The average decrease in blood pressure produced by the so-called "first clonidine dose" was determined in 18 cats. The average reduction is shown in Fig. 3. The hypotensive effect of clonidine was calculated in per cents of the initial blood pressure (full line). The effect of consecutively increasing doses of clonidine (broken line) was calculated in per cents of the original blood pressure value. As it is seen from Fig. 3, the first injection of 20 μg/kg of clonidine caused a 30% fall in blood pressure; however, when the animal had already received 10 μg/kg clonidine, the same 20 μg/kg dose caused but a 15% vasodepression. The relationship of the two curves clearly demonstrates the extent of tachyphylaxis.

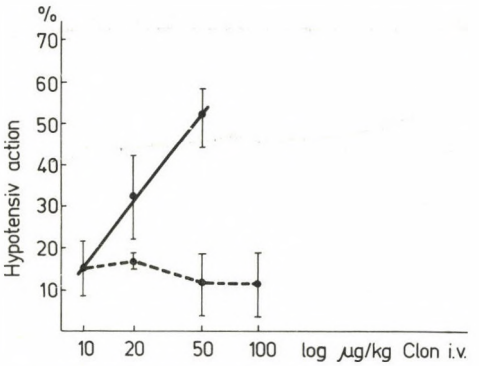

Fig. 3. Development of tachyphylaxis to the hypotensive effect of clonidine. Abscissa: log of clonidine dose, μg/kg i.v. Ordinate: extent of vasodepression in per cent of the initial blood pressure value. —: Curve of the effect of the first clonidine dose (mean of 18 animals); ---: effect of consecutive clonidine doses (mean of 8 animals ±S.E.)

Modification of the blood pressure effect of clonidine after administration of indirectly acting sympathomimetics

The clonidine tachyphylaxis shown in Figs 1 and 2 was similar to the tachyphylaxis evoked by indirectly acting sympathomimetic amines such as tyramine (*Winder* et al. 1948; *Day* and *Rand* 1963), amphetamine and methamphetamine (*Takasaki* et al. 1973). The permanent contraction of the nictitating membrane is also indicative of this resemblance (*Fleckenstein* and *Burn* 1953; *Fleckenstein* and *Stöckle* 1955). It is assumed that clonidine too acted by a mechanism similar to that of indirect

sympathomimetic amines; for instance, a rapid tachyphylaxis against amphetamine counteracted the blood pressure effect of clonidine. Fig. 4 shows the tachyphylaxis developed to amphetamine. The first dose of amphetamine, 1 mg/kg, increased blood pressure by 60 mm Hg, while further 2—4 mg/kg doses caused a negligible elevation amounting to 10—15 mm Hg only. A consecutive intravenous injection of 100 μg/kg of clonidine caused a slow and permanent decrease of 20 mm Hg only. Comparison of this effect with the effect of 50 μg/kg of clonidine shown in Fig. 2 reveals that tolerance to amphetamine markedly inhibits the blood pressure effect of clonidine. No inhibition can be seen in the amphetamine-induced contraction of the nictitating membrane which keeps on to contract to the maximum under the effect of clonidine. The same results were obtained in experiments performed with ephedrine or tyramine.

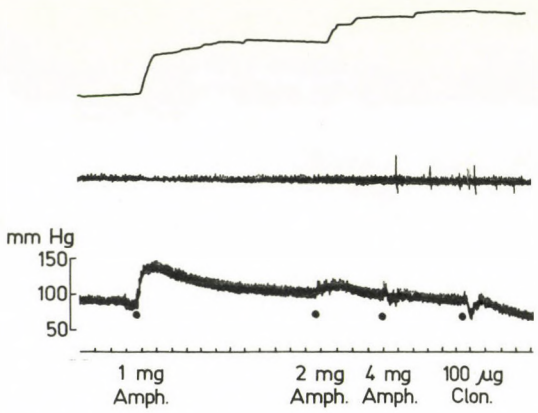

Fig. 4. Tolerance to amphetamine modifies the effect of clonidine. Cat, 3.2 kg. Intraperitoneal anaesthesia with 35 mg/kg of choralose +30 mg/kg of urethane. From top to bottom: nictitating membrane, respiration, blood pressure, time in minutes. Amph: Amphetamine; Clon: clonidine

Figure 5 shows the dose–response curve of the so-called "first clonidine dose" (full line). The line —.— shows the dose–response curve of the first dose of clonidine administered after the development of tyramine tachyphylaxis. Calculation of individual points was done as in Fig. 3. As seen, once a tachyphylaxis had developed to either tyramine or amphetamine the hypotensive effect of clonidine will be greatly reduced; clonidine, when given alone in a dose of 50 μg/kg, decreased blood pressure by 50% but the same dose only caused a decrease of 10% after the development of tachyphylaxis to tyramine.

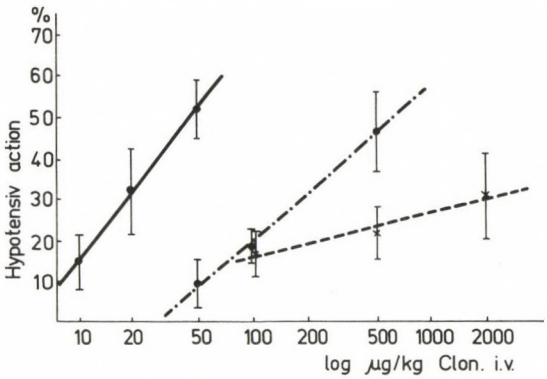

Fig. 5. Influence of tyramine and amphetamine tachyphylaxis on the hypotensive effect of clonidine. Abscissa: log of clonidine dose, µg/kg. Ordinate: degree of vasodepression in per cent of the initial blood pressure value. —: Curve of the effect of the first clonidine dose (mean of 18 animals); —.—: dose-effect curve of the first of clonidine taken after tyramine tachyphylaxis (each point corresponds to the mean of 3 animals); x—x: dose-effect curve of the first dose of clonidine after amphetamine tachyphylaxis (each point represents the mean of 3 animals ±S.E.)

Modification of the blood pressure effect of clonidine in animals pretreated with cocaine, bretylium and reserpine

Cocaine is known to alter the effect of sympathomimetic amines (*Tainter* and *Chang* 1927; *Tainter* 1929; *Fleckenstein* and *Stöckle* 1955; *MacMillan* 1959; *Lindmar* and *Muscholl* 1961; *Trendelenburg* 1961). Two hundred µg/kg of tyramine increased blood pressure by 70 mm Hg. Tyramine had been chosen as test-substance because no tachyphylaxis develops to this indirectly acting amine unless it is administered five or six times. Three pretreatments with 1.7 mg/kg of cocaine abolished the hypertensive action of tyramine; under similar conditions, 100 µg/kg of clonidine first increased then slowly decreased blood pressure. The degree of hypotension was smaller than that caused by 100 µg/kg of clonidine alone. Contraction of the nictitating membrane persisted.

Bretylium, a drug blocking peripheral adrenergic neurones (*Boura* and *Green* 1959), also prevented the blood pressure decreasing effect of clonidine. Such an experiment is shown in Fig. 6. A dose of 2 mg/kg of bretylium inhibited but did not completely counteract the effect of 50 µg/kg of clonidine. Five mg/kg of bretylium, however, fully abolished the vasodepressor effect of a high dose (200 µg/kg) of clonidine. Under such conditions even 1 mg/kg of clonidine caused a short and transient decrease in blood pressure. The nictitating membrane contraction remained unaltered.

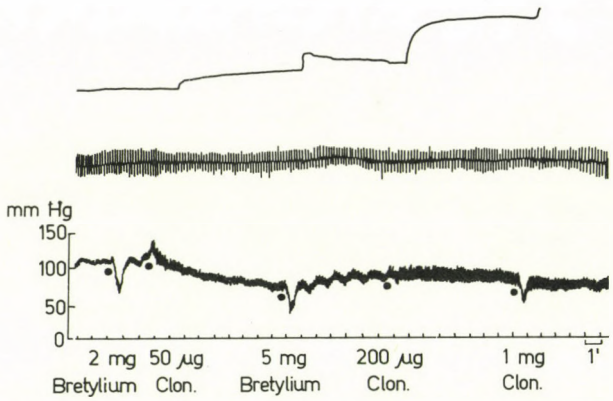

Fig. 6. Bretylium counteracts the vasodepressor effect of clonidine. Cat, 2.7 kg. Intraperitoneal anaesthesia with 35 mg/kg of chloralose +300 mg/kg of urethane. From top to bottom: nictitating membrane, respiration, blood pressure, time in minutes Clon: Clonidine

Pretreatment with reserpine also modified the effect of sympathomimetic amines (*Trendelenburg* et al. 1962; *Burn* and *Rand* 1958; 1960; *Fleming* and *Trendelenburg* 1961; *Trendelenburg* 1963; *Carlsson* et al. 1957). An experiment of this type performed on a cat pretreated with 5 mg/kg of reserpine subcutaneously 24 hours prior to the investigation is shown in Fig. 7. Clonidine in a dose of 100 µg/kg increased blood pressure by 60 mm Hg and kept it at this level permanently.

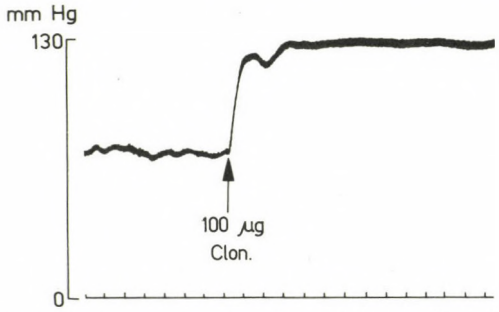

Fig. 7. Effect of clonidine on blood pressure in reserpine-pretreated animal. Cat, 3.5 kg. Intraperitoneal anaesthesia with 20 mg/kg of chloralose +150 mg/kg of urethane. The animal received 5 mg/kg of reserpine subcutaneously 24 hours prior to the experiment

Effect of noradrenaline infusion on clonidine tachyphylaxis

Cocaine (*Callingham* and *Cass* 1962; *Lockett* and *Eakins* 1960; *MacMillan* 1959; *Trendelenburg* 1963), bretylium (*Hertting* et al. 1962) and reserpine all deplete catecholamines and inhibit their re-uptake. These effects are counteracted by noradrenaline (*Burn* and *Rand* 1960).

Figure 8 shows an experiment after the development of clonidine tachyphylaxis, when 100 µg/kg of clonidine only elevated blood pressure. The cat received

a noradrenaline infusion at a rate of 10 μg/kg/min for 25 minutes. Subsequently, blood pressure fell below the pre-infusion level. Intravenous injection of 100 μg/kg of clonidine at this point of time elicited a long-lasting vasodepression a new but not of such degree as in untreated animals. When compared with the 100 μg dose given prior to the administration of noradrenaline, the effect was definitely reversed.

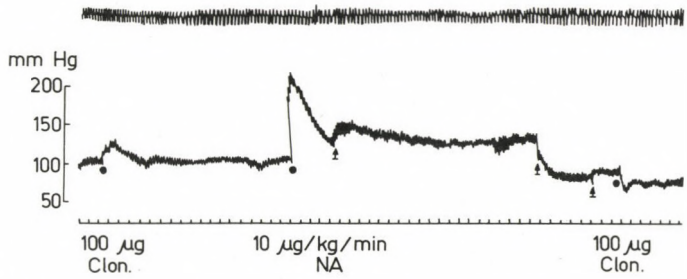

Fig. 8. Effect of noradrenaline infusion on clonidine tachyphylaxis. Cat, 2.5 kg. Intraperitoneal anaesthesia with 35 mg/kg of chloralose + 300 mg/kg of urethane. From top to bottom: respiration, blood pressure, time in minutes. Clon: clonidine; NA: noradrenaline; ↑: break of recording for 30 min

Discussion

As seen from our experiments a rapid and definite tachyphylaxis develops to the vasodepressor effect of clonidine. *Walland* and *Kobinger* (1971) who applied clonidine topically in the mouse eye already found that repeated doses failed to cause any mydriasis. Tachyphylaxis is common with the indirect sympathomimetics such as tyramine (*Winder* et al. 1948; *Day* and *Rand* 1963) or amphetamine (*Takasaki* et al. 1973). The phenomenon is ascribed to noradrenaline depletion (*Lindmar* and *Muscholl* 1961; *Lockett* and *Eakins* 1960), an explanation accepted in the literature. In our experiments the tachyphylaxis developed to indirect sympathomimetics inhibited the vasodepressor effect of clonidine. It is therefore assumed that clonidine acts by a similar mechanism. This assumption has been supported by results obtained on animals pretreated with cocaine, bretylium or reserpine. Cocaine inhibits noradrenaline re-uptake in the rabbit heart and ear (*MacMillan* 1959) and causes noradrenaline depletion (*Callingham* and *Cass* 1962). Bretylium inhibits the uptake of circulating noradrenaline (*Callingham* and *Cass* 1962; *Hertting* and *Axelrod* 1962) liberated by pre- and postganglionic stimulation or administered exogenously. It is well known that reserpine causes noradrenaline depletion and inhibits its re-uptake. Thus, all the enumerated substances exert their effect through a common mechanism of action, which corroborates our assumption that clonidine may act by noradrenaline release and/or uptake in addition to a direct excitatory effect on adrenergic alpha-receptors. The tachyphylaxis might be connected with these processes.

In our experiments, clonidine-tachyphylaxis could be abolished by an infusion of noradrenaline, a finding also stressing the importance of noradrenaline in the clonidine effect.

It could be imagined that the phenomenon of tachyphylaxis would be a peripheral postsynaptic α-receptor stimulating effect with consecutive vasoconstriction which antagonizes the hypotension due to central alpha-receptor stimulation (*Schmitt* et al. 1967). If this is true the tachyphylaxis must be an imitation of it, i.e. a false tachyphylaxis. However, the direct effect of clonidine on the postsynaptic α-receptor of blood vessels is negligible (*Starke* et al. 1974; *P.-Gibiszer* and *Pórszász,* unpublished observations) and certainly cannot figure in the phenomenon of the tachyphylaxis.

Contraction of the nictitating membrane failed to show any sign of tachyphylaxis, a finding verifying again that no tachyphylaxis occurs to the direct alphamimetic effect. Of the indirectly acting sympathicomimetics, amphetamine, for instance, also causes a lasting contraction of the nictitating membrane and, as seen in Fig. 3, there is no tachyphylaxis to this action.

The vasopressor effect of clonidine after reserpine pretreatment can be ascribed to a direct stimulation of peripheral alpha-receptors. Similarly, the vasopression seen after the development of tachyphylaxis is the result of peripheral alpha-receptor excitation. This has been shown by our pilot experiments in which the sympathetic efferent activity exhibited parallel reflex.

Tyramine, ephedrine and amphetamine are indirect sympathomimetics exhibiting tachyphylaxis. The development of this tachyphylaxis interferes with the hypotensive action of clonidine. Thus, more indirect mechanisms may figure in the mechanism of action of clonidine, even though a direct postsynaptic alphamimetic effect cannot be excluded.

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MECHANISM OF CHLORPROPAMIDE ANTIDIURESIS

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A study was made of the effect of chlorpropamide in rats with pituitary stalk lesion. It was found that the drug made the oliguric interphase more pronounced and protracted, while in the lasting polyuric phase in the absence of endogenous ADH it influenced water intake only moderately. Enhancement of the effect of exogenous ADH was observed after a 2-day chlorpropamide pretreatment. The results support the view that the presence of a small amount of endogenous ADH is necessary for chlorpropamide-antidiuresis. The drug presumably brings about the decrease in diuresis *via* ADH mobilization, but its peripheral effect is also enhanced.

In 1966 it was discovered by chance that chlorpropamide moderates the polyuria in vasopressin-sensitive diabetes insipidus (*Arduino* et al. 1966; *Reforzo-Membrives* et al. 1966). This effect of the drug has been confirmed in numerous cases (*Reforzo-Membrives* et al. 1968; *László* et al. 1970; *Miller* and *Moses* 1970a; *Radó* et al. 1970; *Reimold* 1970; *László* and *Czakó* 1971; *Uhlich* et al. 1971; *Wales* and *Fraser* 1971; *Schneider* et al. 1972; *Murase* and *Yoshida* 1973). Some authors have, however, questioned the antidiuretic effect of chlorpropamide (*Hocken* and *Longson* 1968; *Ehrlich* and *Kooh* 1969; *Kumar* et al. 1969; *Sack* and *Katznelson* 1969; *Ettinger* and *Forsham* 1970; *Bruns* et al. 1972) so that its clinical value has still to be clarified. We have therefore investigated the mechanism of chlorpropamide antidiuresis in rats.

Methods

The first part of the experiments was carried out on 30 female white R-Amsterdam rats, weighing 180—200 g, and fed a standard diet. Diabetes insipidus was induced in 10 animals by destroying the pituitary stalk electrolytically with a Horsley-Clarke apparatus under ether anaesthesia. The rats were subsequently placed into individual cages, and their daily fluid intake and urinary output were measured. After the fourth postoperative day, 5 mg chlorpropamide per 100 g body weight was administered subcutaneously twice daily for 7 days. Untreated rats with stalk lesion and non-operated animals served as controls. The latter group was divided into a sub-group of 10 animals where no treatment whatever was performed, and a second sub-group of 10 rats, where 5 mg chlorpropamide per 100 g body weight was administered twice daily. On completion of the experiment the operated animals were killed by decapitation and the location and extent of the lesion were checked histologically. Animals with incomplete stalk lesion were omitted from evaluation. The details of processing and evaluation were reported earlier (*László* et al. 1962).

In the second series of experiments we have studied whether chlorpropamide was able to influence the effect of exogenous ADH. There experiments were carried out on 20 female white Wistar rats, weighing 180—230 g, fed a standard diet. For 8 hours prior to the experiment the animals were starved, but received water and libitum. Prehydration and anaesthesia were performed by the method of *de*

Wied (1960). Next, with the aid of a cannula inserted into the bladder, the quantity of urine dripping out of the bladder in 10 min was measured. Then, with the insertion of appropriate control periods, the quantities of urine excreted in 10 min after the administration of three logarithmically increasing doses of lysine-vasopressin were determined, and expressed as percentages of the value for the control period. For details see *László* and *Kovács* (1968). On completion of the titration, the rats were treated subcutaneously with 5 mg chlorpropamide per 100 g body weight twice daily for 2 days. Two hours after the early morning treatment on the third day, the decrease of diuresis induced by the same amount of ADH was again examined.

Results

As Fig. 1 shows fluid intake of the non-operated, untreated control animals was 20—40 ml daily and did not fluctuate significantly and in the control group it was not influenced by chlorpropamide. In the stalk-lesioned but untreated group, fluid consumption showed the typical three-stage curve (*Kovács* et al. 1962). At first, the intake was enhanced, this was followed by a relative decrease for 1—2 days and finally, the polydipsia again became marked. Under the effect of chlorpropamide, water intake curve changed considerably in the animals with stalk lesion; oligodipsia became more pronounced and did not attain the level of untreated animals with stalk lesion by the 10th day of treatment. It is also seen in Fig. 1 that chlorpropamide failed to normalize the fluid intake in the phase of prolonged polydipsia in the rats with stalk lesion which did not possess an ADH-reserve; their spontaneous fluid consumption lay between the values for the non-operated control group and the untreated stalk lesioned group. Daily urinary output exhibited similar changes, and hence is not shown diagrammatically.

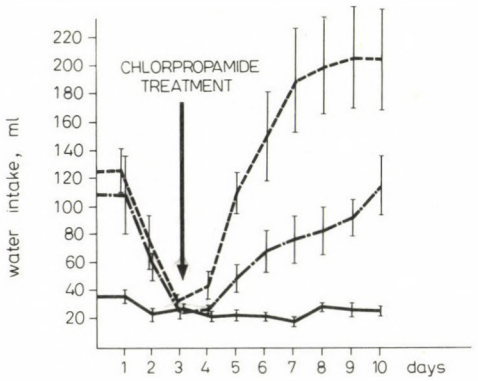

Fig. 1. Effect of chlorpropamide on daily water intake of rats pituitary stalk lesion. ———: Chlorpropamide-treated, stalk-lesioned animals (10 rats); —.—: untreated, stalk-lesioned animals (10 rats); ———: untreated control (10 rats)

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Figure 2 illustrates that the 2-day chlorpropamide pretreatment markedly decreased the changes in diuresis induced by lysine-vasopressin in all the three doses, and thus brought about an enhancement of the effect of ADH. In other words, ADH-sensitivity was increased by the treatment. The difference was most pronounced in the case of the lowest dose of lysine-vasopressin.

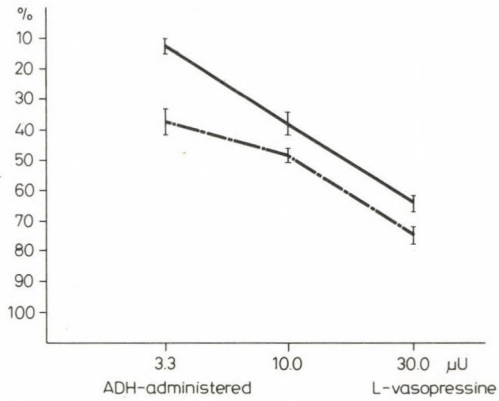

Fig. 2. Effect of chlorpropamide on ADH sensitivity of alcohol-unaesthetized rats; ———: untreated control (10 rats); —.—: chlorpropamide-treated animals (10 rats)

Discussion

Chlorpropamide has long been used for the treatment of diabetes mellitus, and many authors have dealt with the mechanism of its antidiuretic action. In connection with this a number of concepts have been put forward, *viz.*

(i) a direct vasopressin-like effect on the renal tubules;

(ii) enhancement of ADH-release;

(iii) sensitization of the distal renal tubules to ADH, otherwise present in ineffective amounts;

(iv) increase in the concentration of cAMP in the tubular and pancreatic beta cells.

The direct tubular effect is supposed by the experience that in diabetes insipidus patients chlorpropamide fails to affect solute secretion; it decreases significantly the free water clearance. The concept was further supported by the fact that chlorpropamide does not induce antidiuresis in vasopressin-resistant renal diabetes insipidus (*Arduino* et al. 1966; *Meinders* et al. 1967, 1969; *Froyshov* and *Haugen* 1968; *Hocken* and *Longson* 1968; *Ehrlich* and *Kooh* 1970; *Radó* et al. 1970; *Reimold* 1970; *Webster*

and *Bain* 1970; *László* and *Czakó* 1971; *Liberman* et al. 1973). At the same time it decreases urinary output in healthy individuals (*Hocken* and *Longson* 1968; *Reforzo-Membrives* et al. 1968) and in psychic polydipsia (*László* et al. 1970; *Radó* et al. 1970; *Lameire* et al. 1971). The beneficial effect of the drug has been observed in water diuresis, in the case of repression of the endogenous vasopressin (*Meinders* et al. 1969), in familial diabetes insipidus (*Froyshov* and *Haugen* 1968) and in congenital vasopressin deficiency. These data support the possibility of a direct tubular point of attack. Animal experiments did not clarify whether the mechanisms of antidiuretic action of vasopressin and chlorpropamide are the same. Some workers found that chlorpropamide enhances the water permeability of the frog bladder (*Danisi* et al. 1970; *Lozada* et al. 1972; *Ozer* and *Sharp* 1973), whereas others could not support this (*Ingelfinger* and *Hays* 1969; *Mendoza* 1969; *Uhlich* et al. 1971; *Wales* and *Fraser* 1971; *Mendoza* and *Brown* 1974). Application of a very small amount of vasopressin, which in itself was ineffective, led to a considerable enhancement of the effect of the drug on the frog bladder, while in the presence of a large dose of vasopressin the drug was not able to increase the water permeability of the frog bladder (*Ingelfinger* and *Hays* 1969; *Mendoza* 1969; *Mendoza* and *Brown* 1974). Experiments on Brattleboro rats with congenital diabetes insipidus led to similar results (*Berndt* et al. 1970; *Miller* and *Moses* 1970b; *Uhlich* et al. 1971).

On the above basis it is assumed that chlorpropamide has no direct antidiuretic activity, and that the presence of a small amount of endogenous ADH is necessary for its effect to be exerted.

Our own examinations too agree with this concept. Chlorpropamide treatment led to a marked reduction of the daily fluid intake of rats with pituitary stalk lesion only when these still possessed an ADH-reserve. In the period of prolonged polyuria, a moderate effect was only observed.

Another possibility is that the antidiuretic action of chlorpropamide is exerted by an enhancement of ADH-release, or a potentiation of the effect of endogenous vasopressin.

Enhancement of ADH-release and of urinary ADH excretion by chlorpropamide treatment was first observed by *Moses* et al. (1973). We obtained a similar result by determination of the ADH content of the plasma with a biological method (*Czakó* et al. 1975).

The question is still open whether chlorpropamide is capable of enhancing the effect of exogenous ADH in the distal tubules. In 1971 it was reported that the increase in urinary osmolality and the decrease in free water clearance under the action of ADH were more significant when the drug was given during chlorpropamide treatment (*Lameire* et al. 1971). These data have since been confirmed by other authors (*Moses* et al. 1973; *Murase* and *Yoshida* 1973). On the other hand, *Ettinger* and *Forsham* (1970) found no change in the antidiuretic effect of intravenously administered vasopressin after oral chlorpropamide pretreatment. We too observed that rats reacted more sensitively to exogenous ADH if its administration had been preceded by 2-day chlorpropamide treatment.

Thus, the possibility cannot be ruled out that chlorpropamide enhances the effect of endogenous ADH on the distal tubules. This was supported by our finding that in stalk-lesioned rats the oliguric phase accompanied by endogenous ADH release was more pronounced under the effect of chlorpropamide. As to the concepts on the role of cAMP, the antidiuretic effect of ADH is known to be exerted *via* an increase of the concentration of cAMP in the tubular cells, probably by means of the activation of adenylcyclase (*Orloff* and *Handler* 1964; *Ozer* and *Sharp* 1973). A similar mechanism is assumed for chlorpropamide, with the difference that it increases the concentration of cAMP not only in the tubular cells, but also in the pancreatic beta cells, and hence contributes to the enhancement of the effect of ADH (*Lozada* et al. 1972).

It may be concluded that the presence of a small amount of endogenous ADH is necessary for the antidiuretic effect of chlorpropamide. Its diuresis-decreasing action is probably exerted by a mobilization of the ADH reserves. The possibility cannot be excluded, however, that the small quantity of vasopressin, which in itself does not cause a change in diuresis, may potentiate its peripheral effect.

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Recensiones

E. BÜLBRING, M. F. SHUBA (Eds)

Physiology of Smooth Muscle

Raven Press, New York 1976. X + 448 pages, with 244 figures and 12 tables. Price: $ **35.50**

The volume contains the papers presented at a Symposium on the Physiology of Smooth Muscle, held in Kiev (USSR), in October, 1974, a satellite symposium connected with the 26th International Congress of Physiological Sciences. The wide scope of material covered in the volume reflects the current activity in smooth muscle research all over the world. In recent years a great progress has been achieved in the understanding of cellular mechanisms controlling ion distribution and ion movements, in voltage clamp experiments, ionic current membrane excitation, the origin of rhythmic spontaneous activity, the ultrastructure of smooth muscle cells and nerve terminals and the characteristic mechanisms of action of neurotransmitters.

The symposiums provided a unique opportunity for an exchange and discussion of informations from many countries otherwise not easily accessible. The book will be of interest to physiologists, pharmacologists, biochemists and biophysicists.

K. LISSÁK

B. SAMUELSSON, R. PAOLETTI (Eds)

Advances in Prostaglandin and Thromboxane Research

Volumes 1 and 2. Raven Press, New York 1976. Volume 1: XVI + 506 pages, with 254 figures and 100 tables; Volume 2: XVI + 521 pages, with 123 figures and 65 tables. Price: Volume 1: $ **45.00**; Volume 2: $ 47.00

This first two volumes of the series Advances in Prostaglandin and Thromboxane Research contain most of the papers presented at the International Conference on Prostaglandin, held in Florence, Italy, in May, 1975. Volume 2 includes abstracts of the more than 700 poster presentations. Research in the field of prostaglandins has greatly increased during the past decade, and the basic biological facts already dominate the clinical sciences.

In these two volumes, all aspects of the field have been presented, such as organic chemistry, biochemistry, pharmacology, physiology, pharmaceutics and numerous clinical disciplines, as reflected well by the contents of the two volumes.

Contents of Volume 1: Biosynthesis and Metabolism; Assay Methods; Prostaglandin Synthetase Inhibitors; Prostaglandin Dehydrogenase; Chemistry, Receptors; Cyclic Nucleotides; Nervous System; Cardiovascular System; Respiration.

Contents of Volume 2: Gastrointestinal System; Reproductive Physiology I and II; Pregnancy Interruption; Platelets; Inflammation.

The conference was introduced by a lecture of *Samuelsson* surveying the history of prostaglandins discovered more than 40 years ago. Rapid progress of the analytical methods ensued after clarification of the metabolic transformations of the various prostaglandins and the use of mass-spectrometric and radioimmunological methods. The new term "thromboxane" was accepted and introduced during the Florence conference. The new term marks a novel group of compounds derived from the endoperoxides with an oxane ring structure; they have pronounced biological effects.

This new series of volumes will provide an unavoidable source of information for organic chemists, biochemists, pharmacologists, physiologists and students of the most different medical disciplines.

K. LISSÁK

F. W. AHNEFELD, C. BURRI, W. DICK, M. HALMÁGYI (Eds)

Mikrozirkulation

Klinische Anästhesiologie und Intensivtherapie.
5. Band. Springer—Verlag, Berlin, Heidelberg, New York 1974. XI + 207 pages, with 126 figures and 8 tables. Price: DM 24.—; US $ 9.80

The volume contains two main parts: the first one deals with the pathology of microcirculation, while the second part with its clinical and therapeutic aspects.

The chapters of microcirculatory pathology are based upon observations performed with the most up-to-date research methods. A modern, systematic account is given of the pathological microcirculatory processes concerning the interaction between metabolism, breathing and circulation.

Electronmicroscopic demonstration of the morphologic characteristics of endothelial lesions in the course of development of stasis and thrombosis are of particular interest. Analysis of microcirculation with a new, rheologic approach has a prominent part in the volume.

Measurement of tissue O_2 tension is the best means to demonstrate disturbances of tissue perfusion, i.e. of microcirculation. The electronmicroscopic findings prove the submicroscopic–morphologic consequences of tissue anoxia.

Changes of tissue pH were estimated by polarography, thus the connection of alterations of tissue pH and microcirculation has exactly been proved.

The contributors describe in detail the principles of estimation of capillary microcirculation with the aid of microsurgical methods. These allow to measure the actual state of capillary microcirculation in the organs such as the kidneys, lungs and the brain.

The chapter reviewing the special morphological characteristics and pathophysiological aspects of pulmonary microcirculation is also of great interest. The authors give a detailed description of special non-respiratory pulmonary functions and analyse the pathomechanism of intrapulmonary platelet aggregation and of the shock-lung, processes which raise some haemodynamic and O_2 diffusion problems.

The second part of the volume deals with the clinical aspects of microcirculation. The principle is that to improve microcirculation in the case of shock is to induce beneficial alterations in organs flow as well as to initiate haemodilution. An evident aim of this treatment is to relieve tissue hypoxia by administering drugs which inhibit blood clotting and by improvement of the rheologic status. Each drug inhibiting the platelet aggregation or relieving the vasoconstriction acts in this direction. In the cases of endotoxin-shock, administration of heparin, Trasylol and the maintenance of appropriate oxygenation are of primary importance.

Experimental data support the clinical observation that beta-blockers have a beneficial effect in refractory shock. These results corroborate the idea that beta-blockers might improve the disadvantageous haemodynamic effects of a prolonged adrenergic stimulation having mainly pulmonary and splanchnic manifestations. In the treatment of septic shock, glucagon, steroids and digitalis have an important role. The last chapter reviews the application of drugs which are known to modify the rheologic parameters of blood, and the authors give a rheologic approach of the therapeutic aspects of plasma and blood viscosity as well as the inhibition of red cell aggregation.

The volume will be of use for all those interested in intensive care as well as those working in shock or microcirculatory research.

M. TEKERES

J. M. UNSELD

Blutersatz durch stromafreie Hämoglobinlösung

Anaesthesiology and Resuscitation. Vol. 85.
Springer-Verlag, Berlin, Heidelberg, New York 1974. VIII + 90 pages, with 17 figures. Price: DM 32.—; US $ 13.10

It is an interesting problem of blood transfusion to find a solution having a good oxygen-transporting capacity and being free of blood-group and cross-reactions. Stroma-free haemoglobin solution has an ideal oxygen-transporting capacity and is free of blood-group reactions. This monograph reviews the experimental data on this solution with the final aim to study its therapeutic application.

As observed in pigs, the stroma-free haemoglobin solution has a high oxygen-binding capacity. Haemoglobin and albumin are of nearly the same molecular weight, thus their plasma expanding characteristics are similar. Infusion of haemoglobin solution to normovolaemic, conscious animals resulted in an increase of urine excretion without affecting the clearance of creatinine. Haemoglobin disappeared from the plasma quickly, its half-life being 2.5 hr.

Effects of the haemoglobin solution and of a 5% solution of human albumin have been compared in anaesthetized normovolaemic animals. Haemoglobin increased mean arterial blood pressure, and diminished the total peripheral resistance considerably and cardiac output, pulse volume and heart rate slightly. Five per cent albumin solution increased cardiac output and pulse volume while it decreased the total peripheral resistance. Mean blood pressure and heart rate did not change after albumin administration. These findings indicate that albumin increased blood volume and caused haemodilution, while haemoglobin induced pharmacological changes despite the fact that it increased the circulating blood volume and decreased the haematocrit value. Neither haemoglobin nor albumin affected blood pH oxygen consumption.

Oxygen content of the plasma increased after haemoglobin infusion and a measurable arteriovenous oxygen difference could be detected. In a range of 30–50 Torr, the dissociation curve of plasma infused with stroma-free haemoglobin solution did not differ from that of normal human blood. The curve displayed a shift to the left at decreased pO_2 values. Above 160 Torr, plasma haemoglobin is only partially saturated with oxygen in consequence of the slight methaemoglobinaemia. Stroma-free haemoglobin disappeared from plasma by the 10th–15th hr after its administration and its half-life estimated in anaesthetized pigs was about 3.5 hr. Only 7–15% of the haemoglobin was excreted by the kidneys.

A major proportion of the infused haemoglobin was taken up by the reticuloendothelial system, where it was metabolized to bilirubin and bound to different tissues.

Haemoglobin solution and human albumin had similar effects on renal function. Urine excretion was increased without any significant change in creatinine or ^{51}Cr-EDTA clearance and in electrolyte excretion. No sign of a renal damage was found.

The effect of haemoglobin solution was compared with retransfusion of the blood withdrawn in haemorrhagic shock experiments at a mean blood pressure of 40 Torr maintained for 3 hr. Haemoglobin had a beneficial effect by increasing cardiac output above the preshock level and by decreasing pulse volume, heart rate and peripheral resistance to the control level. Within two hours after haemoglobin treatment the shock-induced metabolic acidosis had normalized and haematocrit increased, indicative of haemodilution. Oxygen uptake increased and the arteriovenous oxygen difference of whole blood decreased but the cardiovascular failure returned 3 hours later.

Retransfusion of blood had a similar effect with the exception that it caused a lasting correction of the shock-induced pathological changes, without a relapse.

After both haemoglobin infusion and retransfusion renal function had normalized within 3 hours and urine volume, osmotic clearance and electrolyte excretion returned to preshock values. Creatinine clearance and the osmolality U/P ratio remained low, pointing to an impairment of the renal concentrating capacity. These changes are consequences of the hypoxaemic damage induced by shock since they persisted even after retransfusion. A slight increase in urea level might be the only side-effect of haemoglobin infusion, because this increase could not be measured after retransfusion.

Three hours after its infusion the stroma-free haemoglobin disappeared from the plasma. This caused a decrease in cardiac output and an increase in total peripheral resistance. A second haemoglobin infusion or preferably a transfusion of blood was then needed. Thus, haemoglobin infusion will ensure survival until the arrival of appropriate blood.

The stroma-free haemoglobin solution with its high oxygen binding capacity seems to have advantages over other plasma expanders or plasma substituents. Since it does not have any specific antigen properties one must not estimate blood groups or perform cross-reaction tests. The risk of transfusion hepatitis is also avoided, as the solution can be subjected to reliable sterilization processes.

The experimental data of this monograph raise hopes that stroma-free haemoglobin will prove beneficial in shock therapy.

M. TEKERES

E. N. A. BIRÓ (Ed.)

Proteins of Contractile Systems

FEBS, Vol. 31. Proceedings of the Ninth Meeting of the Federation of European Biochemical Societies, Budapest 1974. Akadémiai Kiadó, Budapest 1975. VIII + 228 pages, with 113 figures and 32 tables

The volume presents the papers contributed to S-1 Symposium of the 9th FEBS Meeting, introduced by *E. N. A. Biró*. The book consists of three parts. Part 1: ATPase mechanisms and actin-myosin interactions; Part 2: Regulatory systems of the I filament; Part 3: Myosin: phosphorylation, assembly. The 20 papers are as follows: 1. The magnesium ion dependent ATPase of myosin *(C. R. Bagshaw, J. F. Eccleston, D. R. Trentham)*; 2. Ligand induced changes in thiol group reactivity of fast and slow myosins *(M. C. Schaub, J. G. Watterson, M. Pfister, P. G. Waser)*; 3. Affinity chromatography of myosin on columns containing immobilized actin or ATP analogues *(I. P. Trayer, R. C. Bottomley, H. R. Trayer)*; 4. Contraction of glycerinated muscle fibres, myofibrils and actin threads induced by water-soluble myosin fragments *(A. Oplatka, J. Borejdo, H. Gadasi, R. Tirosh, N. Liron, E. Reisler)*; 5. Influence of ions and of ATP on the conformation of HMM as studied by proteolysis *(L. Szilágyi, I. Kurrenoy, M. Bálint, E. N. A. Biró)*; 6. Light meromyosin and associated proteins: Their functional properties, interactions with contractile proteins *(M. Kalamkarova, E. Kofman, W. Nankina)*; 7. Molecular mechanism of regulation of muscle contraction by the Ca–troponin system *(S. Ebashi, S. Ohnishi, K. Maruyama, T. Fujii)*; 8. Interactions among the proteins of thin filament *(W. Drabikowski, R. Dabrowska)*; 9. Ca–protein and protein–protein interactions in the regulation of the actin–myosin interaction by the troponin–tropomyosin system *(J. Potter, P. Leavis, J. Seidel, S. Lehrer, J. Gergely)*; 10. Creatine kinase and M-line structure *(T. Wallimann, D. C. Turner, H. M. Eppenberger)*; 11. Tropomyosin: Correlation of amino acid sequence and structure *(D. Stone, J. Sodek, P. Johnson, L. B. Smillie)*; 12. The amino acid sequence of troponin I from rabbit white skeletal muscle *(J. M. Wilkinson, R. J. A. Grand)*; 13. Theory of protein secondary structure and its application to muscle proteins *(O. B. Ptitsyn, A. V. Finkelstein, V. I. Lim)*; 14. Phosphorylation of the proteins of the myofibril *(S. V. Perry, H. A. Cole, M. Morgan, A. J. G. Moir, E. Pires)*; 15. Platelet myosin phosphorylation: Studies on the kinase, substrate and effect of phosphorylation *(R. S. Adelstein, J. L. Daniel, M. A. Conti, W. Anderson, jr)*; 16. Some differences in the properties of the myosin preparations with and without C-protein *(I. Kakol)*; 17. Morphogenesis of synthetic myosin filaments *(I. Pinset-Härström, J. E. Morel)*; 18. Platelet contractile proteins: Subcellular localization, physical state and functional importance *(N. Crawford)*; 19. Aggregation of myosin extracted from slime mold and amoeba *(J. D. Haese, H. Hinssen)*; 20. Comparative studies on natural and synthetic actomyosins from the slime mold and cross striated muscle *(H. Hinssen, J. D. Haese)*.

The book presents an up-to-date review of the biochemistry of contractile proteins and will be a useful source of information for every biochemist, biophysicist and physiologist working in this field.

E. VARGA

E. J. HIDVÉGI, J. SÜMEGI, P. VENETIANER (Eds)

Biochemistry of the Cell Nucleus. Mechanism and Regulation of Gene Expression

FEBS, Vol. 33. Proceedings of the Ninth Meeting of the Federation of European Biochemical Societies, Budapest 1974. Akadémiai Kiadó, Budapest 1975. 464 pages

This book consists of 49 selected papers presented in two Symposia of the Ninth Meeting of the Federation of European Biochemical Societies held in Budapest. The biochemistry of cell nucleus and the mechanism and regulation of gene expression had developed considerably in recent years and is receiving much attention from those working in these and related fields. An opportunity to keep abreast with progress was afforded at the Budapest Congress, when eminent molecular biologists of high international reputation reported on the latest advances.

Among the many specialized fields described and discussed in the papers were: synthesis and structure of messenger RNA; synthesis and structure of ribosomal RNA; nuclear–cytoplasmic interactions; prokaryotic and eukaryotic DNA-dependent RNA polymerases; transcriptional signals in nucleic acids, and structure and function of DNA and chromatin.

The significance of this volume can be demonstrated by a quotation taken from the Introduction written by Prof. *Harris Busch:* "The massive amount of new and exciting data presented at this Symposium reflects the increasingly clear picture of nuclear function available to modern science. It is indeed a splendid springboard for future major advances in this field. Increasingly interesting technology has become available as well as a broad expansion of information in many fronts in biology."

A. TIGYI

F. ANTONI, A. FARAGÓ (Eds)

Post-Synthetic Modification of Macromolecules

FEBS, Vol. 34. Proceedings of the Ninth Meeting of the Federation of European Biochemical Societies, Budapest 1974. Akadémiai Kiadó, Budapest 1975. 175 pages

This book contains 14 papers on the post-synthetic modification of macromolecules given at the first international symposium on this important field of biochemistry. The papers about the problem of mRNA methylation and on phosphorylation of proteins convince the reader of the great significance of post-synthetic modifications.

After the Introduction by *E. Borek,* 9 papers are presented in the first chapter which deals with the strain-specific modification and restriction of DNA in bacteria, DNA methylation in nuclei, studies using a purified DNA methylase, the methylation of DNA and radiation damage, the bacterial RNA methyltransferase mutants, the modifications of tRNAs and the significance of these modifications.

The second chapter is concerned with side-chain modifications of nuclear and ribosomal proteins. Two papers deal with histone modification in cell cycles and with the role of histone-lysine methylation in cellular differentiation, growth and development. Two papers discuss the phosphorylation of ribosomal proteins and of nucleolar preribosomal particles and one deals with the phosphorylation of non-histone chromosomal proteins in cells.

The book is recommended to students, research workers and clinicians interested in molecular biology.

J. MOLNÁR

J. GERGELY, G. A. MEDGYESI (Eds)

Antibody Structures and Molecular Immunology

FEBS, Vol. 36. Proceedings of the Ninth Meeting of the Federation of European Biochemical Societies, Budapest, 1974. Akadémiai Kiadó, Budapest 1975. 170 pages

The 36th volume of the FEBS meetings is devoted to the subjects Antibody Structure and Function and Lymphocyte Receptors and Antibody Synthesis, as two major topics. The volume contains 17 lectures, a List of Contents and a Subject Index.

In the first part a paper by *J. D. Capra* et al. contains new comparative data on the variable region of human Ig H-chains, with a definite identification of four hypervariable regions. These regions are responsible for the idiotypic determinants as well as for the antibody specificity. The study of *A. C. Wang* in connection with the evolution of genes of the Ig V-region concentrates on the role of regulatory gene duplication in the accumulation of differently expressed genes. *J. C. Jaton* et al. investigating the V domains of homogeneous rabbit pneumococcus antibodies suggest that the group *a* allotypic markers are evolved from V region subgroups encoded by separate germ line genes. Experiments of chain recombination showed full binding activity and idiotypic specificity only in the homologous recombinants. The lecture of *F. M. Poulsen* et al. deals with the conformational properties of normal rabbit IgG. *J. R. Clamp* presents data about the oligosaccharide units of human immunoglobulins. *F. Franek* investigated the binding features of the early and late anti-DNP antibodies and found them to have a prominent role in giving shape or flexibility to the late antibody molecules in combination with multivalent antigens. *J. Gergely* and *G. A. Medgyesi* presented findings on the preferential reassociation of autologous and heterologous H and L chains. A preferential interaction is determined mainly by constant sequences. In the serological activity of monotypic IgM cold agglutinins, J-chains play an important role while L-chains do not. *D. Beale* offered new data about the fragmentation and reduction of porcine IgM suggesting conformational differences between human and porcine IgM polymers. *R. S. Nezlin* et al. localized RL allotypic antigenic determinants in the constant region of the kappa L-chains. *O. V. Rokhlin* and *R. S. Nezlin* determined the ratio of RL^1 and RL^2 allelic variants of kappa chains of rat Ig in hybrid nonimmune animals. The enhanced activity of the RL^1 gene may result from the heterozygosity of the allelic V genes. The paper of *H. Bazin* et al. presents some new data on LOU rat immunocytomas and their secretions, on IgE catabolism and rat allotypy. In the paper of *G. A. Medgyesi* et al. the interaction of rat Igs with complement is discussed, demonstrating the complement binding activity of the IgM class, IgG_1 and IgG_{2a} subclasses and the lack of fixing activity of subclass IgG_{2c}.

In the second part, a study on the control of antibody formation is presented by *A. R. Williamson* et al., discussing the commitment of the precursor cell, the control of expression of V–C gene pairs, the translational control of H-chain synthesis, the Ig-mRNA–H interaction, and the membrane-bound receptor antibody. A study regarding combined hybridization and structural analysis of mouse L-chain mRNA is presented by *T. H. Rabbitts* et al. *R. M. E. Parkhause* and *E. R. Abney* argue against any known Ig subunit functions as surface receptors on murine T cells, while they support the IgD as B cell receptor. *L. Jaroskova* et al. offer evidence of IgM being the primary receptor for antigen recognition on B cells in the fetal pig. *G. Sármay* et al. present data on the interaction of aggregated IgG with Fc receptors of lymphocytes and macrophages. The Clq binding groups of the Fc fragments are not identical with the IgG receptors.

Summarizing, the 36th volume of FEBS presents original papers with up-to-date results. It informs about new developments, trends, experimental approaches in the most exciting fields of theoretical immunology. Contributions of prominent scientists and teams make the book valuable to every reader interested in recent developments of antibody structure, function and molecular immunology.

I. KÉTYI

MARIA WOLLEMANN (Ed.)

Properties of Purified Cholinergic and Adrenergic Receptors

FEBS, Vol. 37. Proceedings of the Ninth Meeting of the Federation of European Biochemical Societies, Budapest 1974. Akadémiai Kiadó, Budapest 1975. 144 pages

This booklet consists of 8 papers read at the 1974 Budapest Meeting of the Federation of European Biochemical Societies. Three papers deal with fine structure, biochemical and physical properties of the acetylcholine receptor isolated from the electric organs of *Torpedo* and *Electrophorus*. The rest, collected in Chapter II, is devoted to the adrenergic receptors, with special emphasis on the relations between the receptor and adenylate cyclase activity.

An outstanding morphological analysis of the isolated acetylcholine receptor is presented by *Nickel* (Konstanz, GFR). In addition to a number of freeze-fractured specimens, standard transmission electron microscopy of ultrathin sections and the results of negative staining are included, both in the normal state and after α-bungarotoxin binding. Negative staining has proved useful in analyzing fine structural alterations of the membranes and membrane particles. Unfortunately, some of the electron micrographs are unreasonably small; this, in conjunction with the typographical technology of the photoprint procedure results in a slightly awkward appearance.

Heilbronn's paper, written with *Mattsson* and *Elfman*, stresses the diversity between acetylcholine receptor and acetylcholinesterase. The receptor is an acid glycoprotein consisting of two subunits, with apparent molecular weights of 38,000 and 42,000.

The third paper in this series (by *Raftery, Bode, Vadlen, Michaelson, Deutsch* and *Moody*) describes the fractionation of electroplax membrane particles. The main emphasis is upon fractions 4 to 10 (enriched in acetylcholinesterase activity) and upon fractions 16 to 30, enriched in acetylcholine receptor activity. Using the technique of negative staining, acetylcholinesterase-rich membrane particles are shown to be surrounded by a 100 A halo of subparticles, probably associated with the membrane by electrostatic bonding. Receptors were found to conform with the traditional 80 Å doughnut-form; the particles cover the membrane surface. By re-association with phospholipids, isolated purified receptors were reconstituted to yield excitable membranes.

Knowledge of beta adrenergic receptors and adenylate cyclase is summarized in a good review article by *Lefkowitz* (Durham, North Carolina). The relationship between aminergic receptors and adenylate cyclase activity in mammalian heart muscle is analyzed in detail by *Wollemann* (Szeged, Hungary). She arrives at an interesting conclusion at the level of molecular anatomy. In accord with the fluid mosaic model, the receptor–enzyme complex is visualized as consisting of two proteins: the beta receptor lying at the outside (a peripheral protein in *Singer*'s terminology), whereas the enzyme adenylate cyclase is postulated to float within the lipid backbone of the membrane.

The role of thyroid hormone in the foundation of catecholamines and adenylate cyclase is discussed by *Will-Shahab, Wollenberger* and *Schulze* (Berlin, DDR), whereas the sensitivity of thyreotrophic stimulation of adenylate cyclase to adrenergic antagonists is described by *Marshall, v. Borcke, Shardlow* and *Malan* (London). The last paper (by *Pfeuffer* and *Helmreich*, Würzburg, GFR) deals with the signal transfer from the receptor to the enzyme in erythrocyte membranes. The informational value of this non-edited summary, lacking both experimental data and references, is strikingly low as compared with the rest of the volume.

It is regrettable that the papers of *Cohen* and *Changeux* (Paris) and of *De Robertis* (Buenos Aires), read at the FEBS meeting, are missing.

In spite of the apparently overhasty editorial work, the booklet will be a useful aid for the neurobiologist. A good subject index is attached.

B. CSILLIK

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РЕЗЮМЕ

НЕКОТОРЫЕ СВОЙСТВА ПРОГРЕССИВНОГО АНТИТРОМБИНА ЧЕЛОВЕКА

Л. А. ПАЛОШ, Г. БЛАШКО и Р. МАЧОВИЧ

Эффект некоторых одно- и двухвалентных катионов на реакцию тромбин–антитромбина исследовался in vitro. Установили, что 0–0,1 мол хлористого натрия или калия не влияли на активность тромбина или антитромбина, а в более высокой концентрации уменьшали активность тромбина. Хлористый кальций или магнезий при концентрации от 0 до 0,05 мол повышали энзимную активность, а при их более высокой концентрации активность уменьшалась. Инактивация тромбина антитромбином была ускорена при концентрации хлористого кальция или магнезия выше 0,04 мол.

Антитромбин инактивировался при pH 7,3 и 65 °C в течение нескольких минут, а гепарин не защищал его от тепловой денатурации. Инактивация тромбина антитромбином не происходила при 0 °C, но присутствие гепарина стимулировало взаимовлияние между тромбином и антитромбином.

ЭФФЕКТ ЛИПИДНОЙ НАГРУЗКИ И КАРБОН-ТЕТРАХЛОРИДА (CCl_4) НА ВЫДЕЛЕНИЕ ЖИРОВОЙ КИСЛОТЫ ИЗ ЖИРОВОЙ ТКАНИ, ИЗОЛИРОВАННОЙ IN VIVO У СОБАК

И. СЛАМКА, П. ШАНДОР, Е. КОЛТАИ и Е. КОВАЧ

Под влиянием липидной эмульсии (Липофундин ^{R}), введенной в системную циркуляцию у собак, выделение жировой кислоты из жировой ткани, изолированной in vivo, увеличивалось с $0{,}51 \pm 0{,}096$ мкЭкв/100 г/мин до $3{,}18 \pm 0{,}48$ мкЭкв/100 г/мин. Если липидная эмульсия вводилась в артерию жировой ткани, выделение жировой кислоты повышалось с $0{,}34 \pm 0{,}053$ мкЭкв/100 г/мин до $1{,}67 \pm 0{,}25$ мкЭкв/100 г/мин. Этот эффект могли наблюдать и в денервированной жировой ткани; в этом случае выделение жировой кислоты повышалось с $0{,}40 \pm 0{,}053$ мкЭкв/100 г/мин до $1{,}16 \pm 0{,}14$ мкЭкв/100 г/мин.

Когда CCl_4, растворенный в липидной эмульсии, вводился в системную циркуляцию, значительного изменения не происходило, и величина была $0{,}45 \pm 0{,}063$ мкЭкв/100 г/мин. А когда CCl_4, растворенный в Липофундине вводился в артерию жировой ткани, выделение жировой кислоты увеличивалось с $0{,}34 \pm 0{,}053$ мкЭкв/100 г/мин до $1{,}01 \pm 0{,}18$ мкЭкв/100 г/мин, и в денервированной ткани с $0{,}40 \pm 0{,}053$ до $0{,}96 \pm 0{,}016$ мкЭкв/100 г/мин.

ВЗАИМОВЛИЯНИЕ МЕЖДУ ЦАМФ И АДРЕНОРЕЦЕПТОРАМИ В РЕГУЛЯЦИИ ФУНКЦИИ ПОЧКИ И СЕКРЕЦИИ РЕНИНА

К. ГАЛ и И. ФОРГАЧ

Авторами исследовался эффект d-цАМФ, введенного в почечную артерию в дозе 1,0 мг/мин, на гемодинамику почки и секрецию ренина у нормотензивных, изонатремических, наркотизированных собак и у собак, предварительно обработанных селективными ингибиторами альфа- и бета-рецепторов. Было установлено, что цАМФ возбуждал секрецию ренина, влияя непосредственно на югстагломерулярный аппарат, и не гемодинамическим путем или через внутрипочечные адренорецепторы.

МЕТАБОЛИЗМ КАТЕХОЛАМИНОВ В МОЗГЕ И ИЗБЕГАТЕЛЬНЫЙ РЕФЛЕКС У КРЫС

А. ХРАШЕК, А. ПАВЛИК и Э. ЭНДРЁЦИ

Избегательный условный рефлекс вырабатывался в „shuttle-box" 50 сочетаниями у белых крыс-самцов, которые потом на основе исполнения заданий были разделены на группы. Метаболизм мозговых катехоламинов измерялся в коре, гипоталамусе, гиппокампе и стволе мозга через 16 часов после внутрижелудочкового введения ^{3}H-норадреналина. Сравнивая с умеренно или слабо учащимися животными, содержание радиоактивного катехоламина значительно уменьшалось у крыс с высоким учебным исполнением. Самое большое снижение наблюдалось в гиппокампе и гипоталамусе, меньшее в стволе мозга и не находилось изменения в коре.

В следующем опыте введение меченого норадреналина производилось через 2 дня после того, как животные были разделены по учебе. Не нашли значительной дифференции в метаболизме катехоламинов мозга среди животных с различным исполнением заданий.

По мнению авторов существует функциональная связь между увеличением метаболизма норадреналина в течение учебы и организацией целенаправленной поведенческой реакции.

ГЕМОДИЛЮЦИЯ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ КАРДИОГЕННОМ ШОКЕ

Т. БАРАНКАИ и Ш. НАДЬ

Исследовался эффект изоволемической гемодилюции декстраном у собак в кардиогенном шоке. Установили, что в кардиогенном шоке минутный объем увеличивался под эффектом гемодилюции, но это повышение не компенсировало снижения транспорта кислорода вследствие уменьшения способности крови связывать кислород.

Снижение величины гематокрита ниже 30% оказывается особенно вредным в кардиогенном шоке.

ЭКСПЕРИМЕНТАЛЬНАЯ МОДЕЛЬ ДЛЯ ИЗУЧЕНИЯ ТРИПТИЧЕСКОЙ ГЕМОРРАГИЧЕСКОЙ ЭНТЕРОПАТИИ У СОБАК

М. ГЕРГЕЙ

Авторами вырабатывалась простая модель для изучения энтеропатии, характеризующей шоковое состояние у собак. Вехрняя брыжеечная артерия была перфундирована норадреналином (2 мкг/кг/мин) в течение 1 часа через сердечный катетер, введенный в соответствующее место при рентгеновом контроле. Перфузия вызывала фармакологическую окклюзию артерии вследствие локальной стимуляции альфа-рецепторов, и таким образом в короткое время образовывалась триптическая геморрагическая энтеропатия без гиповолемии или операционного вмещательства.

ФАРМАКОЛОГИЧЕСКИЕ ИССЛЕДОВАНИЯ ПО НЕЙРО-МЫШЕЧНОЙ ПЕРЕДАЧЕ ВАЗОМОТОРНОЙ РЕГУЛЯЦИИ

Й. ПОРСАС, К. П.-ГИБИСЕР и Й. ПОРСАС ЙР.

На 150 наркотизированных хлоралозом + уретаном кошках исследовались почечная симпатическая эфферентная активность и ее изменения, наступающие при раздражении центральной культи блюждающего, седалищного и локтевого нервов и влияние фармаконов (диазепам, тофизопам, фентоламин, дигидроэрготамин, хлорпромазин, резерпин, клонидин, атропин, метизергид и фениндамин) на основную активность и симпатические рефлексы.

На основе эфферентной симпатической активности и изменений симпатических рефлексов можно сделать заключение о функциональном состоянии вазомоторного центра.

Метизергид и фениндамин не тормозят симпатической эфферентной активности и не оказывают влияния на симпатические рефлексы. Эти исследования исключают возможность того, что в вазомоторном нейроне серотонин или гистамин являются трансмиттерами. Опыты, проведенные атропином, исключают, что мускарин-подобный эффект ацетилхолина играет роль в симпатических ингибиторных или стимулирующих рефлекторных процессах.

Из исследованных фармаконов только диазепам и клонидин тормозили эфферентную симпатическую активность. Клонидин был более селективным и влиял в более низкой дозе (20 мкг/кг), чем диазепам (0,5 – 1 мг/кг).

Альфа-блокирующие агенты интенсивнее тормозили висцерально-симпатическую ингибиторную рефлекторную дугу, чем сомато-симпатическую. Ингибиторный трансмиттер, по всей вероятности, был норадреналин.

Диазепам и тофизопам уменьшали симпатические стимулирующие рефлексы, а клонидин и фентоламин стимулировали их. Другие фармаконы были неэффективными. Что касается трансмиттеров, на основании этих экспериментальных данных не могли дать однозначащего ответа.

ТАХИФИЛАКСИЯ К ГИПОТЕНСИВНОМУ ЭФФЕКТУ КЛОНИДИНА И ВОЗМОЖНЫЙ МЕХАНИЗМ ЭТОГО ЭФФЕКТА

К. П.-ГИБИСЕР и Й. ПОРСАС

Опыты производились на кошках под хлоралозовым-уретановым наркозом. К гипотензивному эффекту клонидина развивалась тахифилаксия: в случае низких доз (10 – 20 мг/кг) медленнее, а при высокых дозах (50 мг/кг) быстрее. Что касается контракции мигательной мембраны, не развивается быстрая тахифилаксия. Это явление похоже на тахифилаксию, развивающуюся при даче посредственных симпатомиметических анионов. Когда на гипотензивный эффект клонидина развивалась тахифилаксия, последующие дозы только повышали кровяное давление. Инфузией норадреналина можно прекратить тахифилаксию. Из этих результатов авторы делают вывод, что гипотензивный эффект клонидина наступает посредственным путем, т. е. через метаболизм норадреналина.

МЕХАНИЗМ АНТИДИУРЕЗА, ВЫЗВАННОГО ХЛОРПРОПАМИДОМ

Л. ЦАКО, Е. НАДЬ и Ф. А. ЛАСЛО

Авторами исследовался эффект хлорпропамида на обмен воды у крыс с нарушением ножки гипофиза. Установили, что под влиянием фармакона олигурическая интерфаза более выраженная и удлиненная, в то время как в длительной полиурической фазе, при отсутствии эндогенного антидиуретического гормона, он только умеренно влиял на прием воды у крыс. Повышение эффекта эксогенного антидиуретического гормона наблюдалось после 2-дневной обработки хлорпропамидом. Результаты поддерживают то объяснение, что хлорпропамидовый антидиурез нуждается в присутствии небольшого количества эндогенного антидиуретического гормона. Хлорпропамид, по всей вероятности, уменьшает диурез мобилизацией антидиуретического гормона, и в то же время повышает также периферическое влияние антидиуретического гормона.

СТЕФАНЦОВ, Б. Д., ГОНЧАРОВА, Л. С.: К вопросу о надежности в деятельности некоторых парных образований продольговатого мозга животных. Физиолог. Ж. СССР. **1968,** 54: 398 – 405.

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Physiologia

HOMOSYNAPTIC AND HETEROSYNAPTIC INTERACTIONS IN THE NUCLEUS TRACTUS SOLITARII

By

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The interactions of potentials evoked by stimulation of low-threshold vagal afferents were studied in the nucleus tractus solitarii, at the level of the obex in cats under chloralose-pentobarbital anaesthesia. The peak latency of the negative wave was 6.28 ± 0.56 msec in case of ipsilateral stimulation and 6.92 ± 0.49 msec in case of contralateral stimulation. The response evoked from the ipsilateral side was greater than that from the contralateral side, their ratio was 4 : 3. The evoked response was sensitive to asphyxia, its amplitude exhibited frequency-dependence and fell to half of the original value at about 30 cps. The evoked response did not show post-tetanic potentiation. The homosynaptic stimulus train was followed by a post-excitatory depression manifesting not only with a diminished amplitude but also with a lengthening of latency.

The responses to bilateral stimulation ($B_{\mu V}$) were greater than those evoked separately, but smaller than the sum of the two ($S_{\Delta V}$). A negative correlation was found between $S_{\mu V}$). and $\Delta S_{\Delta V}$ ($\Delta S_{\mu V} = B_{\mu V} - S_{\Delta V}$). The relationship could be described by the regression equation:

$$\Delta S_{\mu V} = -0.369\ S_{\Delta V} + 8.635$$

The majority of interactions was occlusional in character. Addition was observed in few cases; facilitation could not be produced.

Highest response to bilateral stimulation could only be produced within an interval of ± 1 msec; at ± 2.5 msec the summation ceased. A further increase of the inter-stimulus time resulted in inhibition that reached the maximum at 5 msec. The original amplitude of the response tested returned by around 30 msec. The time of restoration depended on the size of the reaction evoked by the conditioning stimulus. The inhibitions observed in the nucleus tractus solitarii have been brought into relation with the prolonged positivity that accompanies the negative component of the evoked potential.

Several papers have dealt with the medullary projections of vago-aortic and baroreceptor afferentation (*Andersen* and *Berry* 1956; *Porter* 1963; *Stefantsov* and *Gontcharova* 1968; *Urabe* et al. 1968; *Humphrey* 1967; *Crill* and *Reis* 1968; *Miura* and *Reis* 1969; *Seller* and *Illert* 1969; *Gabriel* and *Seller* 1970; *Biscoe* and *Sampson* 1970; *Kumada* and *Nakajima* 1972), but data are scarce on the characteristics of medullary interactions. The changes elicited by stimulation in blood pressure (*Such* and *Obál* 1969; *Such* 1970), sympathetic nervous system (*Such* et al. 1972) and EEG pattern (*Such* et al. 1976) raised the question of the role played by the nucleus tractus solitarii. This nucleus is the first relay station of the reflex arch, and its role in determination of the character and extent of homo- and heterosynaptic interactions to merit a detailed investigation.

Methods

The experiments were performed on 25 cats anaesthetized with a mixture of chloralose and pentobarbital (50 mg/kg and 10 mg/kg, respectively) given by the intraperitoneal route. After insertion of a tracheal cannula the cervical vagal nerves were prepared and cut on both sides. The central stumps were then led to the dorsal side through the neck muscles. Thereafter, the animals were fixed in a stereotaxic apparatus, and the obex was explored after sucking off the caudal part of the cerebellum. The electical activity of the region of the nucleus tractus solitarii was recorded by means of laquer-insulated tungsten electrodes 20—30 μ in diameter. The area investigated was located 1.0 mm lateral from the obex and 0.8–1.2 under the surface. Indentification was made on the basis of stereotaxic coordinates (AP; −13.5 −14.7; *Berman* 1968), the morphology as well as the phase relations and the latency of potential (*Porter* 1963). The electrical sings were followed on a DISA oscilloscope by means of a differential amplifier and stored on magnetic tape by using an FM adapter. Evaluation was made partly from films, and partly from curves averaged with a 512-channel analyser.

The vagal nerves were stimulated with a two-channel stimulator. In case of single stimuli, the reaction was tested with 1 cps using an impulse width of 0.1 msec. The voltage of the stimuli varied according to the actual experimental conditions. A high-frequency isolator unit was used to diminish stimulus artefacts.

Results

Characteristics of the evoked potentials

Responses in the region investigated could be elicited by stimulation of both vagal nerves. The evoked potential consisted of a high-amplitude negative wave followed by a prolonged low-amplitude positive one 15–25 msec in duration. The negative component was often preceded by a small negativity of about 2.5 msec latency that presumably meant the incoming afferent volley (Fig. 1). Peak latency of the great negative potential was 6.38 ± 0.56 msec in case of ipsilateral stimulation and 6.92 ± 0.49 msec in case of contralateral stimulation. The voltage necessary to evoke this potential varied between 1 and 2 V. A change of the voltage caused the potential rapidly to reach the maximum amplitude; a further increase in the voltage usually failed to influence the morphology of the potential. The amplitude of responses elicited from the homolateral side was generally higher, the ratio of the responses being about 4 : 3.

The evoked response was sensitive to asphyxia and disappeared 1—2 min after discontinuing the artificial respiration. The amplitude of the response was frequency-dependent (Fig. 2) and fell to half the original value by about 30 cps. This indicates that the recording had been made from postsynaptic elements, as intramedullary afferent fibres follow the stimulus frequency up to 100—150 cps without any decrease in amplitude. (*Humphrey* 1967; *Seller* and *Illert* 1969). Figure 2 shows the frequency-dependence of the responses evoked by ipsilateral and contralateral vagal stimulation. As it can be seen, the differences in amplitude persisted throughout the whole frequency range investigated.

Another method to differentiate between pre- and post-synaptic responses would be the phenomenon of post-tetanic potentiation (*Hughes* 1958). This, however, could

Fig. 1. Potential recorded from the nucleus tractus solitarii after vagal stimulation. Recording: 1 mm laterally from the obex and 0.8 mm vertically from the surface. Stimulus parameters: 0.2 cps, 0.1 msec and 3 V. Calibration: 10 msec and 50 μV

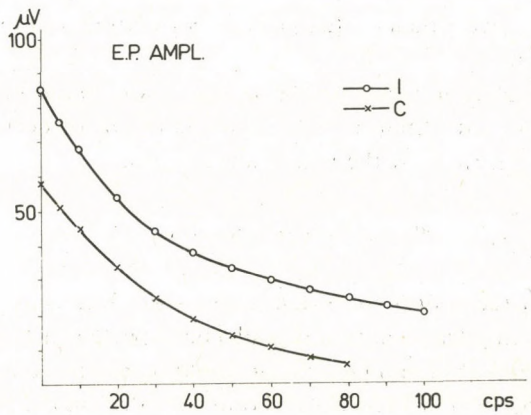

Fig. 2. Frequency-dependence of the amplitude (EP AMPL) of potentials evoked by ipsilateral (I) and contralateral (C) vagal stimulation in the nucleus tractus solitarii. Stimulus parameters: 0.1 msec and 4 V

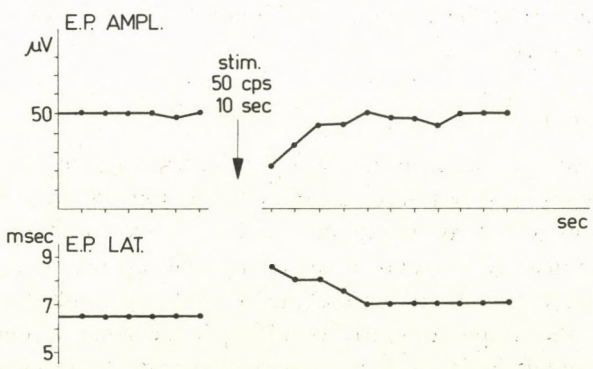

Fig. 3. Effect of homosynaptic tetanization on amplitude (EP AMPL) and latency time (EP LAT) of evoked potentials. Testing at 1-sec intervals, tetanization (50 cps, 10 sec) at arrow

not be demonstrated and after homosynaptic conditioning even inhibition was observed (Fig. 3) that manifested itself not only with a diminished amplitude but also with lengthening of latency. The inhibition ceased in about 5 sec after tetanization with 50 cps for 10 sec (Fig. 3). Heterosynaptic conditioning did not influence the effect of homosynaptic test-stimuli at 1 cps.

Spatial summation

The responses to bilateral stimulation were generally higher than those elicited separately but, generally they did not reach the sum of the two. Bilateral interactions of the two vagi were studied in the case of reactions of different magnitude. For this purpose, separate as well as simultaneous stimulation of the vagal nerves were applied with 1 cps for 20 sec. The series obtained in this way were averaged and their amplitude were determined. The sum of the amplitudes of separate responses ($S_{\mu V}$) was subtracted from the magnitude of responses to bilateral stimulation ($B_{\mu V}$). The differences ($\Delta S_{\mu V}$) were then evaluated with correlation and regression analysis, in relation to the sums.

A negative correlation ($r = -0.828$, $p < 0.1\%$) was found between the magnitude of separately-evoked responses ($S_{\mu V}$) and the extent of occlusion ($\Delta S_{\mu V}$). The relationship can be described by the regression equation,

$$\Delta S_{\mu V} = -0.369 \cdot S_{\mu V} + 8.635$$

The line depicted in the coordinate sytem crosses the X-axis at a value of 25 μV (see Fig. 4); this means that facilitation could be expected in the case of separate responses below 12.5 μV. Still, no facilitation could be produced in the experiments, and addition was observed in a few cases (points situated on the X-axis).

At a given voltage, the highest response to bilateral stimulation was obtained in the case when the peaks of potentials evoked from the right and left side coincided in time or differed by ± 1 msec only (Fig. 5). Longer temporal differences led to a rapid diminution of the amplitude of simultaneous responses, and summation ceased at ± 2.5–3 msec.

Temporal interactions

When the interval between individual impulses was lengthened over 3 msec, the amplitude of the test responses decreased as compared with the control. Fig. 6 shows the effects of heterosynaptic and homosynaptic conditioning stimuli. The vertical axis demonstrates the percentage changes of test reactions. After an initial summation, the responses begin to separate at 3—4 msec, and the first component will be fixed at the amplitude corresponding to the conditioning stimulus. The full line shows the effect of ipsilateral conditioning on the reactions evoked from the contralateral side, and the broken line the effect of contralateral conditioning

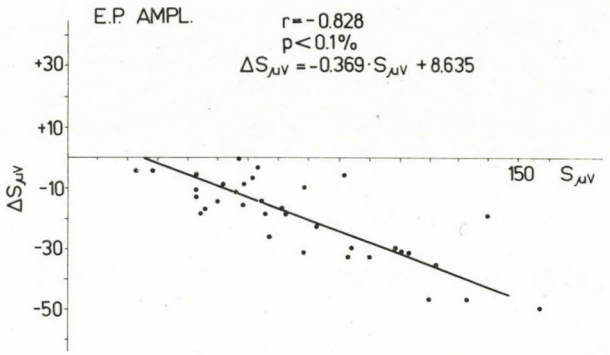

Fig. 4. Spatial interactions of potentials evoked by vagal stimulation. $S_{\mu V}$: sum of the amplitude of responses to stimulation of right and left vagal nerves. $\Delta S_{\mu V}$: differences between amplitudes of potentials evoked by bilateral stimulation ($B_{\mu V}$) and the sum ($S_{\mu V}$) of the separately evoked responses. r: correlation coefficient, equation of regression line. P: probability index of "r". For details see text

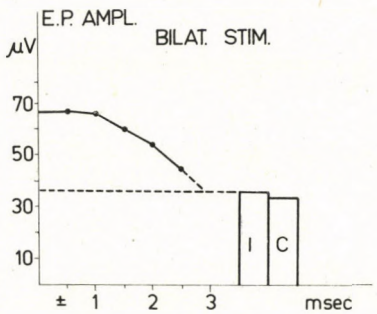

Fig. 5. Temporal relations of bilateral interactions. I: amplitude of potentials evoked from ipsilateral side. C: amplitude evoked from contralateral side. The extent of summation is highest in the ±1 msec interval; thereafter, it steeply diminishes

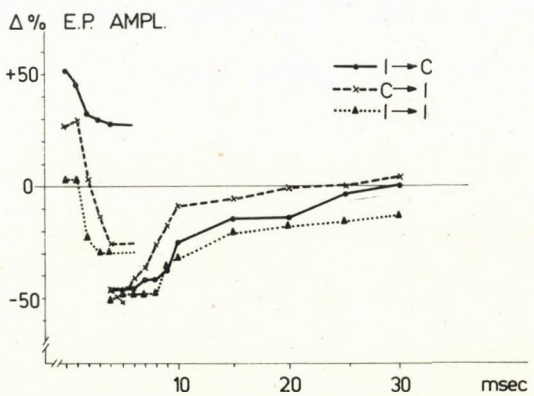

Fig. 6. Effect of homosynaptic (I→I) and heterosynaptic (I→C, C→I) conditioning impulses on amplitude of evoked potentials. The ordinate shows the percentage differences ($\Delta\%$) of the reactions tested. For details see Text

on ipsilaterally elicited responses. As it can be seen, the responses evoked from the contralateral side were 25% weaker than those elicited from the ipsilateral side. The dotted line shows the homosynaptic interactions. In this case two pairs of electrode were placed on the nerve; the distal electrode was used for conditioning while the central one for testing. The response evoked from the distal electrode was smaller than that elicited from the central one. Inhibition was greatest around 5 msec; thereafter diminished it gradually with the increase of the interval-distance between individual impulses. The greatest inhibition occurred in the case of homotopic conditioning (dotted line); its restoration was not complete even at 30 msec. On the average, ipsilateral conditioning (full line) inhibited the response evoked from the contralateral side 8% more than did contralateral conditioning in respect of reactions evoked from the ipsilateral side (broken line). These differences can however attributed to the fact that ipsilateral stimulation evoked a biologically greater response since, if the potential evoked from the contralateral side would be greater, the inhibitory effect of contralateral stimulation ought to become more effective.

Discussion

Interactions of baroreceptor afferents in the nucleus tractus solitarii have been dealt with by *Gabriel* and *Seller* (1970). These authors found a 25—30% stronger response to bilateral than to unilateral stimulation. They explained the non-linear summation by the fact that different baroreceptor afferents partly relayed to the same post-synaptic neurones, but failed to deal with the rules and dynamics of summation. In the present experiments the extent of summation was shown to be a function of the magnitude of separate reactions, a relation that can be described by a regression equation. Even though the majority of the interactions lies within the range of occlusion, the summation may sometimes reach the level of addition. In the case of bilateral stimulation summation only appears a stimulus interval of about ± 2.5—3 msec. Our data indicate that, due to the strong convergency, there is no, or only an insignificant, subliminal fringe in the first relay station, which is a condition for the development of facilitation. Thus, other components of the reflex arch, and most probably the interneurones, are responsible for the facilitation phenomena observed during vasomotor reflex processes (*Such* 1970).

Throughout this paper the term "summation" — that actually means addition — is used in a general sense, for characterizing the common responses. By no mean do we consider it as a quality, summations not being additive or, if so, on rare occasions only. Belonging to summation phenomena are considered by us also facilitation, addition, occlusion as well as overlapping, since all these events differ only in the extent of convergence or divergence (*Such* 1972).

In the course of temporal interactions no post-tetanic potentiation could be obtained in the region investigated. Though *Biscoe* and *Sampson* (1970) mentioned

that the negativity showed post-tetanic potentiation in the region corresponding to AP: 15—17 Horsley-Clarcke's coordinates, they failed to publish a figure or any detailed data in this connection. The post-excitatory inhibition recorded in our experiments was the highest in the case of homotopic conditioning. The extent of inhibition as well as its restoration depended on the magnitude of the response to conditioning stimulus, the frequency of stimulation and the duration of the preceding impulse train.

As to the nature of the intrasolitarial inhibition, one may assume that the changes in membrane potential following the excitatory state, were responsible for it. The time-relations of the positivity following the negative component of the evoked potential are in agreement with the duration of post-excitatory inhibition evoked by paired impulses. Indicative of such an assumption are also the observations of *Gabriel* and *Seller* (1970), according to wich the interactions of baroreceptor nerves are not influenced by either strychnine or picrotoxin. A similar post-excitatory depression at cortical and subcortical levels was observed in the case of optic and acoustic stimuli (*Marshall* 1949; *Clare and Bishop* 1952; *Rosenblith* et al. 1950). Investigations with microelectrodes revealed the decreased excitability to be connected with hyperpolarization of the neuronal membrane.

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INFLUENCE OF PHENOBARBITAL PRETREATMENT ON BILIARY ROSE BENGAL EXCRETION IN RATS

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Plasma concentration, hepatic uptake and biliary excretion of intravenously administered rose bengal was determined in rats pretreated with phenobarbital (50 mg/kg daily, for four days). After an initial (0—16 min) rapid fall in plasma rose bengal concentration caused by hepatic uptake of the dye, the curves in control and pretreated rats did not differ from each other either after administration of a small (5 mg/kg) or a large (50 mg/kg) dose. Hepatic rose bengal concentration was significantly lower in pretreated animals than in the control group. Since liver weight was higher in the phenobarbital pretreated animals than in the controls, the total amount of rose bengal taken up by the liver did not differ in the two groups. The biliary excretion of low dose (5 mg/kg) rose bengal was significantly higher in phenobarbital pretreated than in the control rats but with doses of 50 mg/kg and 100 mg/kg no difference was observed. These doses of rose bengal diminished the increased bile flow caused by phenobarbital.

According to *Klaassen* (1968) phenobarbital pretreatment in rats enhances the fall of plasma concentration of intravenously given bromsulphophthalein (BSP), 3,6-dibromphthalein disulphonate (DBSP) and indocyanine green (ICG). The decrease is also more marked in human patients pretreated with phenobarbital (*Gógl* and *Jávor* 1971).

BSP is excreted with bile after conjugation with glutathione, whereas DBSP (*Javitt* 1965) and ICG (*Wheeler* et al. 1958) are eliminated in unchanged form. It is likely that phenobarbital acts by enhancing the hepatic uptake and/or by increasing biliary excretion. The aim of the present study was to clarify this problem. In the experiments rose bengal was used, a compound which is not metabolized in the liver (*Jirsa* and *Raban* 1962), but is taken up by the hepatic cells and excreted by active transport with the bile (*Mendeloff* 1949; *Taplin* et al. 1955) the dye is known to compete with other organic anions for the biliary excretory sytem (*Fischer* and *Varga* 1974).

Material and methods

^{131}I-rose bengal (500 mCi/mmol) was purchased from Radioisotopes Production and Distribution Centre, Swierk, unlabelled rose bengal from Fluka A. G., Buchs SG.

Male Wistar rats of 250—300 g body weight were used. The animals were fasted 16–20 hours prior to the experiment, water was given ad libitum. Under intraperitoncal urcthan (1.2 g/kg) anaesthesia a median laparotomy was performed, the common bile duct was canulated with polyethylene tubing

(PE—10), and the bile collected in two periods, between 0 and 16 and between 16 and 36 minutes following administration of the dye. Diluted ^{131}I-rose bengal dissolved in physiological saline and given into the femoral vein in a volume of 0.1 ml/100 g body weight. The left carotid artery was cannulated with polyethylene (PE—50) tubing and blood samples were taken at various intervals following dye administration, 500 IU heparin was given intravenously before the experiment. Body temperature of the rats was maintained at 37 °C by a bulb. At 36 min after the administration of rose bengal the liver was removed and weighed.

Blood content of the liver sample was determined according to *Holzer* et al. (1956). The quantity of rose bengal in hepatic blood was subtracted form the total quantity of dye found in the liver. The volume of the biliary tree was estimated according to *Barber-Riley* (1965); it amounted 5.3 μl/g liver on the average.

The radioactivity of bile, blood and liver samples was measured in a gamma scintillation counter equipped with a well-type crystal (Type NK-150, Gamma). The rose bengal concentration of plasma was calculated by measuring the radioactivity of blood samples and determining the hematocrit value.

The inducing effect of phenobarbital was established by the increase in liver weight, shortening of hexobarbital (70 mg/kg) sleeping time and by the increase of microsomal protein and cytochrome P-450 content of the liver. Microsomal protein was determined according to the method of *Lowry* et al. (1951) and the cytochrome P-450 content of the liver by the method of *Klinger* (1973).

In the Tables average values $\pm$S.E. are given. Significance was calculated by the *t*-test The constants k and half time ($t_{1/2}$) of decrease of rose bengal concentration in plasma were calculated using the equation $y = y_0 e^{-kt}$.

Results

Effect of phenobarbital pretreatment on the liver

As a result of phenobarbital pretreatment, the liver mass calculated for 1 kg body weight increased by 22.9%, hexobarbital sleeping time was shortened by 56.6%, the amount of microsomal protein was enhanced 1.5 fold and the cytochrome P-450 content increased double the value measured in the control animals (Table I).

Hepatic uptake of rose bengal

The initial rapid fall in the plasma concentration of intravenously injected 5 mg/kg of rose bengal is due to uptake by the liver (Fig. 1). The half time of the initial rapid fall was 2.07 min in control rats and 2.21 min in phenobarbital pretreated animals. After a 50 mg/kg dose the initial fall in plasma concentration was less marked. The difference in the half time of plasma concentration between the control and treated animals was not significant statistically (control groups 2.64 min; treated group, 2.88 min).

In some experiments the rats were given a dose of 50 mg/kg intravenously, killed by bleeding and rose bengal concentration in the liver was determined. The concentration reached the peak in 10—12 min, then gradually decreased in both the control and the phenobarbital pretreated animals (Fig. 2). These data also support the assumption that the initial rapid fall in the plasma concentration of rose bengal was due to hepatic uptake of the dye.

Table I

Liver weight, hexobarbital sleeping time, cytochrome P-450 and protein content of microsomal fraction of the liver in control and phenobarbital pretreated rats (n = 20)

	Weight of liver g/kg b.w.	Hexobarbital sleeping time, sec	mμ mol cytochrome P-450/mg protein	Microsomal protein mg/g liver
Control	32.3 ± 1.62	1376 ± 225	0.676 ± 0.042	21.4 ± 2.02
Pretreated with phenobarbital	39.7 ± 2.96*	397 ± 59.5**	1.32 ± 0.03**	31.0 ± 4.00*

* = $p < 0.05$
** = $p < 0.01$

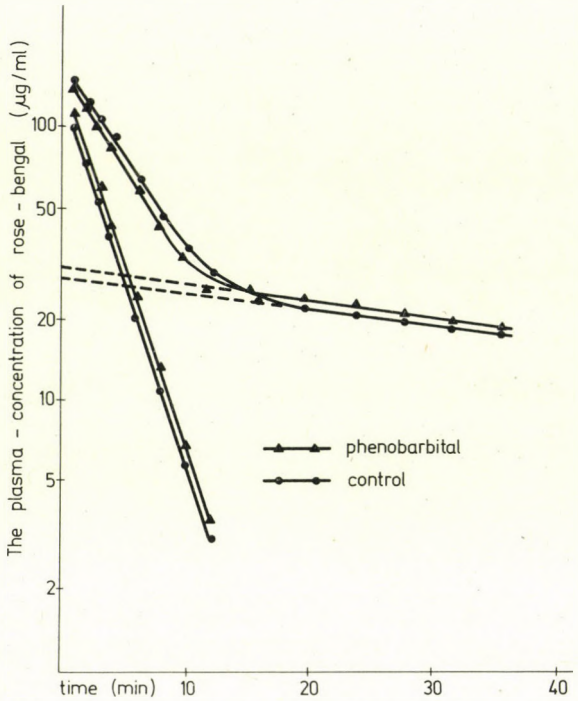

Fig. 1. Rose bengal concentration of plasma in control and phenobarbital pretreated rats. Each point represents the average value for 10 animals. The dose of rose bengal was 5 mg/kg intravenously. The k value of the curve is 0.335 for the controls and 0.314 for rats pretreated with phenobarbital

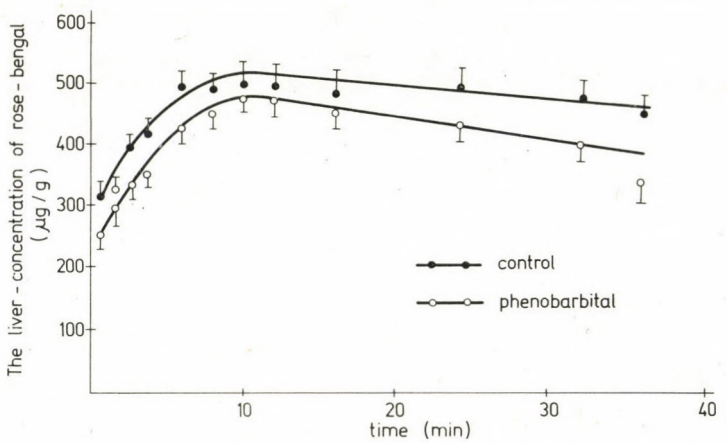

Fig. 2. Rose bengal concentration in the liver after a dose of 50 mg/kg. Each point shows the average value for 6—8 animals

Table II

Rose bengal concentration in the liver of control and phenobarbital pretreated rats
(n = 10)

Dose of rose bengal	Liver concentration of rose bengal μg/g		Amount of rose bengal in liver mg/kg b.w.	
	control	pretreated	control	pretreated
5 mg/kg	109 ± 6.1	85.0 ± 4.9*	5.53 ± 0.34	3.32 ± 0.33
50 mg/kg	451 ± 43.1	333 ± 24.2*	14.6 ± 1.42	13.2 ± 0.95

* = $p < 0.05$

Table III

Biliary flow, biliary concentration and excretion of rose bengal in control and phenobarbital pretreated rats
(n = 10)

Dose of rose bengal mg/kg	Bile flow μl/kg/min		Liver concentration of rose bengal μ/ml		Biliary excretion of rose bengal μ/kg/min	
	control	pretreated	control	pretreated	control	pretreated
5	48.3 ± 3.92	76.3 ± 4.18*	897 ± 67	830 ± 59	43.4 ± 4.12	63.3 ± 5.87**
50	40.6 ± 3.83	50.0 ± 3.81	3330 ± 383	3010 ± 198	135 ± 14.3	151 ± 14.1
100	34.4 ± 3.29	42.3 ± 3.76	4410 ± 420	4010 ± 402	156 ± 14.9	170 ± 15.0

* = $p < 0.05$
** = $p < 0.01$

At the end of the experiment, at 36 min after administration of rose bengal, its concentration in the liver was lower in the pretreated than in the control rats (Table II). However, the hepatic rose bengal content in the two groups did not show a significant difference, because the liver mass calculated for 1 kg body weight was increased in the pretreated rats.

Biliary excretion of rose bengal

By increasing the dose of rose bengal, its amount excreted with the bile in the 16—36 min period increased both in the control and in the pretreated rats (Table III). The excretion rate (63.3 μg/kg/min) of a 5 mg/kg dose in the phenobarbital pretreated was significantly higher ($p < 0.01$) than in the control group (43.3 μg/kg/min). On the other hand, the biliary excretion of 50 and 100 mg/kg rose bengal did not differ markedly in the two groups. Bile flow was higher in the pretreated animals than in the controls. The concentration of rose bengal in bile was slightly lower in the pretreated than in the control rats. The difference, however, was not significant ($p > 0.05$). The effect of phenobarbital to increase the bile volume was more marked with lower than with higher doses of the dye (Table III). This may be ascribed to the more marked effect of high doses in decreasing the bile flow.

Discussion

The effect of phenobarbital on the three main steps of hepatic transfer uptake, biotransformation and transport into the biliary capillaries is not known. The drug has been shown to enhance the microsomal and non-microsomal drug metabolism in the liver (*Conney* 1967; *Remmer* and *Merker* 1963; *Orrenius* et al. 1965). In rats pretreated with phenobarbital the biliary excretion of BSP was increased (*Klaassen* 1969; 1970/a) due partly to the increased conjugation of BSP with glutathione (own unpublished data). The glutathione conjugated BSP has a greater affinity to the excretory system than the free dye (*Varga* et al. 1974). However, as a result of phenobarbital induction the excretion of non-metabolizing DBSP (*Klaassen* 1970/a) and ICG (*Gógl* and *Jávor* 1971) was also increased. According to the investigations of *Levi* (1973) ligandin, one of the protein binding organic anions can be induced by phenobarbital and therefore the hepatic uptake of different organic anions increases. *Klaassen* (1970) concluded from indirect evidence that the hepatic uptake of ICG was enhanced after phenobarbital treatment.

Earlier we have shown that after low doses of rose bengal only 11.2% could be found in the extrahepatic organs, the rest was in plasma, liver and bile (*Fischer* and *Varga* 1974/a). The initial fall in plasma concentration of intravenously given rose bengal was caused by its hepatic uptake; this was proved by direct measurements (Tables I, II, III). Phenobarbital pretreatment failed to increase the hepatic uptake

of either low or high doses of rose bengal in any period following its administration. The difference in hepatic uptake after low and high doses of rose bengal can be explained by the limited storing capacity of the liver (*Fischer* and *Varga* 1974/a).

According to *Klaassen* (1969, 1970/a) only those inductors increase the biliary excretion of BSP which enhance the bile flows as well. *Heart* et al. (1969) showed that after phenobarbital pretreatment the enhanced bile flow plays the main role in the increase of biliary excretion of organic anions. The present results also support that hypothesis. The biliary concentration of rose bengal did not differ in the control and the phenobarbital pretreated animals. The increased biliary excretion of rose bengal after phenobarbital induction went parallel with the bile flow. At a low dose (5 mg/kg) of rose bengal the bile flow was markedly higher in the pretreated rats ($p < 0.01$) than in the controls. On the other hand, with larger doses (50 and 100 mg/kg) there was much less difference between the two groups. This can be explained by the observation that phenobarbital increases first of all the biliary acid independent fraction (*Berthelot* et al. 1969; *Paumgartner* et al. 1971; *Klaassen* 1971), although, *Dhumeaux* et al. (1970) have shown that rose bengal decreased the biliary acid independent fraction of bile in a dose related manner.

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SPATIAL INTERACTIONS OF BLOOD PRESSURE REFLEXES

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Spatial interactions of depressor and pressor reflexes evoked by unilateral and bilateral stimulation of various visceral and somatic afferents were analysed in chloralase-pentobarbital or pentobarbital anaesthetized cats. It has been found that the dynamics of the spatial interactions can be described by regression equations as a function of the size of the responses evoked by separate stimulation. The angle of inclination and the position of the regression line in the coordinate systems is influenced by the type of reflex, the anaesthetic used and the functional state of the animal. At standard conditions the relations are reproducible. Sagittal splitting of the medulla and the pons did not essentially alter the character of the interaction. The forms of the interactions were unaffected by previous stimulations.

It is well known that the manifestations of the spatial interactions may be facilitation and occlusion alike, depending on the size of the separately evoked responses (*Creed* et al. 1932). The properties of the relations have not, however, been analysed profoundly. The first effort to describe quantitatively the vasomotor summation phenomena appeared, to our best knowledge, in the work of *Bittman* and *Raiciulescu* (1964). These authors found a linear correlation between vasopressor responses evoked by separate and simultaneous stimulation of various cortical and deep cerebral structures. As they could not survey the general regularity of the dynamics of the interactions, they have not discussed the question. Neither were the relationships clarified by our earlier investigations (*Such* 1970) in the summation of vasomotor reflexes. We have therefore attempted to outline the general properties of the spatial interactions of the blood-pressure reflexes by analysing the experimental data statistically.

Methods

Experiments were carried out on cats of either sex, weighing 2.5—5.0 kg, anaesthetized intraperitoneally with a mixture of 50 mg/kg chloralose and 10 mg/kg pentobarbital or with 45 mg/kg pentobarbital alone. For eliciting blood-pressure reflexes the vagal, tibial and median nerves and the common carotid arteries were prepared on both sides. The nerves were tied distally and cut. Blood pressure was recorded from the femoral artery by a mercury manometer on smoked paper. The reflexes were elicited by square-pulse stimulation through bipolar silver electrodes attached to the proximal stump of the nerves, or by compression of the common carotid arteries. The cats were immobilized with gallamine and ventilated artificially at a frequency of 16/min. All the experiments were performed on bilaterally vagotomized animals.

The interactions were studied at different reflex intensities. To analyse a given interaction, practically identical blood-pressure reflexes were elicited by separate stimulation of two afferent channels and these reactions were compared with the effects evoked by simultaneous stimulation of the same nerves with unchanged stimulus parameters. The results were interpreted by comparison and statistical analysis (*Brownlee* 1965) of the sum of separately evoked responses and the difference of the simultaneously evoked ones from the appropriate sum. This way of evaluation is essentially in accord with the sherringtonian principle.

Results

A) *Interactions of depressor reflexes*

a) Tibial nerve stimulation

In Fig. 1 the dynamics of the spatial interactions of the vasodepressor reflexes induced by stimulation of the tibial nerves is illustrated. The dots mean data from the same animal anaesthetized with 50 mg/kg chloralose +10 mg/kg pentobarbital. The abscissa shows the sum (C) of blood pressure decreases evoked by separate stimulation of the right and left tibial nerves, the ordinate the difference (ΔC) between responses evoked by bilateral simultaneous stimulation (B) and the sum of the separately evoked ones. In the formula $\Delta C = B - C$ if the "ΔC" values are positive,

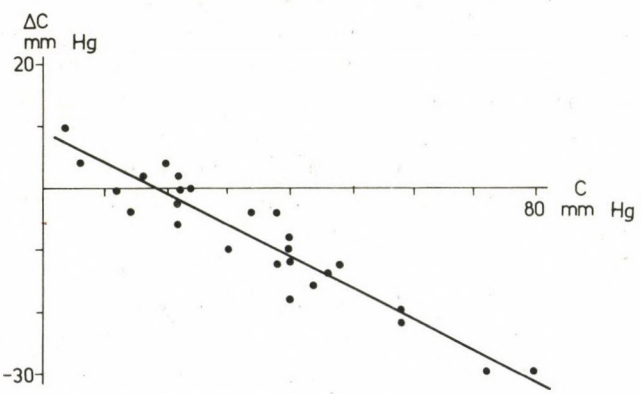

Fig. 1. Cat, 3.9 kg, 50 mg/kg chloralose + 10 mg/kg pentobarbital intraperitoneally. Interactions of depressor reflexes evoked by stimulation of tibial nerves at reflex-reactions of various magnitudes. Frequency of stimulation: 10 cps, impulse duration: 0.1 msec. "C": sum of the blood-pressure responses evoked by separate stimulations of the central stumps of the right and left tibial nerves. "ΔC": difference between responses evoked by bilateral simultaneous stimulation and the sum of the separately evoked ones. The values are expressed in mmHg. For the results of the correlation and regression analysis, see Table I, No. 5

the interactions are facilitatory, and if they are negative, the interactions are occlusive in character. The abscissa represents the addition line. Consequently, every dot in the system is the result of three data. During the experiment the frequency (10 cps) and pulse duration (0.1 msec) was constant, only the voltage was changed. The duration of stimulation was 10 sec in every case. When testing a given interaction, all stimulus parameters were constant.

The distribution of the dots showed a negative correlation. The relation can be described by a regression equation (Table I, No. 5). The line intersects the abscissa at about 20 mm Hg, which means that facilitation can be expected at separate responses smaller than 10 mm Hg, although occlusion occurs in this interval too. The interactions with more intensive reactions manifest themselves with increasing occlusion. Under identical experimental conditions the regression lines constructed on the basis of data obtained from different animals have practically the same position. Nevertheless, the position of a regression line in the coordinate system is significantly influenced by the functional state of the animals. In pentobarbital (45 mg/kg) anaesthesia (Fig. 2), the angle of inclination is steep (Table I, No. 8) and the regression line intersects the abscissa at higher "C" values. As a result, more

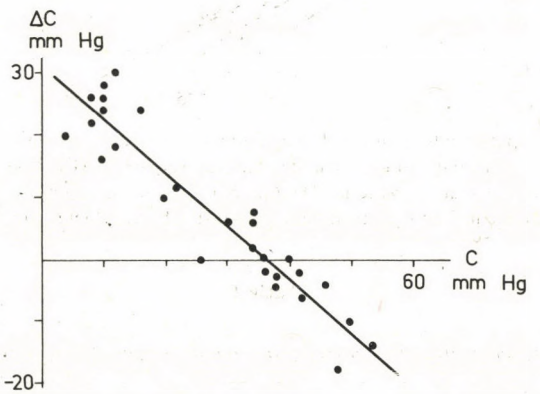

Fig. 2. Cat, 2.9 kg, 50 mg/kg pentobarbital intraperitoneally. Interactions of depressor reflexes evoked by tibial nerves stimulations. In pentobarbital anaesthesia the regression line is steeper and is shifted to the right as compared with the one shown in Fig. 1 (Table I, No. 8)

dots occur in the facilitatory area. Results obtained with tibial nerve stimulation in chloralose-pentobarbital and pure pentobarbital anaesthesia are summarized in Fig. 3, where only the regression lines are presented. The lines Nos 4 and 5 originate from two different animals anaesthetized with chloralose-pentobarbital. There are no significant differences in their angle of inclination, so the lines can be regarded parallel. Although between the two parallel lines the displacement proved statistically appreciable (see the corresponding numbers in the Tables) the difference still cannot

be considered physiologically important. More significant changes could be demonstrated in pentobarbital anaesthesia (broken lines in Fig. 3). The lines Nos 7 and 8 are steeper than the former and shifted to the right. Their regression coefficients differ from those obtained in chloralose-pentobarbital anaesthesia. Though the coefficient of line No. 6 did not change essentially, the line is significantly shifted to the right, it intersects the abscissa near 40 mm Hg. It can be seen in Fig. 3, that the pentobarbital anaesthesia the possibility of facilitation increases considerably. This is clearly demonstrated by the length of portions of the regression lines lying over the abscissa.

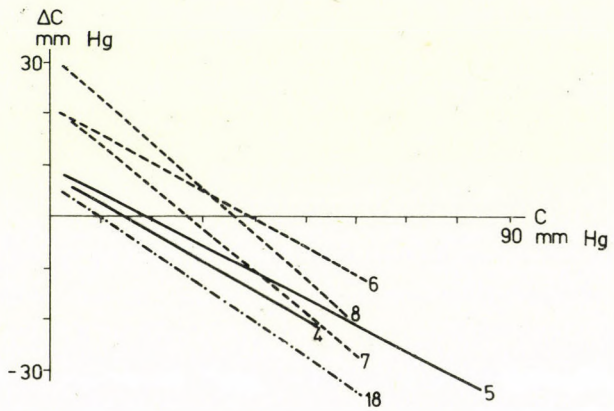

Fig. 3. Summation of spatial interactions of the depressor reflexes of somatic origin. Lines Nos 4 and 5 were constructed from data obtained under chloralose-pentobarbital anaesthesia; lines Nos 6, 7 and 8 under pure pentobarbital anaesthesia. The line No 17 shows interactions after spliting the lower brain stem. For statistical data, see in the Tables. Details in text

Table I

Correlation and regression analysis of the interactions of blood-pressure reflexes

No.	Reflexes	n	r	p_r%	b	a	95% conf.
1.	vagus depressor (chloralose-pentobarbital)	37	−0.83	< 0.1	−0.56	11.09	14.91
2.	vagus depressor (chloralose-pentobarbital)	20	−0.80	< 0.1	−0.64	12.48	9.58
3.	vagus depressor (pentobarbital)	35	−0.95	< 0.1	−0.95	31.27	7.63
4.	tibial depressor (chloralose-pentobarbital)	29	−0.86	< 0.1	−0.57	8.41	7.52
5.	tibial depressor (chloralose-pentobarbital)	26	−0.94	< 0.1	−0.50	9.28	6.83

Table I

Correlation and regression analysis of the interactions of blood pressure reflexes

No.	Reflexes	n	r	p_r%	b	a	95%conf.
6.	tibial depressor (pentobarbital)	44	−0.73	< 0.1	−0.55	21.22	13.17
7.	tibial depressor (pentobarbital)	36	−0.91	< 0.1	−0.82	21.89	9.26
8.	tibial depressor (pentobarbital)	28	−0.94	< 0.1	−0.85	31.04	9.10
9.	vagus pressor (chloralose)	21	−0.79	< 0.1	−0.44	23.73	12.56
10.	carotid pressor (chloralose-pentobarbital)	20	+0.65	< 1.0	+0.92	9.42	20.49
11.	median pressor (chloralose-pentobarbital)	28	−0.81	< 0.1	−0.28	11.82	16.67
12.	median pressor above 70 mm Hg "C" values	11	−0.96	< 0.1	−0.62	52.55	9.08
13.	median pressor under 70 mm Hg "C" values	18	+0.02	> 90.0	–	–	–
14.	median pressor (pentobarbital)	24	−0.58	< 1.0	−0.30	9.24	7.79
15.	median pressor (chloralose-pentobarbital)	21	−0.50	< 2.0	−0.20	7.32	11.22
16.	vagus depressor after splitting the medulla (chloralose-pentobarbital)	7	−0.92	< 1.0	−0.91	5.57	6.99
17.	tibialis depressor after splitting the medulla (chloralose-pentobarbital)	21	−0.91	< 0.1	−0.68	6.92	8.48
18.	carotid pressor after splitting the medulla (chloralose-pentobarbital)	9	+0.72	< 5.0	+0.74	0.18	5.02

The numbers correspond to those in the Figures

n: registrated triads; r: correlation coefficient; p_r%: probability index of correlation coefficient; b: regression coefficient; a: constant term; 95% conf: confidence band.

Table II

Comparison of regression lines

No. of line	P_b	b′	a′	$p_{a'}$
4–5	> 30	−0.52 −0.52	6.96 9.87	< 1.0
5–7	< 0.1	—	—	—
5–8	< 0.1	—	—	—
5–6	> 60	−0.52 −0.52	9.93 20.56	< 0.1
1–2	> 60	−0.57 −0.57	11.43 10.59	> 60
1–3	< 0.1	—	—	—
1–9	> 20	−0.52 −0.52	9.68 27.04	< 0.1
11–14	> 80	−0.28 −0.28	11.88 8.64	> 10
11–15	> 30	−0.27 −0.27	9.96 11.20	> 60
14–15	> 40	−0.22 −0.22	7.05 8.30	> 10
5–17	< 2	—	—	—
4–17	> 20	−0.63 −0.63	10.44 5.91	< 1.0
1–16	> 20	−0.57 −0.57	11.53 1.24	< 1.0
2–16	> 20	−0.69 −0.69	14.06 2.81	< 0.1
3–7	> 10	−0.88 −0.88	29.53 23.92	< 0.1
10–18	> 30	+0.82 +0.82	− 5.49 − 0.39	< 2.0

Important comparisons. The numbers of the lines correspond to those in Table I and the Figures p_b: probability index of the deviation of the slopes; b′: common slope of the parallel lines; a′: new intercepts; $p_{a'}$: probability index of the deviation of the parallel lines.

b) Vagal nerve stimulation

The relations were in full aggreement with those presented above. In chloralose-pentobarbital anaesthesia (Fig. 4, line Nos 1 and 2) the angle of inclination of the regression line is small, it intersects the abscissa at about 20 mm Hg. The data of line No. 1 were obtained from 3 animals; in spite of this the two lines practically coincide. In pure pentobarbital anaesthesia (Line No. 3) the regression coefficient is significantly higher and the shift of the line to the right is expressed.

Thus, there is no fundamental difference between the spatial interactions of depressor reflexes whether they are of somatic or visceral origin. The regression lines in both cases lie in a similar way in the coordinate system. Comparison of lines for the chloralosed animals (Lines Nos 4 and 5 and Nos 1 and 2) show that they are parallel, the mean for groups falls on the same line, so they can be replaced by a general regression line ($\Delta C = -0.54 + 9.67$) The data obtained in pentobarbital anaesthesia show greater differences, so they cannot be summarized by a general regression line. The increase of the regression coefficients and the shift of the lines to the right is, however, unambiguous with both types of depressor reflex.

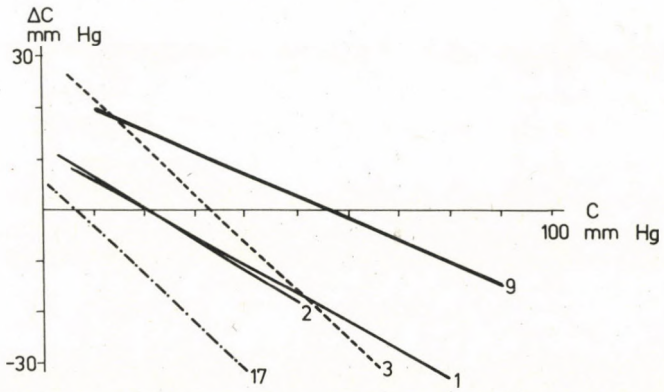

Fig. 4. Summation of spatial interactions evoked by stimulation of central vagal stumps. Interactions of depressor reflex3s in chloralose-pentobarbital (lines Nos 1 and 2) and in pure pentobarbital (No. 3) anaesthesia, and after splitting the medulla (No. 16). Line No. 9 represents interactions of vagal pressor reflexes under deep chloralose anaesthesia. For details, see text

B) *Interactions of pressor reflexes*

a) Vago-pressor reflexes

It has been shown earlier (*Pórszász* and *Such* 1958, *Such* and *Pórszász* 1960) that deep chloralose anaesthesia results in a complete blocking of the depressor mechanisms and under such circumstances vagal nerve stimulation elicits a pressor

response. Bilateral interactions of vagopressor reflexes show a negative correlation (Table I, No. 9). The angle of inclination of the regression line is somewhat smaller than those of the vagal depressor reflexes (Fig 4, Lines No. 9), but the regression line is considerably shifted to the right. This accounts for the greater facilitatory tendency of these pressor reflexes.

b) Interactions of pressor reflexes evoked by compression of the common carotid arteries

Fig. 5 shows the distribution of the interactions registrated with various separately evoked responses. The data were obtained from five cats. As it is seen, in this case the correlation is positive (Table I, No. 10). On increasing the size of the separately evoked responses the degree of the facilitation increases. Occlusion was not found in any case. Considering that the carotid sinus reflex is an inverse process, the positive correlation is obvious. The details of the phenomenon will be considered in the Discussion.

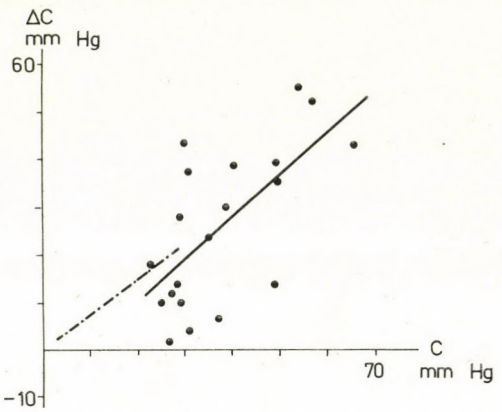

Fig. 5. Interactions of carotid sinus hypertensive reflexes in cats with intact (smooth line) and with split medulla (broken line). Data of regression lines see in Tables under Nos 10 and 18

c) Interaction of pressor reflexes of somatic origin

Pressor reflexes were evoked by stimulation of the median nerves with 20—50 cps, and 1—2 msec pulse duration for 10 sec. In the majority of cases the dots are distributed between +10 and −10 "ΔC" values near the abscissa. Consequently, the angles of inclination of the regression lines are rather small (Fig. 6 lines Nos 11, 14, 15). There were no significant differences between the lines. Their group means are on the same line, so the three lines can be replaced by a general regression line ($\Delta C = -0.25 + 9.1$).

In one of the animals the interaction of the somatic reflexes were followed up to 130 mm Hg "C" values (No. 11). There was no correlation below 70 mm Hg (No. 13), but above that value the regression coefficient became significant showing that in this area the same relations dominate that were observed with depressor reflexes (Fig. 6 line No. 12).

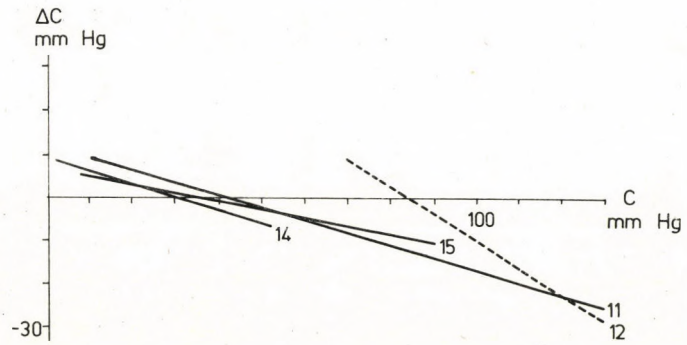

Fig. 6. Interactions of pressor reflexes evoked by median nerve stimulation. Frequency of stimulation: 50 cps, impulse duration: 2 msec. Lines Nos 11 and 15 show distribution of data in chloralose-pentorbarbital, No. 14 in pentobarbital, anaesthesia. There is no significant difference between the three lines. Line No. 12 shows the interactions at "C" values above 70 mm Hg. Data derived from population No. 11. Further details see in text

C) *Effect of division of the lower brain stem on bilateral interactions of blood pressure reflexes*

Analysing the role played by the medullary vasomotor centre in interactions, experiments were done on animals with divided brain stem. Sagittal splitting was carried out in the midline from the upper part of the pons to the C_2 segment of the spinal cord. The experimental data showed that the forms of the interactions persist, only the regression lines are shifted to the left as compared to those of animals with intact medulla (see lines —.—.—. in Figs 3, 4 and 5). According to the morphological examination, the injury did not extend to more than ± 0.5 mm from the midline.

D) *Effect of repeated stimulations on the reproducibility of the blood pressure reflexes*

In the experiments blood pressure returned to the initial value after about one minute after the stimulations. Therefore the reflexes were elicited at one minute intervals, and comparison of the responses was done at identical blood pressure levels. This is important, because the magnitude of the reflex responses depends

on the prestimulation blood pressure level. In the case of pressor reflexes (median nerve stimulation) there is a negative correlation between the blood pressure elevation and the initial value. Between 95 and 160 mm Hg basal blood pressure the pressor reflexes evoked by identical stimulus parameters were scattered along the $Y = -0.43 \times +94.8$ regression line (n:21). In the case of depressor reactions the correlation is positive. The magnitude of the depressor reflexes (vagal nerve stimulation) can be described by the equation $Y = 0.83 \times -53.87$, as a function of the prestimulation blood pressure (n:29). The reproducibility at identical blood pressures, analysing 62 depressor responses elicited by tibial nerve stimulation, was within the $5 \pm 10\%$ range.

Discussion

The spatial summation of blood pressure reflexes was studied by *Martin* and *Stiles* in 1916 but except some few attempts, systematic investigations concerning the general relations of the interactions have not been made. The mostly sporadic and accidental observations were restricted to the qualitative description of some interactions. Still, analysis of the reflex effects of the multiple afferentation has its practical importance. In the intact organism several afferent channels are functioning and the result of their interactions is the prevailing actual response of the cardiovascular system.

The present results describe the general relations of the spatial interactions in connection with the depressor and pressor reflexes of visceral and somatic origin. According to our observations, all reflex types except the carotid sinus hypertensive reflex, show a negative correlation. The interactions manifest themselves with facilitation, occlusion or overlapping, according to the degree of divergence and convergence determined by the intensity of the afferentation, and by the functional state of the preparation. Consequently, the regression lines intersect the facilitatory and occlusive area alike. In connection with the regression lines the question may arise whether they unambiguously characterize the given reflex-interactions. Under similar circumstances the calculated lines are consistent with each other and data obtained in different animals are also comparable. On the basis of our experimental data, characteristic interaction-types can be distinguished. The interactions of depressor reflexes in chloralose—pentobarbital anaesthesia were inclined to occlusion, but in pure pentobarbital anaesthesia the probability of facilitation increased considerably. This latter results should be attributed to the better functional state of the animals rather than to the specific effect of pentobarbital. In respect of pressor reflexes, regardless of the anaesthetics used, addition and facilitation dominate against the occlusion phenomena. Our results explain why in the case of baroreceptor afferentation only occlusion (*Ninomiya* and *Irisawa* 1969; *Angell James* and *Daly* 1970), and in the case of sympathetic summations only facilitation (*Gellhorn* 1959) have been described.

The interactions of carotid sinus hypertensive reflexes have a peculiar feature. As it was shown earlier (*Such* 1970; *Such* et al. 1972) their bilateral interactions are facilitatory in nature. This was supported by *Worthen* and *Peiss* (1972) in contrast to *Sagawa* and *Watanabe* (1965) according to whom the bilateral carotid signals show a simple algebraic summation in the output. In the present experiments occlusion has never been found and the interactions of the carotid hypertensive reflexes showed a positive correlation. The phenomenon can be explained by the inverse character of the reflexes. The strong central overlapping of the tonically active baroreceptor afferents manifest themselves with the occlusive tendency of depressor reflexes. The separate elimination of individual afferent channels decrease only slightly the inhibitory zone, while compression of both carotids practically suspends the inhibition. In the experiments the animals were vagotomized bilaterally, so afferentations from the aortic arch were ineffective.

On the basis of the analysis of the reflex summations following splitting of the medulla and the pons we have to conclude that morphological connexions between the two parts of the lower brain stem have not an important role in the bilateral interactions. The shifting to the left of the regression lines can be explained by the deterioration of the functional state followed the injury.

It might be argued that the preceding stimulation may alter the magnitude of the next reflex. According to *Lukoshkova* and *Pavlik* (1972) the somatic conditioning stimulation facilitates the pressor reflexes while *Niechaj* and *Dyba* (1974) have shown a biphasic — inhibitory and facilitatory — effect lasting about 200 msec after conditioning stimulations of the depressor nerve. Under our experimental conditions the serial stimulations had no influence on each other. The changes in magnitude of the reflex reactions at the same stimulus parameters were produced mainly by blood pressure oscillations. The magnitude of the reflex reactions follows the law of the initial value (*Wilder* 1967). In the case of depressor reactions the correlation is positive, whereas in the case of pressor reflexes it is negative between the basal blood-pressure and the magnitude of the reflex response. Our results agree with the statement of *Kristt* (1975) that in the rat the magnitude of depressor responses evoked by hypothalamic stimulation is directly proportional with the prestimulation blood pressure, while between the pressor response and the basal blood pressure there is an inverse relation.

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CHEMICAL NATURE OF FUNCTIONAL CHOLINORECEPTOR GROUPS OF *LYMNAEA STAGNALIS* NEURONS

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1. The effect of pH and the influence of some alkylating agents on the properties of the cholinoreceptive membrane of the mollusc *Lymnaea stagnalis* neurons have been studied using the microelectrode voltage clamp technique.

2. Lowering below 7.5 of the pH of the bathing solution had to decrease the neuronal responses to ACh. A twofold decrease in cholinoreceptive membrane conductivity was found at the pH 6.7 ± 0.1 ($n = 10$). Raising the pH to 10.6 did not influence the response to ACh.

3. The pH effect is not associated with the influence on the properties of ionic channels but appears to be due to reduction of a functional group at the ChR active site by proton.

4. No highly reactive SH-groups were found at the ChR active site, but some functionally important carboxyl groups have been discovered.

5. The effect of pH is probably connected with reduction of —COO— or imidazol group with a pK_a of about 6.7.

Investigation of the nature of cholinoreceptor (ChR) protein functional groups is important for the understanding of the function of ChR and for choosing the most effective means to influence synaptic transmission. Besides, information on the chemical topography of an active site and its environment may help to classify the ChR's. In the present study we have used two methods to solve the problem: pH variation and chemical modification of proteins *in vivo*.

The properties of some cholinergic agents depend on the pH of the solution (*Michelson* and *Zeimal* 1970; *Bregestovski* et al. 1975). To interpret the results obtained with such compounds one must know the effect of pH on the behaviour of the cholinoreceptive membrane. Moreover, variations of the pH of the solution may help to make a decision about ionization constants (pK_a) of some functional groups participating in the function of ChR. This approach is successfully used for identification of amino acid side chains at enzyme active sites (*Bresler* 1973) and in investigation of the properties of chemoreceptive and electroexcitable membranes (*Hille* 1973; *Machne* et al. 1973).

Chemical modification *in vivo* gives information about the significance of some protein groups in ChR structure. The existence of an important S—S bond near the nicotinic ChR active site in *Electrophorus electricus* electroplax (*Karlin* and *Bartels*, 1966; *Karlin* 1969) and in the frog sartorius muscle end plate (*Lindstrom* et al. 1973; *Ben-Haim* et al. 1973) was shown in experiments using group-specific

reagents with affinity to ChR. A carboxylate anion appears to play an essential role in ChR function (*Chang* et al. 1970; *Edwards* et al. 1970; *Beddoe* et al. 1971); these data have, however, been obtained either with reagents with no affinity to ChR or by methods which do not allow to confirm the specificity of the reagent's effect on ChR.

We have studied the effect of pH and of some modifying agents with affinity to ChR using the method of microelectrode voltage clamp. This method permits to record the changes in membrane conductivity and current when acetylcholine (ACh) is applied.

Methods

The experiments were carried out on completely isolated giant neurons of the mollusc *Lymnaea stagnalis*. The neurons were prepared by enzyme treatment of the ganglionic ring (0.5—1 hr) and then by mechanical isolation with tungsten needles and fine glass pipettes (*Kostenko*, 1972; *Kostenko* et al., 1974). A mixture of trypsin (1.3 mg/ml) and hyaluronidase (2 mg/ml), or pronase (3.5 mg/ml) alone was used.

Giant neurons 130—200 μm in diameter from three ganglia (visceral, right and left parietal) were selected and placed into the experimental chamber of about 0.1 ml volume. Complete replacement of the solution in the chamber was achieved in 2—3 sec. Composition of the standard bathing solution was (mM): NaCl, 50; KCl, 1.6; $CaCl_2$, 4.0; $MgCl_2$, 8.0; Tris, 0.25 g/l; HCl 0.5 M to pH 7.5. The nerve cell was provided with 10—20 MΩ glass microelectrodes filled with 2.5 M KCl. An operational amplifier was used for clamping of the membrane potential (Fig. 1). Currents through the membrane were registered in the circuit of the reference electrode. A micropipette filled with 2 M acetylcholine chloride was placed at the surface on the cell body. As a rule the whole surface of the neurone is sensitive to ACh, but maximum responses are usually recorded in the axon hillock area.

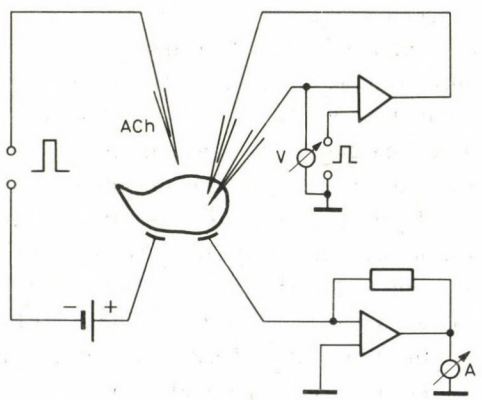


Fig. 1. Experimental block-scheme

The membrane potential was clamped near the resting potential level and the steady-state current—voltage relation of the electroexcitable membrane was recorded. Then the current—voltage relation of the cholinoreceptive membrane was determined by ACh microapplication: the peak current amplitudes through the activated membrane were measured at different levels of the membrane potential. The reversal potential (E_r) of responses to ACh was estimated by extrapolation of a linear part of the current—voltage relation to the potential axis. When the drugs were added to the bathing solution the

change in membrane conductivity was measured by the application of rectangular de- and hyperpolarizing voltage pulses (±10 mV) from the level of holding potential. In 10—15 min after the drug had been washed out, the standard solution was replaced by a solution of a different pH and a new series of measurements was performed.

The external pH was changed in the range 5.8—10.6. Perfusion with acid solutions lasted 7—8 min, since a more prolonged influence leads to irreversible changes in the electrical properties of native membranes. All the experiments were performed at room temperature (18—20 °C).

Results and discussion

The influence of external pH on the properties of electroexcitable and cholinoreceptive membranes

The current—voltage relationships of the electroexcitable membrane, determined at different pH_s are shown in Fig. 2. It is clearly seen that lowering of the external pH reduced the neurone resting potential, while raising the pH increased it. The average shift of the resting potential was 14±0.5 mV/pH unit. In the pH range studied, the changes of passive membrane conductance amounted to less than 20%. The shift of the resting potential was probably due to changes of the membrane surface charge (*Mozhaeva* and *Naumov*, 1972) or to an alteration of chloride permeability.

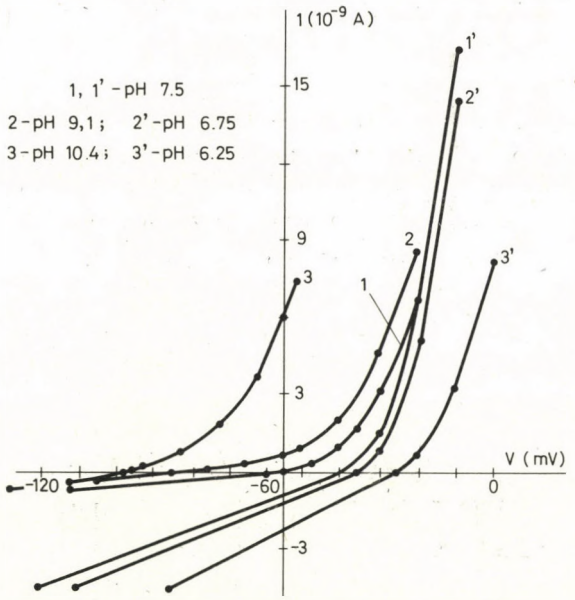


Fig. 2. Steady-state current—voltage relations of an isolated neuronal electroexcitable membrane at different pH values

Investigating the cholinoreceptive membrane properties, it was found that lowering of the pH of the bathing solution below 7.5 leads to a decrease in neuronal sensitivity to ACh (Fig. 3a). At pH 5.8—6.0 responses to ACh disappeared almost completely. The changes of pH from neutral to 10.6 did not influence the sensitivity of the cholinoreceptive membrane (Fig. 3b);

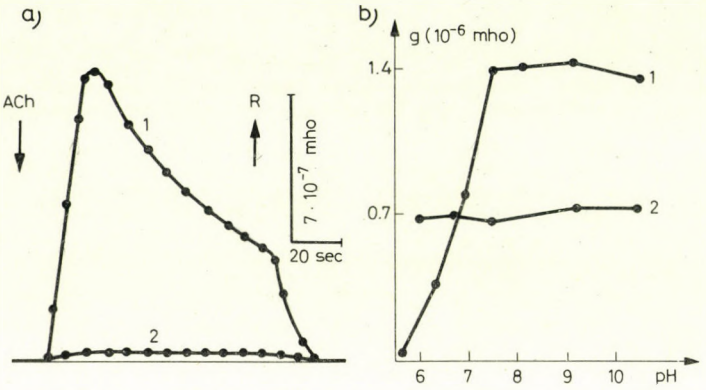


Fig. 3. Responses of an isolated neurone to ACh and KCl at different pH values of the bathing solution. a) Conductivity of cholinoreceptive membrane, activated by $1 \cdot 10^{-6}$M ACh at pH 7.5 (1) and 5.8 (2), as calculated from changes in current amplitude in response to standard membrane potential shifts (± 10 mv). b) pH-dependence of membrane conductivity. Bath with $1 \cdot 10^{-6}$M ACh (1) and $1 \cdot 10^{-2}$M KCl (2) added

What are the main causes of the phenomenon?

The increase in proton concentration affects the degree of ionization of

— some functionally important groups of the ChR active site (Fig. 4);

— polar groups in ionic channels of the cholinoreceptive membrane (Fig. 4).

To clarify which of these two suppositions is correct, the following experiments were performed.

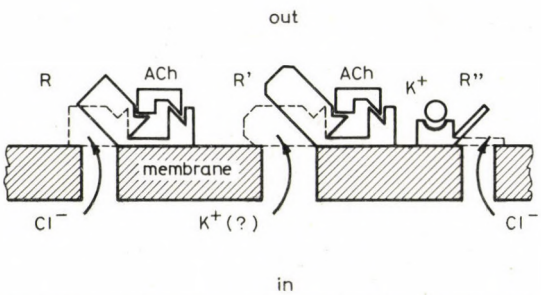


Fig. 4. Schematic representation of the mollusc neuronal membrane. R′ and R″ — cholinoreceptors with associated chloride or potassium channel. R‴ — a K^+ "receptor" of membrane, associated with a chloride channel

The site of the pH effect: cholinoreceptor or ionic channel?

It has recently been shown by *Kislov* and *Kazachenko* (1974), that in the main part of giant neurons of the right and left parietal ganglia of *Lymnaea stagnalis*, ACh increases the membrane permeability to chloride ions only. These authors also found that an increase in membrane chloride conductivity occurs at raising the external potassium ion concentration (*Kislov* and *Kazachenko* 1975), showing that the mollusc neurons possess a system of chloride channels activated by K^+. Reversal potentials at ACh application and at elevating the K^+ concentration in the bathing solution have the same value. It may therefore be suggested that chloride channels activated by ACh or K^+ are of a similar structure (*Amin* 1974).

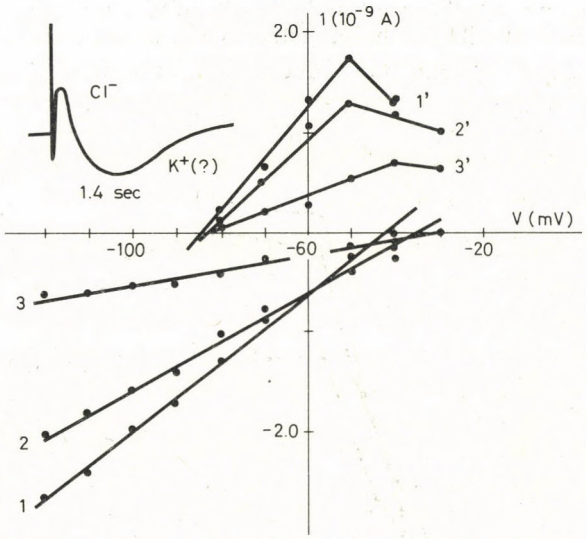


Fig. 5. Current—voltage relations of fast (1, 2, 3) and slow (1′, 2′, 3′) components of responses to ACh microapplication. 1,1′ — pH 7.5; 2,2′ — pH 6.65; 3,3′ — pH 6.25

If the function of chloride channels were dependent on the pH of the solution one should expect a decrease of membrane responses to K^+ on lowering the pH. The responses of the neurone to elevated K^+ concentrations appear, however, to be practically constant at any pH value (Fig. 3 b, curve 2). Thus, we may suggest that the pH does not influence chloride ionic channel functional groups.

The chemical heterogeneity of some neurons of *Lymnaea stagnalis* allowed to test this suggestion in another set of experiments. Some giant neurons of the visceral ganglion are known to generate biphasic responses to ACh (Fig. 5). A fast depolarizing component arises due to the increase in chloride permeability.

The ionic mechanism of the slow hyperpolarizing component has not been studied, but judging from the value of E_r (—55 ÷ —80 mV), potassium channels are activated ($E_K = -60 \div -80$ mV). This seems very likely as some *Aplysia* neurones have been shown to respond to ACh with two-component potential changes and the slow hyperpolarizing component is a potassium one (*Kehoe* 1972). Thus, it was possible to examine the pH effect on the same neurone with two types of ionic channels (cation and anion selective) activated by ACh. The results of such an experiment are shown in Fig. 5. It is seen that on lowering the pH both components decreased in parallel. Responses were completely eliminated at the pH 6.0. The values of cholinoreceptive membrane conductivity of both response phases halved at the same pH, 6.8 (Fig. 6, curves 1 and 2). On adding ACh to the bathing solution, the same results were obtained (Fig. 6, curve 3). The mean dissociation constant (pK_a) of the functionally important ionizing group was in all the experiments equal to pH 6.7 ± 0.1 ($n = 10$). Thus, the pH effect is not a consequence of some change in the ionic transport system, but is due to reduction of some group at the ChR active site by proton.

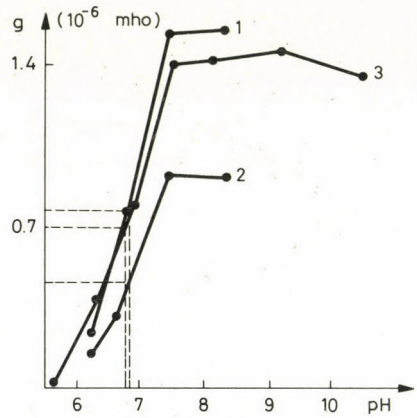
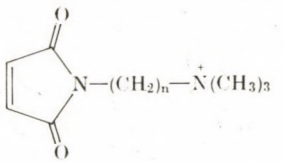


Fig. 6. pH-dependence of cholinoreceptive membrane conductivity at microapplication (1, 2) or addition to bath (3) of ACh. pH values corresponding to a 50% decrease of responses are shown by dashed line

The curve of ACh action dependence on pH has a monotonous form. It is reasonable to suppose that one type of chemical group has a pK_a of about 6.7. As the apparent pK_a of an ionizing group depends on its surroundings in a protein molecule, it is impossible to determine the type of chemical group from the pK_a value alone. It is necessary to take into consideration the imidazole of histidine (pK_a 6.0), COOH (pK_a 2—4.5) and SH-groups (pK_a 8—10). To distinguish between these groups we used a number of modifying group-specific reagents with affinity to ChR.

Do SH-groups take part in the ChR active site formation?

N-ethylmaleimide and other maleimide derivatives are known to be highly specific reagents towards SH-groups (*Cohen* 1968). We used several N-alkylderivatives of maleimide containing a quaternary nitrogen atom with the general formula.



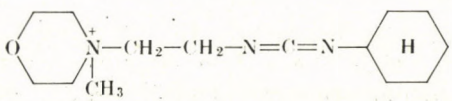
n = 2, 3, 4, 5, 6

These compounds seem to have an affinity to ChR due to the presence of a quaternary nitrogen atom. All the compounds appeared to be capable of activating the cholinoreceptive membrane. The derivative with n=2 was 25—300 times and the others were 1,000—1,500 times less effective than ACh. None of the reagents blocked cholinoreceptive membrane conductivity irreversibly after 10—15 min treatment at pH 7.5.

These data suggest that highly reactive SH-groups are absent in the vicinity of the ChR active site. S—S bonds play an essential role in the function of ChR. After their reduction by dithiothreitol, all the derivatives of maleimide blocked the response to ACh irreversibly.

Identification of COOH groups in the ChR active site

We studied the effect of one of carbodiimides which contains a quaternary nitrogen atom on the mollusc neurone cholinoreceptive membrane.



Water-soluble carbodiimides are known to react mainly with carboxyl (and phosphate) groups (*Khorana* 1953; *Riehm* and *Scheraga* 1966; *Hoare* and *Koshland* 1967). The specificity of carbodiimides towards COOH-groups increases at low pH. As a result of COOH modification an extremely reactive derivative of O-acyl isourea is formed. Further transformation of this derivative depends on the availability of neighbouring nucleophilic groups (*Riehm* and *Scheraga* 1966; *Hoare* and *Koshland* 1967). If there are no such groups in the vicinity, hydrolysis of O-acyl isourea with regeneration of —COOH usually takes place. The addition of a nucleophile to the incubation medium stabilizes the irreversible modification of carboxyl groups (*Hoare* and *Koshland* 1967). This permits to distinguish the modification of —COOH

from other protein groups, as the presence of nucleophiles in the raction mixture does not influence the alkylation of other groups by carbodiimides.

The carbodiimide studied (CDI) appeared to induce at $4 \cdot 10^{-3}$ M an almost complete elimination of neurone responses to ACh (Fig. 7). After CDI had been washed out the sensitivity to ACh was rapidly restored. A 6—8 min treatment of the neurone with a mixture of CDI and glycine methyl ester ($4 \cdot 10^{-3}$ M) caused a 1.5—2 decrease in the response to ACh (Fig. 8). The degree of inhibition did not change during 1—1.5 hours of washing. Glycine methyl ester itself did not induce any change in the neuronal sensitivity to ACh. Lowering the pH of the bathing solution below 7.5 enhanced the action of the reactive mixture of CDI and glycine methyl ester (Fig. 8). The pH-dependence of the effect and the irreversible character of the block on simultaneous treatment with CDI and the ester suggest the modification of carboxyl but not of any other protein groups.

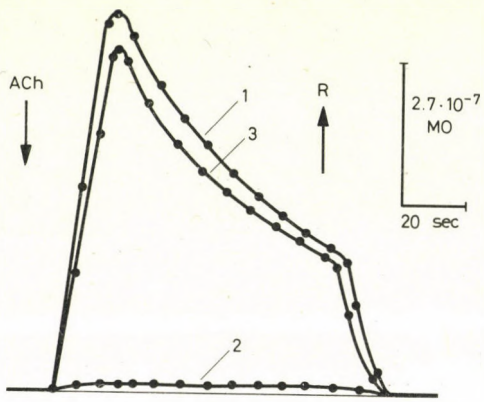


Fig. 7. CDI influence on responses of an isolated neurone to ACh $1 \cdot 10^{-6}$M. 1) control; 2) ACh effect in the presence of CDI $4 \cdot 10^{-3}$M; 3) ACh effect 10 min after CDI was washed out

Neither CDI nor its mixture with glycine methyl ester induced any alterations in the resting membrane potential or in current—voltage relation of the neuronal electroexcitable membrane. ACh $8 \cdot 10^{-6}$ M significantly protected the cholinoreceptive membrane from the action of the reagents (Fig. 9). Thus, the CDI effect is apparently directed to a carboxyl group localized in or near the ChR active site. This group is very important for the activation process.

The pH-dependence of ACh action on *Lymnaea* neurones is probably associated with reduction of the same carboxyl group by external proton. Still, participation of some amino groups in the pH effect cannot be excluded.

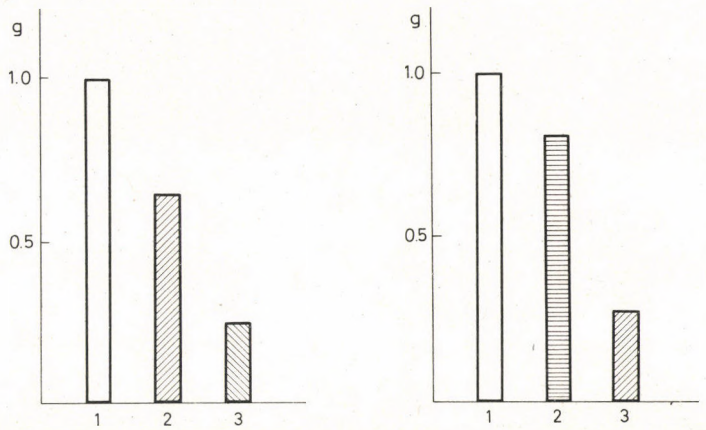


Fig. 8. Irreversible block induced by mixture of CDI and glycine methyl ester at different pH. 1. Increase in conductivity under the effect of $1 \cdot 10^{-6}$M ACh at pH 7.5 in control; 2. Response after 10 min treatment of the neurone with a mixture of $4 \cdot 10^{-3}$M CDI and glycine methyl ester ($4 \cdot 10^{-3}$M), pH 7.5; 3. The same as in 2, but the pH of the reaction mixture was 6.0. Response to ACh is recorded at pH 7.5. The values for conductivity are averaged, n = 8–10 neurones

Fig. 9. Effect of ACh as a protector against the mixture of CDI and glycine methyl ester. 1. Neuronal response to $1 \cdot 10^{-6}$M ACh in control. 2. Response to $1 \cdot 10^{-6}$M ACh after treatment with mixture of the same reagents in the presence of $8 \cdot 10^{-6}$M ACh. 3. Response to $1 \cdot 10^{-6}$ ACh after treatment with a mixture of CDI and glycine methyl ester.

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Acta Physiologica Academiae Scientiarum Hungaricae, Tamus 49 (3—4), 231—235 (1977)

Pharmacologia

ANTIARRHYTHMIC EFFECT OF DISOPYRAMIDE INJECTED INTO THE SINUS NODAL ARTERY

By

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(Received February 13, 1975)

In anaesthetized dogs, the antiarrhythmic drug disopyramide was injected directly into the sinus nodal artery in doses that ranged from 10 to 200 μg/ml. Lower concentrations of the drug induced a non-significant increase in sinus rate of atropinic origin and were incapable of blocking atrial fibrillation induced by 10 μg/ml of acetylcholine injected into the sinus nodal artery. Only concentrations higher than 100 μg/ml of the drug, which caused bradycardia, proved to be effective in preventing the arrhythmia. It is concluded that the antidysrhythmic effect of disopyramide in sinus arrhythmia is a consequence of a direct negative chronotropic effect opposite to its known atropinic effect.

Disopyramide, introduced as an antiarrhythmic agent by *Mokler* and *Van Arman* (1962), has been assayed in different experimental arrhythmias and found to be two times more potent and less toxic than quinidine (*Katz* et al 1963; *Desruelles* et al. 1967; *Gardenas* et al. 1971). Disopyramide has an atropinic and a direct effect (*Sekiya* and *Vaughan-Williams* 1963; *Lisin* and *Carlier* 1973; *Deshpande* and *Mokler* 1967); the latter effect overlaps anticholinergic actions and reveals itself with an opposite, dose-dependent action in A–V conduction (*Pérez-Medina* et al. 1975).

The purpose of present work was to study the chronotropic effect of disopyramide injected directly into the sinus node artery and to determine which of the two mentioned effects of the drug was responsible for abolishing a sinus-provoked rhythm disturbance.

Methods

Eight mongrel dogs weighing 11 to 18 kg were anaesthetized with 30 mg/kg sodium pentobarbital. After tracheal intubation, ventilation was maintained with a Harvard pump. The chest was opened through the right fourth intercostal space and the heart exposed and suspended in a pericardial cradle. After injecting 3 mg/kg heparin I. V. the sinus nodal artery was dissected and cannulated at its origin from the right coronary artery, following the technique described by *James* and *Nadeau* (1962). Acetylcholine (10 μg/ml) was injected into the sinus nodal artery three times at the beginning, with an interval of 5 minutes between injections. With this dose atrial fibrillation ensued and at the same time served to ascertain sinus tissue perfusion. Once this was accomplished, 10, 50, 100 and 200 μg/ml of disopyramide

were successively injected into the artery and three minutes after each dose, acetylcholine (10 μg/ml) was applied. This sequential design served to determine the required dose capable of inhibiting the arrhythmogenic response induced by acetylcholine. Both drugs were dissolved in Ringer solution pH 7.4 at 37 °C. One ml of the solution was injected each time into the sinus nodal artery via a three-way stopcock. Perfusion was maintained between injections with blood bypassed from a femoral artery. Arterial pressure in the other femoral artery was monitored continuously and maintained over 90 mm Hg by intravenous infusion of 5% dextrose solution. An electrogram and a tachogram from successive R waves of the ECG, were monitored in a 4 channel oscilloscope and recorded by a RM-45 Nihon-Kohden polygraph.

Results

In 36 trials an injection of 10 μg/ml of acetylcholine provoked a high rate atrial fibrillation with a mean duration of 74 ± 19 seconds. In 28 trials the arrhythmia was preceded by sinus arrest and initiated by an ectopic impulse (Fig. 1). Injection of 10 and 50 μg/ml disopyramide into the sinus nodal artery, increased the sinus rate by 14 and 17%, respectively ($p < 0.05$), but the drug was incapable of blocking the atrial fibrillation induced by acetylcholine. In 5 experiments, 100 μg/ml caused a non-significant rise, while in three cases this dose depressed the sinus rate. In the first group the arrhythmia was not abolished, but in the other it was prevented by disopyramide. With the two higher doses, sinus rate decreased by 12 and 22% and in 8 experiments each dose blocked the acetylcholine fibrillation (Fig. 2).

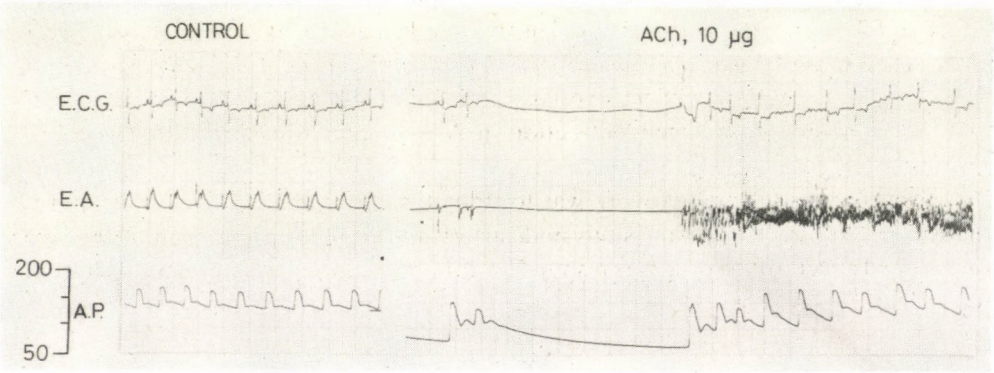

Fig. 1. Effect of 10 μg of acetylcholine injected directly into the sinus node artery. The upper tracing is a standard lead II electrocardiogram, the middle one is an atrial electrogram, and the lower tracing corresponds to arterial pressure. Paper speed, 25 mm/sec .

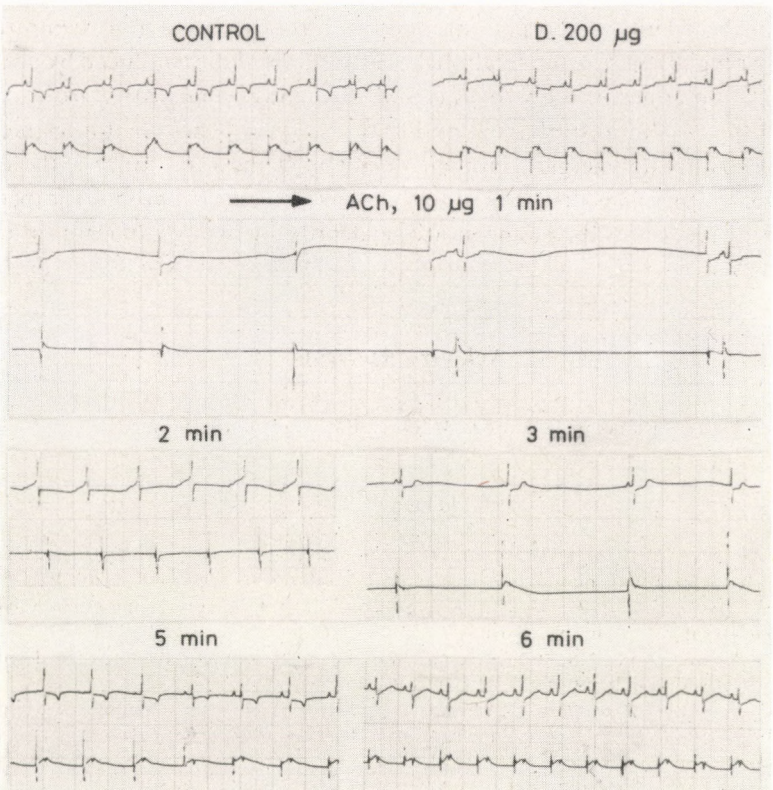

Fig. 2. Antifibrillatory effect of 200 µg disopyramide injected directly into the sinus nodal artery before the injection of 10 µg acetylcholine. The upper tracing is a lead II electrocardiogram. The lower tracing is an atrial electrogram. Acetylcholine provokes impulse arrest and a probable A–V node escape rhythm, but fibrillation is blocked

Discussion

Based on the finding that disopyramide in therapeutic doses shortens escape time and produces a slight increase in sinus rate, the drug has been recommended by *Befeler* et al. (1973) for the treatment of the sick sinus syndrome. Although atrial fibrillation provoked by acetylcholine injection into the sinus node artery has no resemblance to the sick sinus syndrome, it may serve a double purpose: to evaluate the effect of an antiarrhythmic drug on an experimental atrial rhythm disturbance, caused not only by a vagal effect but also by an adrenergic influence (*Hashimoto* et al. 1968), and to study the chronotropic action of the drug on sinus tissue.

From the results of the present study it is concluded that concentrations of 100 μg/ml or higher are needed to abolish the atrial fibrillation induced by acetylcholine. Only those concentrations of the drug that lower sinus rate, are capable of preventing the rhythm disturbance; and only the lower doses elicit a characteristic tachycardia of anticholynergic origin, but do not block the appearance of the arrhythmia. Based on these results, it is inferred that the antiarrhythmic properties of disopyramide rest on its direct chronotropic depressing action, and not on its atropinic effect.

Acknowledgements

The technical assistance of Miss *Mirta Martinez*, BSc, is greatly appreciated.

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EFFECT OF PROSTAGLANDINS E_1 AND $F_{2\alpha}$ ON NEUROMUSCULAR TRANSMISSION IN THE FROG SARTORIUS MUSCLE

By

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(Received February 26, 1976)

End plate potentials (e.p.p.s.) and miniature end plate potentials (m.e.p.p.s.) were recorded intracellularly at the neuromuscular junction of the frog sartorius muscle. Addition of as little as 8.5×10^{-8}M PGE_1 reduced the mean m.e.p.p. frequency. The mean amplitude of m.e.p.p.s was not changed, the mean amplitude of the e.p.p.s and the quantum content of the transmitter released by a nerve impulse was slightly reduced. A decrease in mean m.e.p.p. frequency was also seen in response to the administration of 8.5×10^{-8}M $PGF_{2\alpha}$. The mean amplitude of e.p.p.s and m.e.p.p.s and the quantum content remained unchanged. The possible presynaptic mode of action of PGs in the preparation is discussed.

A release of prostaglandins (PGs) has been shown to take place in the rabbit heart (*Wennmalm* 1971), in the rabbit ileum (*Ferreira* et al. 1972) and guinea-pig ileum (*Botting* and *Salzmann* 1974) at rest as well as after stimulation. There is evidence to indicate that PGs modify both adrenergic (*Hedqvist* 1970a; *Baum* and *Shropshire* 1971; *Illés* et al. 1973) and cholinergic transmission (*Wennmalm* and *Hedqvist* 1971; *Baum* and *Shropshire* 1971; *Hadházy* et al. 1973; *Illés* et al. 1974) in these organs.

PG release in response to nerve or chemical stimulation has been shown in the rat phrenic nerve-diaphragm preparation (*Ramwell* et al. 1965; *Laity* 1969) and the question arose whether PGs had some role in the modulation of cholinergic transmission in skeletal muscle. Therefore, the effect of PGs (PGE_1 and $PGF_{2\alpha}$) has been studied on the spontaneous and stimulation evoked release of the transmitter at the neuromuscular junction of the frog sartorius muscle.

Methods

The sartorius nerve-muscle preparation from winter Rana temporaria frogs was used in the experiments. The muscle was stretched on the bottom of a Perspex chamber and bathed in 3 ml of saline solution of the following composition (mM): NaCl, 114; KCl, 2.5; $CaCl_2$, 0.5; $MgCl_2$, 5; $NaHCO_3$, 2.2. The nerve was placed on stimulating electrodes in a side chamber in moist air. The temperature of the bath was 22—25 °C.

Stimulus pulses of 50 μsec duration at a frequency of 0.3 Hz using supramaximal voltage were delivered through an isolation transformer to the muscle nerve. End plate potentials (e.p.p.s) and miniature end plate potentials (m.e.p.p.s) were recorded intracellularly with glass microelectrodes filled with 2.5 mk KCl and having resistances of 5—8 MΩ. Electrodes were connected to the input of a high impedance preamplifier, the output of which was fed into an oscilloscope.

0.5 mM Ca^{2+} and 5 mM Mg^{2+} decreased the amplitudes of the focally recorded e.p.p.s to about 3 mV, and in this way the possibility of a non-linear summation of unit potentials was reduced (*Martin* 1955). Quantum contents *(m)* were estimated as the ratio of the average amplitude of the evoked e.p.p.s to the average amplitude of the m.e.p.p.s recorded in each experiment. 20 e.p.p. and at least 100 m.e.p.p. amplitudes were used for the statistical estimates of *m*.

PGE_1 and $PGF_{2\alpha}$ (Chinoin Pharmaceutical Works, Budapest) were diluted from 0.5—1 mg/ml stock solutions in 99.7% ethanol and 3 mM Na-acetate respectively. The stock solutions were dissolved further in saline solution and added to the organ bath in a volume of 0.09 ml. Drug contact time was 10 min for both PGE_1 and $PGF_{2\alpha}$.

Results

The effect of PGE_1 on neuromuscular transmission is illustrated in Fig. 1 and results of 7 experiments are summarized in Table I. Figure 1 shows e.p.p.s and m.e.p.p.s under control conditions, in the presence of PGE_1 (8.5×10^{-8}M), and after washing out the drug. The e.p.p. amplitude fluctuated from one trial to another according to the quantal hypothesis. Addition of as little as 8.5×10^{-8}M PGE_1 reduced the mean m.e.p.p. frequency by 36.9% Mean amplitude of m.e.p.p.s did not change, the mean amplitude of e.p.p.s and the number of quanta of transmitter released by a nerve impulse *(m)* were reduced by 18.8% and 20.2%, respectively. In several experiments, after washing out the PGE_1, m.e.p.p. frequency returned to its initial value or even exceeded it (Fig. 1).

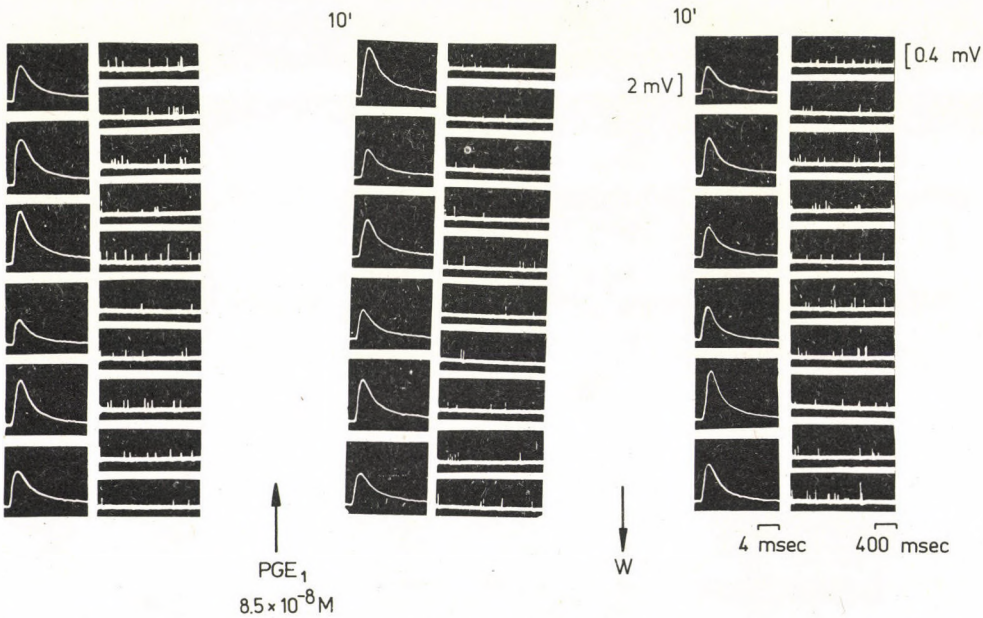

Fig. 1. Inhibition of m.e.p.p. frequency by PGE_1 in frog sartorius nerve-muscle preparation. Subsequently recorded individual e.p.p.s and m.e.p.p.s are shown before (left panel) and during (middle panel) exposure to PGE_1 (8.5×10^{-8}M). Right panel: 10 min after washing out (W) PGE_1. Preparations were stimulated supramaximally with 50, μ impulse duration, and with a frequency of 0.3 Hz

A reduction by 44.1% of mean m.e.p.p. frequency was seen in response to the administration of 8.5×10^{-8}M $PGF_{2\alpha}$ (Table I). The mean amplitude of e.p.p.s and m.e.p.p.s and the quantum content remained unchanged. The action of $PGF_{2\alpha}$ (8.5×10^{-8}M) on m.e.p.p. frequency was also reversible after washing out the drug.

Table I

Effect of PGE_1 and $PGF_{2\alpha}$ on transmission in frog sartorius nerve-muscle preparation

Drug	Number of experiments	Amplitude m.e.p.p., mV (M ± S.E.)	Frequency m.e.p.p., Hz (M ± S.E.)	Amplitude e.p.p., mV (M ± S.E.)	Quantum content e.p.p., (M ± S.E.)
–	7	0.21 ± 0.02	2.71 ± 0.45	2.39 ± 0.33	11.50 ± 1.15
PGE_1 8.5×10^{-8}M	7	0.21 ± 0.02	1.71 ± 0.31**	1.94 ± 0.30**	9.18 ± 0.81*
–	6	0.18 ± 0.01	2.11 ± 0.44	3.16 ± 0.35	18.69 ± 3.00
PGF_2 8.5×10^{-8}M	6	0.17 ± 0.01	1.18 ± 0.28*	2.74 ± 0.42	16.38 ± 2.80

* $p < 0.02$
** $p < 0.001$

Discussion

The major finding of this study is the reduction of m.e.p.p. frequency in the presence of both PGE_1 and $PGF_{2\alpha}$ at low (8.5×10^{-8}M) concentrations. At the same time, m.e.p.p. amplitudes were unchanged and e.p.p. amplitudes only slightly reduced or unchanged, indicating that PGs do not influence substantially the sensitivity of the subsynaptic membrane to the transmitter or the quantum content of the synchronously released transmitter. Higher concentrations of PGs were not tested since results obtained at concentrations higher than the possible physiological ranges are supposed to be of minor importance.

It has been shown that caffeine and other xanthine derivatives increase the frequency of m.e.p.p.s without influencing the depolarization-sensitive release of ACh (*Hofmann* 1969; *Gotgilf* and *Magazanik*, to be published). It might be assumed that these drugs increase the concentration of free Ca^{2+} in the axoplasm, which in fact regulates the frequency of m.e.p.p.s. In addition it was suggested that xanthines are unable to affect Ca^{2+} entry into nerve terminal during depolarization which in turn induces release of the transmitter.

It may therefore be supposed that PGs in contrast to the effect of xanthines, decrease the concentration of free calcium in the axoplasm and have only a small if any effect on the influx. Such a mechanism of action on pCa under resting conditions may be connected with the stimulation of intracellular sites of active transport or sequestration of Ca (*Baker* 1972).

This hypothesis needs experimental support. It has, however, been reported that the inhibitory effect of PGs on adrenergic transmission depends on the concentration of Ca^{2+} in the medium (*Hedqvist* 1970b) and small increase in Ca^{2+} was capable of antagonizing the inhibition by PGs of sympathetic transmission (*Stjärne* 1973). It was therefore suggested that PGs may inhibit Ca^{2+} influx induced by depolarization into the axoplasm (*Hedqvist* 1970b) or facilitate Ca^{2+} efflux from intraaxonal sites (*Stjärne* 1973). It seems likely that PGs influence the level of free Ca^{2+} also in cholinergic nerve endings of the frog sartorius muscle.

Our results are partly at variance with those of *Ginsborg* and *Hirst* (1971) who found that PGE_1 was inactive on neuromuscular transmission in the frog nerve-sartorius muscle preparation. The failure of these authors to demonstrate the effect of PGE^1 on the frequency of m.e.p.p.s may be ascribed to the higher concentration of Ca used in their experiments.

The absence of a definite effect of PGs on e.p.p. and m.e.p.p. amplitude is in good agreement with the results of *Marco* and *Coceani* (1973) who reported that PGE_1 at high concentrations (3×10^{-5}M) failed to affect twitch tension of the frog sartorius muscle stimulated through its nerve or directly.

Acknowledgement

The authors are indebted to Dr *M. A. Kamenskaya* and Dr *F. Vyskocil* for helpful criticism of the manuscript.

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Recensiones

H. HUDDART

The Comparative Structure and Function of Muscle

Volume 53 in the International Series of Monographs in Pure and Applied Biology.
Pergamon Press, Oxford, New York, Toronto, Sydney 1975.
VIII + 397 pages, with 266 figures and 49 tables.
Price: £ 12.85; US $ 30.00

The text of the monograph is based upon a series of lectures given by the author at the University of Lancester in the last seven years, and it follows a natural division into structure, electrophysiology and excitation and mechanical activity. The book attempts to offer an overall picture of what happens in muscle during excitation, contraction and relaxation, and how its structures are involved in these processes.

The vast literature of the past decade on invertebrate and vertebrate muscle her made it possible to examine muscle structure and function in a comparative manner. The methodological possibilities allow to study the muscle in a multilateral way. The fine structural information from electronmicroscopy and biochemical research revealed much about subcellular control systems within muscle cells; new biophysical techniques like X-ray diffraction and nuclear magnetic resonance have provided much information about the components of muscle and their interactions at the muscular level, and the traditional physiological studies provided new data about electrochemistry and muscle mechanics.

The book is divided in three section. Within each section, all muscle types are discussed from a comparative aspect, in as wide a range of animals as the literature allows. SECTION 1. The Structure of Muscle, discusses; 1, The Fine Structure of Skeletal Muscle; 2, The Fine Structure of Cardiac and Visceral Muscle; 3, The Innervation of Muscle. SECTION 2. The Electrical Activity of Muscle presents; 4, The ionic Basis of the Resting Potential; 5, The Action Potential and the Activation of Muscle; 6, Electrical Activity and Electrochemistry of Invertebrate Skeletal Muscle; 7, Electrical Activity of Invertebrate and Vertebrate Cardiac Muscle; 8, The Electrical Activity and Electrochemistry of Visceral Muscle. SECTION 3. The Mechanical Activity of Muscle treats; 9, The Mechanics of Muscle; 10, Excitation-Contraction Coupling and Relaxation.

The book is supplemented with a short glossary of fine structural and physiological terminology and is completed with an author and subject index. The work offers an up-to-date survey of the structure and function of vertebrate and invertebrate muscle, and will facilitate the work of postgraduate students of morphology, biophysics, biochemistry and physiology.

K. LISSÁK

Cell Cycle and Cell Differentiation
Results and problems in cell differentation Vol. 7.

Eds.: J. REINERT and H. HOLTZER

Springer-Verlag, Berlin—Heidelberg—New York 1975.
331 pages with 92 figures. Price $ 28.30

The book deals with different aspects of cell cycle and cell differentiation written by 18 contributors The topics are as follows. Myogenesis: A cell lineage interpretation (S. R. Dienstman and H. Holtzer); The organization of red cell differentiation (H. Weintraub); The cell cycle, cell lineage and neuronal specificity (R. K. Hunt); Neurogenesis and the cell cycle (C. H. Phelps and S. E. Pfeiffer); The cell cycle and the cell differentiation in the Drosophila ovary (R. C. King); The cell cycle and cellular differentiation in insects (P. Lawrence); Nuclear transplantation and the cycle reprogramming of gene

expression (J. B. Gurdon); Morphogenesis during the cell cycle of the prokaryote; Caulobacter crescentus (N. B. Wood and L. Shapiro); Cell division and the determination phase of cytodifferentiation in plants (Meins F); The cell cycle and tumorigenesis in plants (A. C. Braun); Cell cycle and liver function (R. Tsanev); Histones, differentiation and the cell cycle (Th. W. Borun); Cell changes in Neurospora (R. E. Nelson, C. P. Selitrennikoff and R. W. Siegel); Subject index.

The book offers an excellent insight into fundamental processes.

G. TELEGDY

D. NOVIN, W. WYRWICKA, G. A. BRAY (eds):

Hunger

Basic Mechanisms and Clinical Implications. Raven Press,
New York 1976. XV + 494 pages, with 170 figures and 36 tables. Price $ 28.50

The chapters in this volume are based on the papers presented at a conference held in Los Angeles on January 15 to 17, 1975. The conference was organized under the auspices of the Brain Research Institute of the University of California, Los Angeles. The volume provides a comprehensive survey of basic and clinical research on hunger. The chapters discuss the role of neurotransmitters involved in hunger, and revise the classic view of hypothalamic "centres" controlling hunger and satiety.

The contents of the volume are: I. Neurochemistry and Neuroanatomy of Hunger; II. The Role of Nutrients and Energy Metabolism in the Control of Food Intake; III. Developmental and Motivational Properties of Hunger; IV. Set-Point Theory and the Relationship of Weight Regulation and Food Intake; V. Short-Term Regulation of Feeding: Patterning, Peripheral, and Visceral Mechanism; VI. Hunger and Obesity in Man; VII. Conclusion.

The book will be of interest for physiologists, pharmacologists, psychologists and for workers in the various clinical sciences.

K. LISSÁK

W. HASCHKE

Grundzüge der Neurophysiologie

VEB Gustav Fischer Verlag, Jena 1976.
151 pages, with 56 figures and 6 tables. Price: in the GDR,
14.80 M; abroad, 22.— M.

The book has been published in the series "Bausteine der modernen Physiologie". It provides for students and postgraduate students of medicine, biology, psychology and others interested in the bases of neurophysiology a really satisfactory up-to-date summary of neurophysiological knowledge.

The book is divided in the following 14 chapters: 1. Introduction; 2. Excitation-physiology; 3. The neurone; 4. Spread of excitation in the neuronal junction; 5. Function principles in the CNS; 6. Electrophysiology of the CNS; 7. Mechanisms which determine general activity; 8. Sleep; 9. Limbic system; 10. Emotion, motivation; 11. Central nervous sensory mechanisms; 12. Motor regulation; 13. Learning and memory; 14. Higher nervous activity. The book concludes with a short bibliography, author and subject index.

K. LISSÁK

ABSTRACTS

of the lectures delivered at the 41th Congress

of the

HUNGARIAN PHYSIOLOGICAL SOCIETY

Szeged, July 7–10, 1975

INDEX

Effect of cortexolone on the feedback action of dexamethasone

ZSUZSANNA ÁCS and E. STARK

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Cortexolone (1 mg/100 g b.w.) administered prior to dexamethasone (50 μg/100 g b.w.) reduced the suppressing effect of dexamethasone on stress-induced ACTH release, but failed to affect the decrease in the resting corticosterone level. Cortexolone was found to bind to cytoplasmic corticosteroid receptors in some regions of the rat brain.

The data are in favour of the assumption that, similarly to other well-known corticosteroid effects, the corticosteroid feedback action depends on the binding of the hormons to specific receptors in the target cells. Assuming that cortexolone acts by displacing dexamethasone from its cytoplasmic receptor site, the data seem to support the view that the site and/or the mechanism of dexamethasone suppression of resting ACTH secretion are different from those depressing the stress-induced ACTH release.

Biological effect of in vitro labelled ^{125}I prothrombin

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Prothrombin was prepared according to Bagdy and labelled in vitro with ^{125}I by means of chloramine-T. The biological effect of the preparation was investigated in prothrombin-free plasma, measuring the effective concentration, the stability as well as the time of spontaneous degradation.

Circulatory time and elimination of the prothrombin preparation from blood plasma were investigated in animal experiments. The effective concentration and circulatory time of labelled prothrombin were determined in arteficial hypoprothrombinaemia.

On the basis of the data obtained, ^{125}I labelled prothrombin proves a reliable tool for the evaluation of the physiological and biochemical properties of prothrombin.

Changes induced by sexual steroids in lactate dehydrogenase (L-lactate-(NAD)-oxydoreductase, E.C.1.1.1.2.7.) activity of the anterior pituitary in the rat

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Lactate dehydrogenase (LDH) activity of the anterior pituitary was found to be higher in mature female than male rate. The activity decreased after ovariectomy and remained unchanged after orchidectomy. Administration of 1 and 2 μg oestrogen every third day for a period of 18 days increased pituitary LDH activity in both sexes. In this respect, testosterone and progesterone were only effective when given in pharmacological doses. The isoenzyme spectrum changed with the change of enzyme activity. The amount of fractions containing H subunits increased in the case of oestrogen deficiency. M homotetramer synthesis was stimulated by oestrogen. The change in the ratio H:M suggests that oestrogens enhance aerobic glycolysis in the anterior pituitary.

Effect of peripheral autonomic activity on self-stimulation

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It has been shown previously that self-stimulation is always accompanied by definite autonomic changes. A close relationship was found between the changes in level-pressing rats and the extent of the autonomic alterations after application of various drugs. The aim of the present investigations was to study the neural connections between the peripheral and central mechanisms responsible for self-stimulation. Electrical activity of the vagal nerve was recorded from the cervival part of the nerve. Rewarding hypothalamic stimulation was found to suppress vagal activity as a direct effect and to enhance it as an after-effect. Hypothalamic stimulation inducing an immediate increase in the vagal activity was not suitable for self-stimulation. Electrical stimulation of the vagal nerve suspended the lever-pressing behaviour. After bilateral vagotomy, a transitory increase in the rate of lever-pressing was observed. The results presented support the assumption that peripheral autonomic functions are involved in the mechanism of self-stimulation.

Spiroergometric comparison of runs at steady and alternating speed

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Spiroergonometric parameters were measured in random, self-control experiments by means of an on-line computerized Jaeger equipment. The experiments consisted of 8-min runs at steady or alternating speed at a constant distance. The quick "bursts" lasted 5 sec. In other cases, due to the quick periods, the performance fell at 1/3 of the distance between the two steady runs.

Parameters of alternating runs diverged from the theoretical values. The higher stress was indicated by the heart rate and economy of ventilation. The alternating run evoked higher stress in non-competing sportsmen than in distance runners.

Effect of ureteral occlusion on series connected intrarenal resistances in normohydrated and in mannitol-loaded dogs

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Series connected renal vascular resistances were investigated in normohydrated and in mannitol-loaded dogs, under both free flow and stop flow conditions.

1. In the normohydrated dog ureteral occlusion leads to a sharp rise in ureteral and deep venous pressure. The pronounced decrease in afferent resistance surpasses the increase in venular resistance, with a resulting decrease in total vascular resistance and a concomitant increase in RBF. GFR is maintained at about 5/6 of the free flow value.

2. In the mannitol-loaded dog ureteral occlusion leads to a maximum increase in ureteral and deep venous pressure. GFR falls to zero. The increase in postglomerular (efferent + venular) resistance dominates over the decrease in preglomerular (afferent) resistance with a resulting enhancement of total vascular resistance and a concomitant drop in RBF.

3. Under free flow conditions, mannitol-loading does not significantly influence total vascular resistance. Afferent resistance remains unaltered; the slight increase in venular resistances is just compensated by the slight decrease in efferent resistance. Diminution of GFR is due entirely to increased intratubular pressure with no change in glomerular capillary pressure.

Conditioned changes of neuronal activity in the cat's cerebral cortex

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The purpose of the study was to clarify the variants of conditioned multiunit activity shifts caused by direct intracortical electrostimulation of the visual and associative cortex of anaesthetized and immobilized cats.

The activity changes of neuronal pools neighbouring the stimulating electrodes were recorded simultaneously in both areas investigated. The responses to stimulation of the associative cortex consisted of a short inhibitory phase followed by distinct activation. In the visual cortex monophasic responses, including the inhibitory or activation phase only, as well as biphasic ones were characteristic. Association of two intracortical stimuli mostly resulted in the appearance of a new pattern of response, the associated stimuli causing a complete or partial inhibition of the responses induced by the associated stimuli when used separately. The modifications of neuronal activity were preserved during the period after conditioning; thus the neuronal pools of both the visual and associative cortex fixed the conditioned multiunit changes.

Comparison of the physiological characteristics of active-alert induction and traditional relaxation induction of hypnosis

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Induction of relaxation suggestion of sleep and drowsiness, i.e. procedures decreasing the activity level have been used for centuries to induce a hypnotic state. It has been shown (*Bányai, Hilgard* 1974) that a hypnosis-like state can be attained also by methods intended to increase the activity level. These two methods of hypnotic induction lead, however, to different subjective experiences. The traditional procedure results in a state of drowsiness and passivity while active-alert induction produces an extasis-like state.

It was the purpose of the present research to study the differences in polygraphic records of the physiological variables related to these subjective differences, and to elucidate the relationship between the physiological alterations and the sensitivity to suggestion. The experiments performed on university students of high and low susceptibility showed that although the EEG, EMG and ECG changed in accordance with the activity level, the sensitivity to suggestion was increased by both hypnosis-induction procedures.

Interactions between ipsilateral epileptic foci in the association cortex of the cat

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Epileptic foci were produced by application of strychnine or penicillin on the suprasylvian gyrus of superficially anaesthetized cats. The changes of activity of these foci ensuing upon synchronous epileptic activation of primary sensory areas were studied.

Several types of interaction were observed:

1. Foci in the Vis I and Vis II areas inhibited seizure activity of the g. suprasylvius medius anterior (AMSA). A marked suppresion could be observed both in ictal and inter-ictal periods.

2. A focus in the AMSA depressed the convulsive activity in the motor cortex. This latter, in turn, enhanced seizure activity in polysensory areas.

3. Foci in the primary somato-sensory area depressed foci in the AMSA and posterior ectosylvian gyrus, and exhibited a mutual facilitation with the motor areas.

4. Foci in the posterior ectosylvian gyrus inhibited convulsive activity in the AMSA and, at the same time, evoked seizures in adjacent association areas.

The mutual interactions of seizure foci have several well-defineable types manifesting in both inter-ictal and ictal periods. The results offer a basis for bio-feedback experiments.

Interactions between prostaglandins and oxytocynases

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Oxytocynase activity was measured in human and rat serum, fetal and placental tissue as well as organ homogenates of pregnant and non-pregnant rats. In addition, the effect of various prostaglandins (PGE_2, $PHF_{2\alpha}$, 15-M-$PGF_{2\alpha}$) on the enzyme activity of different organs was studied. The results did not reveal any interaction between prostaglandins and oxytocynases.

Investigation of the threshold parameters of intestinal receptors by operant conditioning

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Continuing their previous studies (XL. Congress of the Hungarian Physiological Society, Debrecen 1974) the authors investigated the criteria by which visceral stimulation influences behaviour. Electrical and mechanical stimulation of the rat's isolated intestinal loop served as a model. The threshold parameters, notably the absolute and differential thresholds were used to characterize the visceral influence on operant behaviour.

Operant responding was conditioned in two series of experiments. In the first, discriminative behaviour—partial extinction—was established by applying the internal stimulus as background signal. The relevant operant response patterns were mapped individually by means of changing the parameters of the stimuli applied. In the second group of rats discriminative experiments were performed by means of conditioning two different operant responses.

Both approaches proved successful for the estimation of the absolute and differential threshold of the interoreceptors. In both series different response patterns could be observed depending on the mechanical or electrical nature of the stimulus. The data presented corroborate the previous observations in that intestinal stimuli, ineffective when applied in a non-contingent way, become highly effective after conditioning and will significantly influence on operant behaviour.

Effect of indomethacin-induced inhibition of prostaglandin synthesis on renal function

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The effect on renal function of indomethacin given in a single intravenous dose of 4 mg/kg was studied in anaesthetized, mildly volume-expanded dogs. Cardiac output was measured on the basis of the Stewart-Hamilton principle, intrarenal circulation by the ^{86}Rb accumulation method.

The renal parameters given in the Table were determined one hour after indomethacin administration.

	Control (n = 10)	Indomethacin (n = 11)	
Blood pressure, mm Hg	120 ±24	143 ±19	x
Cardiac output l/min/m^2	3.61 ±1.3	3.58 ±1.1	
RBF ml/min/100 g	401 ±95	292 ±53	xx
CBF, per cent	79.0 ±2.2	84.0 ±3.2	xx
OMBF, per cent	16.9 ±2.1	13.2 ±2.6	xx
IMBF, per cent	4.3 ±1.7	2.8 ±0.8	XX
GFR ml/min/100 g	67.1 ±15.9	62.7 ±21.5	
Sodium excretion	213 ±149	139 ±112	xx

x = $p < 0.05$ xx = $p < 0.01$

Accepting that indomethacin selectively blooks prostaglandin synthesis without affecting other processes, it is assumed that.

(i) prostaglandins influence both the basal vascular tons of different organs and the distribution of renal circulation;

(ii) prostaglandins may have a role in the physiological regulation of sodium excretion.

Nerve excitation generated by muscle excitation

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Starting from Matteucci's experiments investigations were performed on ischiadic nerve gastrocnemius muscle and on whole leg preparations of the frog in order to study the circumstances under which electrical activity of the muscle was capable of generating excitation in a nerve adjacent to the muscle.

The results were as follows. Excitation could be transmitted through 4 to 6 nerve muscle preparations. Tetanic stimulation enhanced the transmission of excitation from muscle to nerve. This kind of transmission took place also in situ. In general spontaneously generated asynchronous muscle excitation failed to produce nerve excitation, while muscle excitation evoked by a stimulus above the threshold but lower than the supramaximal one was capable of exciting the nerve.

These results together with the findings of other authors suggest that, under certain conditions, the effect of muscle excitation on the nerve adjacent to the muscle may play a part in the regulation of neural and muscle function in connection with excitatory processes.

Transcapillary fluid exchange in hind limb muscles during cardiogenic shock

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Previous experiments revealed that haemorrhagic shock irreversibly damaged the transcapillary fluid exchange in the hind limb muscle of the dog, an effect mainly mediated by sympathetic hyperactivity.

In the present studies cardiogenic shock was used as a model, and capillary exchange was studied in the innervated, autoperfused hind limb muscle of cats by means of isovolumetric pletysmography with continuous determination of the capillary filtration coefficient (CFC).

In cardiogenic shock, as compared to haemorrhagic shock, 1. the elevation of CFC was absent; 2. CFC values diminished parallel with the deterioration of heart function.

Pletysmography is recommended as a useful tool in evaluating the effect of therapy and the microcirculatory state of peripheral organs in patients with cardiogenic shock.

Medullary recycling of sodium and urea in the rat kidney after renal sympathectomy

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Previous micropuncture experiments showed that diuresis and natriuresis ensuing after acute and chronic renal sympathectomy were related to a depression of proximal reabsorption. In the present study the transport of sodium and urea has been investigated in control and unilaterally denervated rats by puncturing late proximal and early distal tubular segments.

Chronic renal sympathectomy brought about marked diuresis and natriuresis without changes in either the total or the single GFR. (TF/P) inulin proximally and distally, (TF/P) urea proximally were significantly decreased and (TF/P) Na and (TF/P) K distally increased in the denervated kidney. Proximal fractional reabsorption of sodium and water was 64.5% in controls and 35.6% in denervated organs. The reabsorbed fraction at the early distal level was 84.4% and 72.8%, respectively; thus, medullary recycling markedly increased in the denervated kidney (control: 19.9%, denervated: 37.2%). Similarly, the medullary recycling of urea was also enhanced after denervation. Denervation natriuresis and increased clearance of urea seemed to be due to a depression of proximal transport that cannot be counterbalanced by the medullary recycling of these substances.

Purification of a lymphocyte factor that increases anaphylactoid inflammation in the rat

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Previous experiments have shown that on exposure to insulin lymphocytes of various species release into the incubation medium a factor increasing anaphylactoid inflammation in the rat. In the present experiments different fractionation techniques were used to separate the inflammatory factor from other materials present in the supernatant. Large amounts of supernatant were obtained from calf lymph nodes. The protein content and the inflammatory activity measured in dextran paw oedema test were determined from the starting material and its different fractions. Specific inflammatory activity of the supernatant was increased to double by molecular filtration on Sephadex G-100 gels, 27 times by ion exchange chromatography on DEAE-Sephadex A-50 column and 1300 times by ion exchange chromatography followed by polyacrylamide gel electrophoresis. The last procedure seemed suitable for the production of highly purified inflammatory factor.

Direct cardiac effects of arachidonic acid

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The direct cardiac effect of arachinodic acid (AA, C 20:4), the natural precursor of prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) was studied on isolated left and right atria, papillary muscle and ventricular strips of the guinea pig heart. Spontaneous (sinus) frequency and contractility of the atrium were increased by AA 10^{-6}—10^{-3} M); excitability, conductivity and maximum driving frequency were decreased. AA had no excitatory effect on ventricular musculature. Indomethacin, a drug inhibiting PG-synthetase activity, decreased the positive inotropic and chronotropic effect on the atrium of AA when used at a concentration of 5×10^{-5} M. These effects of AA were, however, left unaltered by reserpine pretreatment (2.5 mg/kg/day for 4 days plus 5 mg/kg on the firth day intraperitoneally), inhibition of beta receptors (pindolol, 2×10^{-6} M), inhibition of histamine receptors (H_2 receptors; burimamide, 10^{-5} M, or metiamide, 10^{-5} M). In contrast, other unsaturated fatty acids such as oleic acid (C 18:1) or linolic acid (C 18:2) did not augment either the spontaneous frequency or the contractility of the atrium. A similarity was found in the positive inotropic effects of AA and PGE_2 as well as $PGF_{2\alpha}$.

The direct excitatory cardiac effects of AA are assumed to be connected with the enzymic transformation of the molecule into prostaglandin.

Role of myosin-cholinesterase in superprecipitation

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Superprecipitation of natural actomyosin prepared according to Ebshi's method from rabbit skeletal muscle was shown to be accelerated by acetylcholine and cholinesterase inhibitors. Such preparations contain not only actin and myosin but regulatory proteins as well. In the present experiments synthetic actomyosin prepared from purified myosin and actin was used to investigate the direct effect on superprecipitation of myosin-cholinesterase. The change of extinction due to superprecipitation was followed spectrophotometrically at 660 nm, it was linear at protein concentrations from 0.1 to 0.7 mg/ml.

Synthetic actomyosin was prepared from myosin solution incubated with 10^{-6} M physostigmine and actin, 4:1. The period necessary to develop maximal superprecipitation was shorter and the E_{max} higher than in the controls. Additional treatment with physostigmine had no influence on the superprecipitation. The superprecipitation of synthetic actomyosin was also significantly accelerated by 10^{-6} M physostigmine.

It is suggested that the cholinesterase-active groups of myosin have a direct effect on the processes that evoke changes in superprecipitation.

Factors regulating the glycogen level in the rat's submandibular gland

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Guanethidine treatment decreased the glycogen content of the submandibular gland 15 min after administration of the drug. Five hours later the glycogen level in the gland was elevated as compared with the control. As an explanation it is assumed that, during the time of the sympathetic blockade, the increase in the glycogen content is due to the relative parasympathetic predominance. Therefore, atropine was given the animals 15 min before guanethidine; this pretreatment did not however prevent the increase in the glycogen content.

Another explanation for the elevation of the glycogen content would be that during the sympathetic blockade, the glycogen level decreasing effect of the sympathetic nervous system is abolished. Five hours after adrenaline treatment the glycogen content was the same as in the controls. If the rats received a beta-blocking agent 15 min after the adrenaline injection, an elevation was observed in the glycogen level. Thus the reason why the glycogen level of the submandibular gland exceeded control one following sympathetic stimulation may be that the glycogen-diminishing effect of the sympathetic nervous system became dominant under such conditions.

Effect of blood glucose concentration on the activity of liver phosphorylase phosphatase

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The key enzyme of liver glycogen mobilization and rise of blood glucose level is the active phosphorylase that can be activated by hormonal or neural stimulation and dephosphorylated by phosphorylase phosphatase. In the liver homogenate the phosphorylase phosphatase is inhibited by an unknown protein, probably by protein-protein interaction. A close relationship can be demonstrated between the increase in blood glucose and the decrease in phosphatase inhibition. Intravenous and oral glucose load as well as intravenous adrenaline lead to a transient release of phosphatase inhibition, an event resulting in the parallel inactivation of phosphorylase. In alloxan-diabetic animals the phosphorylase phosphatase is active consequently, the phosphorylase is inactive and cannot be activated even by adrenaline. On administration of insulin to normal and diabetic animals the phosphorylase phosphatase will be inhibited and the blood glucose dexrease. This is followed by the appearance of active phosphorylase. Inhibition of phosphorylase phosphatase and the effect of glucose can only be detected in concentrated liver homogenate. After dilution the observed phenomena disappear, presumably due to dissolution of the protein-protein interactions.

Effect of simultaneous bilateral stimulation on locomotion and motivation

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Rewarding and punishing effects induced by unilateral electrical stimulation of subcortical structures were found to correlate with contraversive and ipsiversive locomotor tendencies (*Grastyán* et al. 1968).

In order to avoid the laterality caused by unilateral stimulation, in the present study the hypothesis was tested by applying bilateral stimulation. Simultaneous bilateral stimulation of loci causing contraversive and ipsiversive locomotion (in respect of the stimulated hemisphere) resulted in approach or withdrawal behaviour, respectively. Rage and attack evoked by hypothalamic stimulation were supressed by contralateral stimulation of ipsiversive structures, thus proving the inhibitory character of ipsiversive loci on approach behaviour.

The results support the hypothesis that motivation, emotion and locomotion are highly correlated.

^{14}C-stearic acid turnover under ACTH treatment

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It has been shown that prednisone treatment for 6 days increased ^{32}P incorporation into the organic and inorganic phosphate fractions of the heart muscle and the specific activity of the fat extracted 5 min after the injection of ^{14}C-stearic acid.

To the present experiments it was studied whether ACTH pretreatment would bring about similar changes in the turnover of fats in heart muscle, liver and epididymal adipose tissue as did the application of exogenous corticosteroid.

Lipid turnover in the heart muscle, but not in liver and testicular tissue, changed in a similar way as after pretreatment with corticosteroid. Incorporation after ACTH was more intense in the liver and, after prednisone, in epididymal tissue.

The supernatant fraction of Folch extract that contains non-lipid metabolites displayed the highest activity when the lipids displayed the highest activity. It is suggested that ACTH pretreatment accelerates the lipid metabolism of the heart and liver.

Anti-amphetamine and anticonvulsant activity of some beta-blocking agents

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Pretreatment of mice with propranolol results in a decrease of the hypermotility evoked by amphetamine in the mouse (*Weinstock* and *Speiser* 1974). According to *Madan* and *Barar* (1974) pronethalol, propranolol and pindolol abolish the extensor component of the hind limbs in the maximum electroshock seizure test. The present work was undertaken to establish whether CHINOIN-103, an alkanolamine beta-blocking derivative had any effect on amphetamine-induced hypermotility and an anticonvulsant activity.

CHINOIN-103 was more effective than propranolol in preventing amphetamine hypermotility in mice. Both drugs were used in a dose corresponding to 1/10 of the LD_{50} value. Injection of propranolol after amphetamine increased hypermotility, while CHINOIN-103 decreased it more markedly than did pindolol. In the pentamethylene tetrazole test, CHINOIN-103 afforded 30% protection in a dose corresponding to 1/10 of its LD_{50}.

Energy metabolism of the gastric mucosa in immobilized rats

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Phosphate turnover in the organic and inorganic fractions of the gastric mucosa of immobilized rats was studied by laballed phosphate, and the lactate: pyruvate ratio, the alpha-ketoglutamate content as well as the succinate dehydrogenase activity in the gastric mucosa were estimated.

Labelled P content of both the organic and inorganic fractions increased in the initial phase of immobilization. The lactate:pyruvate ratio increased while the alpha-ketoglutamate content remained unchanged and the activity of succinate dehydrogenase was elevated. At later stages of immobilization the specific activity of labelled phosphate decreased in both the organic and the inorganic fractions.

The results obtained are indicative of an increased rate of phosphorus metabolism in the first phase of restraint. The lactate: pyruvate ratio, however, points to a relative hypoxia. It may be assumed that the circulation could not keep pace with the increased metabolic rate. The decrease of phosphorus metabolism in the later period of immobilization might be a consequential phenomenon.

Metabolic and hormonal consequences of exchange transfusion via the umbilical artery or vein

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Twenty exchange transfusions for hyperbilirubinaemia were performed via the umbilical artery or vein in 17 term and 3 preterm infants of 32—41 gestational weeks. Blood preserved with citrate and dextrose was used and the effect of the route of transfusion on plasma glucose, free fatty acids, insulin and growth hormone concentration was measured during the transfusion and for three hours afterwards. (1)

Infants transfused via the umbilical vein ("vein group") secreted 3.22 ± 0.81 mU/body weight net insulin, more than those transfused via the artery ("artery group"), 1.16 ± 0.45 mU/l, although both groups had similar rises in plasma glucose (from 79 ± 35 mg to 176 ± 23 mg in the vein group and from 73 ± 8 mg to 170 ± 13 mg per dl in the artery group). The glucose disappearance constant was 1.65 ± 0.18 versus 1.31 ± 0.18. Before transfusion the mean FFA level in the artery group (1364 ± 103 μmol/l) was almost twice as high as that in the vein group (782 ± 86 μmol/l). The difference decreased during transfusion, it was constant one hour after the transfusion, and rose afterwards. Mean plasma HGH was 15—20 times more

in the infants than in the donor blood (35 ± 5 or 34 ± 5 ng/ml in infants, 1.7 ± 0.7 or 1.6 ± 0.4 ng/ml donor). The difference decreased during and after the exchange transfusion. HGH secreted in the artery group was twice as high as in the vein group (3.09 ± 0.51 or 5.89 ± 1.25 ng/kg body weight net HGH). The results lead to conclude that the arterial route of exchange transfusion is probably more stressful than the venous route. Exchange transfusion performed via the umbilical vein results in a greater stimulation of insulin secretion causing asymptomatic hypoglycaemia.

1. *Cser Á., Milner, R. D. G.:* Metabolic and hormonal consequences of exchange transfusion via the umbilical artery or vein. Biol. Neonate. In press.

X-ray irradiation-induced thrombocytopoietic serum activity and its effect on ^{75}-Se methionine utilization in the platelets of the mouse

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Platelet count significantly decreased after 500 R total body irradiation in mice, with the minimum reached on the 10th day after irradiation. By this time the serum of the animals exhibited thrombopoietic activity: when administered to normal recipients it increased the platelet count by 50—60% on the 5th day following administration. Utilization of ^{75}-Se methionine by the thrombocytes of normal animals was also significantly enhanced by the serum. The extent of utilization depended on the amount of serum injected; repeated application further increased the utilization rate. Beside total serum, its beta-globulin and the peak II fraction obtained by Sephadex chromatography displayed thrombopoietic activity. Individual fractions obtained by preparative acrylamide gel electrophoresis exerted different effects on the utilization of ^{75}Semethionine.

Effect of veratrine on intracellular Na- and K-concentration of the frog sartorius

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Mean the intracellular Na-concentration of the sartorius muscle incubated in Ringer solution increases by 86% under the influence of 0.1 mM veratrine in 60 minutes. Simultaneously, the mean intracellular K-concentration decreases by 29%. The increase in Na-uptake of the muscle under the influence of veratrine is actually 163% on the average because, simultaneously with Na-uptake, the intracellular water content increases by 33.6% on the average. The mean K-content calculated per dry weight of muscle decreased by 6% during incubation with veratrine for

one hour. Therefore, it is mainly the increase of intracellular water content that can be made responsible for the 29% diminution of K-concentration. These results contradict the generally accepted concept that it is almost exclusively the increase in Na-permeability that is responsible for the depolarizing effect of veratrine. In addition to the increase of Na-transport, the decrease of intracellular K-concentration undoubtedly plays a part in the mechanism of depolarization.

Effect of nitroglycerine on the potassium content of isolated heart mitochondria

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In order to elucidate the mode of subcellular nitroglycerine action, the effect of the drug (5×10^{-4} M) was studied on the intramitochondrial ion milieu at pH 7.0 and 7.4. In these media freshly prepared mitochondria loose potassium. The potassium loss increases with increasing pH in the presence of phosphate; at the same time, the phosphate uptake decreases. Nitroglycerine diminishes the potassium loss seen at pH 7.0 to the value found in a medium of pH 7.4. Nitroglycerine is assumed to prevent the decreasing effect of phosphate on the ratio of ADP:0 by inhibiting potassium release from mitochondria.

Effect of Co_2 inhalation and increased air passage resistance on integrated electrical activity of the rabbit diaphragm

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Anaesthetized rabbits were made to inhale gas mixtures containing varying percentages of CO_2 by means of a trachea cannula with changeable resistance. Integrated electrical activity of the diaphragm was recorded and its mean amplitude determined before and five sec after increasing respiratory resistance. Both the frequency of breathing and the integrated electrical activity of the diaphragm increased following CO_2 inhalation. There was a further increase in electrical activity if the respiratory resistance was augmented and, at the same time, the respiratory rate decreased. Integrated electrical activity (1 min) of the diaphragm decreased in every case.

It is assumed that the decrease of respiratory minute volume observed at increased CO_2 tension and increased resistance of the air passage is the result of opposite changes in the neural mechanisms regulating the contraction force of the diaphragm.

Antidiuretic effect of carbamazepine in the rat

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The antiepileptic drug carbamazepine significantly reduces urine excretion in ADH-sensitive diabetes insipidus patients. The mechanism of action is not fully understood. Experiments were performed to clarify the question.

1. Pituitary stalk lesion was inflicted electrolytically in rats and their fluid intake and urine output were measured. In the lesion-induced interphase accompanied by mobilization of endogenous ADH, carbamazepine further diminished the daily urine excretion and fluid intake and lengthened the oliguric period. The drug did not cause any significant decrease of diuresis in the polyuric, polydipsic phase associated with lasting ADH deficiency.

2. The change in diuresis produced by ADH was studied in alcohol-anaesthetized, hydrated rats. Following carbamazepine pretreatment the response of the animals to exogenous ADH was much more marked and longer-lasting.

The results indicate that the presence of endogenous ADH is necessary for carbamazepine to affect water metabolism. The drug probably exerts its effect by potentiating the existing ADH reserve, but ADH mobilization may also play a part in its mechanism of action.

Spinal integrative work of the frog

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The dendrites of motoneurones in the frog are known effectively to contribute to the integration of the motor output. These cells consist of a population with many dendro-dendritic electronic couplings. Thus, if a few elements of the system are excited, the activity spreads quickly through a large part of the system. Then, the strong and highly synchronous motor output required for jumping will be released to the muscles.

No post-synaptic recurrent inhibition has been found in the frog. There are reports about presynaptically mediated recurrent inhibition. The present studies with intracellular electrodes support the possibility of recurrent presynaptic inhibition. In addition, the findings suggest that other processes which are associated with the spike after-potentials may also have a role in the control of the frequency of firing in the frog motoneurones.

Metabolism-dependence of membrane potential oscillation elicited by veratrine in skeletal muscle. II.

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It has been observed previously that skeletal muscle depolarization as well as the membrane potential oscillation elicited by veratrine are essentially independent processes. Depolarization is a passive process, thus it is not influenced by metabolic inhibitors. Oscillations of the membrane potential, however, are inhibited by every agent capable of influencing the amount of available energy produced by glycolytic metabolism. For instance, if the oscillation of the membrane potential is stopped by certain metabolic inhibitors, the membrane does not repolarize. Hence, only the phenomenon requiring chemical energy ceases but the passive depolarization persists. The significance of the role of energy in bringing about oscillation is supported by the fact that depolarization will be elicited in energy-depleted muscle fatigued by long-lasting electrical stimulation prior to veratrine treatment, but oscillations fail to develop. On the basis of the results obtained, the significance of energy-requiring transport is discussed from the point of view of the mechanism of the oscillation phenomenon.

Effect of phenylephrine and isoprenaline on the circulation in tumour-bearing rats

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The effect of phenylephrine (100—300—1000—3000 ng/100 g/min) and isoprenaline (10—30—100—300 ng/100g/min) infusion was investigated on cardiac output (Evans-blue dilution) as well as on circulation in Guérin carcinoma and in different organs (Sapirstein's isotope indicator fractionation method). Normal rats served as control.

In accordance with previous results, the haematocrit, blood pressure and TPR were decreased, organ blood flow was augmented and circulatory resistance diminished in untreated tumour-bearing animals when compared with untreated controls.

Results of the log-dose-response regression analysis were,

(a) phenylephrine infusion increased blood pressure, vascular resistance in the kidney, gut and skin in tumour-bearing animals; it increased blood pressure and the resistance of carcass in normal rats;

(b) isoprenaline infusion decreased blood pressure in tumour-bearing rats; it increased cardiac output, decreased blood pressure, the resistance of brain, myocardium, gut and skin in normal animals;

(c) vascular resistance of Guérin carcinoma calculated for unit weight was considerably elevated by phenylephrine, but not by isoprenaline infusion.

It seems that in tumour-bearing rats the organs and the vessels of the tumour are more responsive to alpha stimulation and less sensitive to beta stimulation. The difference is probably due to the general vasodilation observed in untreated tumour-bearing animals.

Effect of insulin on various inflammatory models

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Insulin has been shown to increase the anaphylactoid reaction, anaphylaxis and delayed hypersensitivity. In the present experiments the effect of insulin on acute, non-immune inflammatory responses was studied.

Carrageenin paw oedema and turpentine pleurisy were decreased by the hormone. The inhibition was dose-related and more expressed at the early stage of inflammation than six hours after the injection of the irritative agents. Glucose decreased exudation by itself but failed to prevent the anti-inflammatory activity of insulin. The possible significance of the findings is discussed.

Effect of beta-blockers on cold-induced thermogenesis in the newborn rabbit

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Thermoregulatory heat production of the newborn rabbit has been shown to be mediated through beta receptors. The main site of cold-induced calorigenesis is the brown adipose tissue. It has also been postulated that the primary event in brown fat thermogenesis is the depolarization of the cell membrane by noradrenaline, and that any agent preventing depolarization might inhibit calorigenesis.

The aim of the present study was to clarify whether the membrane-stabilizing action of beta-blockers is an important factor in cold-induced thermogenesis *in vivo*, and whether this effect is mediated via beta blockade. Propranolol (±Prop), the cardio-selective practolol (Pract) or the membrane-stabilizing dextroisomer pro-

pranolol (+Prop) were injected in doses of 1.0, 2.25 and 5 mg/kg to 3—6-day old rabbits (n=98) and O_2 consumption (O_2), body temperatures (colon T_c; brown fat T_b; muscle T_m), plasma FFA and blood glucose level were measured at ambient temperatures (T_a) of 35 °C and 25 °C before and after administration of the drugs. VO_2 at T_a 35 °C was not influenced by either agent. ±Prop and Pract abolished cold-induced thermogenesis in 2.25 and 5 mg/kg doses ($p<0.001$) while +Prop depressed it only slightly ($p>0.1$). Heat production in brown adipose tissue (T_b) followed the same pattern. ±Prop lowered blood glucose level in a dose-related manner while Pract and +Prop were ineffective. All three agents tended to depress the cold-induced increase of circulating FFA to different extents.

It is concluded that *a)* in the newborn rabbit, cold-induced thermogenesis is mediated through $beta_1$ receptors, and *b)* the anticalorigenic action *in vivo* of beta-blockers at the dose levels used seems to be independent of their membrane stabilizing effect.

Mouse erythrocyte binding receptor of human B lymphocytes

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26.5±8.6% of human peripheral lymphocytes will form rosettes with mouse erythrocytes. A significant correlation can be observed between the number of mouse erythrocyte rosette-forming lymphocytes and the immunoglobulin-bearing cells. Lymphocytes forming rosettes with mouse erythrocytes were isolated by Ig-bearing cells which do not possess sheep erythrocyte binding receptors and cannot be stimulated by phytohaemagglutin.

The mouse erythrocyte binding receptor cannot be inhibited with antihuman globulin, but can be inhibited with pokeweed mutagen and destroyed with trypsin.

Drug-induced modifications of somato-sympathetic reflexes

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In anaesthetized (urethane), immobilized (gallamine) and arteficially ventilated cats electrical stimulation of the sciatic nerve with impulse trains of 16 V and 0.3 msec elicits contractions of the nictitating membrane and an elevation of arterial blood pressure. The magnitude of the responses is frequency-dependent. Short (2 sec) trains result in fast transient responses. Pressor responses to long (2 min) trains, with 16—32—64 cps frequencies in particular, consist of an initial transient high amplitude and a following low steady plateau. The time of increase, the maxi-

mum amplitude of the transients and the magnitude of the steady state component of the reaction were evaluated. The frequency threshold is lower and the amplitude of pressor reflexes higher with longer stimulation than during short trains of identical frequency.

It has been investigated, how and to what extent the characteristics of these reflexes are influenced by various drugs.

Phosphatases in the sarcoplasmic fractions of skeletal muscles

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In the sarcoplasmic reticulum fraction of the slow-twitch soleus and fast-twitch semimembranous muscle of the rabbit, the phosphatases differ in both quantity and activity. Total phosphatase activity is higher in the fast than in the slow muscles, the ratio of p-nitrophenylphosphatase (p-NPPase) to ATPase is 1 : 6, respectively. Ca-activation below 10^{-5} M can only be demonstrated in ATPases; this activation either. The p-NPPase of both muscles can be inhibited by EDTA only slightly, while the ATPase of the fast muscles by 80—90% and that of the slow ones by 10—20%. At concentrations over 10^{-5} M Ca markedly inhibits the p-NPPase of both muscles and the ATPase of the fast muscle. Inhibition of the slow muscle ATPase occurs at higher Ca concentrations only. In experiments, with EDTA, Ca-activation below 10^{-5} M can only be demonstrated in ATPases; this activation (Ca-activated ATPase) is more marked in the fast than in the slow muscle. Thus the role of Ca is different in the two kinds of muscle.

Oxido-reduction state and haemoglobin content in the cat cerebral cortex during haemorrhagic shock

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Haemoglobin content (UV reflectance) and oxido-reduction state (NADH) in the same cortical region were studied by microfluorometry in order to observe the changes which occur in the brain during haemorrhagic shock. The effect of 1.5 min anoxia and 1—3 min direct cortical stimulation was investigated in the control and hypotensive periods one hour after reinfusion. The changes in Hb content were estimated from the cortical reflectance response induced by injecting saline into the lingual artery. At the end of the hypotensive period as well as after reinfusion, ECoG was isoelectric or decreased markedly as compared with the control. Anoxia failed to produce NAD reduction and there was no change in reflectance during cortical stimulation. Saline injection did not affect cortical reflectance. It is assumed that the brain cortex becomes ischaemic during haemorrhagic shock.

Effect of myocardial oedema on left ventricular autoregulation

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It has been shown previously that infusion of noradrenaline, 1.0 μg/kg/min for ten minutes elicits myocardial oedema and increases ventricular wall stiffness in dogs subjected to regional cardiac ischaemia by ligating a major coronary branch. This state can be regarded as an experimental model of heart wall stiffness which increases during human anginal attacks. This model was used for studying the heterometric autoregulation of the left ventricle as well as the autoregulatory phenomena of the coronary vascular bed in myocardial oedema.

In open-chest dogs under chloralose anaesthesia, cardiac output was stabilized by substituting the right heart a roller pump for in order to adjust the load to the left heart at will. Starling-type cardiac function curves were constructed by plotting ventricular work against left auricular pressure.

The results indicate that, simultaneously with the development of myocardial oedema, there is a change in coronary autoregulation and the left ventricular function curves are shifted to the right.

New method for studying cerebral circulation

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Cerebral circulation was studied by the non-diffusible indicator dilution technique. Dilution curves were obtained by injection of high molecular dextran solution into the common carotid of anaesthetized cats and by recording the light intensity at 366 nm reflected from an illuminated area of the anterior suprasylvian gyrus. A certain proportion of both the incident and the reflected light is scattered by the red blood cells present in the examined volume of tissue. The dilution of blood by dextran leads to a decrease of the red cell count and, due the smaller scattering effect, it increases the intensity of the reflected light.

Mean transit time ($\overline{t_a}$) between the common carotid and the examined cortical area was calculated from the time function of the induced reflectance (R[t]). The reciprocal of $\overline{t_a}$ is proportional to the blood flow if the vascular volume does not change.

The usefulness of the method was proved by some tests having a well-known effection cerebral circulation, such as graded hypotension, direct electrical stimulation of the cortex, anoxia, and changes in arterial pCO_2.

Effect of picrotoxin on blood pressure reflexes evoked by stimulation of low threshold cutaneous afferents

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Various cutaneous afferents were stimulated in waking, anaesthetized (chloralose + urethane) and spinal transected (C_2—C_3) cats immobilized by gallamine under arteficial respiration. Combinations of stimulation parameters activating A-fibres only, were applied (0.5—1—2 V, 4 Hz, 0.1 ms, 4 min). Stimulations in waking animals resulted in complex pressor reflexes; upon repetition they gradually became ineffective. No blood pressure responses could be elicited by weak stimulation in anaesthetized and spinal preparations. Administration of picrotoxin in subconvulsant doses led to a transient return of the responsiveness in non-anaesthetized animals, and resulted in the appearance of pressor reflexes in the anaesthetized and spinal group. In these cases marked potentiation of the reflexes was seen after the administration of moderate doses of strychnine. The results suggest that a picrotoxin-sensitive inhibition might play an important part in the integration of vasomotor reflexes evoked by stimulation of cutaneous A-afferents. In addition, they point to the possibility that the intensity of inhibition is considerably increased by anaesthesia and even more so by spinal cord transection.

Mechanism of generation of action potential in Helix neurones: the barium spike

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Spike generation of neurones localized in the visceral left and right parietal ganglia of *Helix pomatia* was examined in physiological (normal) and Na^+-free solutions containing barium ions. In Na^+-free but otherwise normal medium, barium ions caused three kinds of change.

(i) the electrical activity of numerous neurones disappeared;

(ii) the hyperpolarizing after-potential of the calcium-spike generating neurones decreased;

(iii) some neurones which were not capable of generating calcium potential produced barium-spikes.

Thus, the site and mode of action potential generation can change in various ways from neurone to neurone.

Effect of barium ion on various parameters of spontaneous contraction of the Helix heart

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The isometric contractions, and the intracellular action potentials of the Helix ventricle were recorded by means of electro-mechanic transducer and conventional glass microelectrodes. The following parameters of the isometric contraction curves were examined in a medium containing 1—8 mM of barium ions: force of contraction (F_o), mean velocity of force development (S_1), mean velocity of relaxation (S_2), time to peak force (t_1) and relaxation time (t_2).

Parallel with elevation of the extracellular barium concentration, the force of contraction first increased, then decreased; the mean velocity of force development and that of relaxation decreased, though some initial facilitation could be seen as judged from the values of S_1 and S_2 in the presence of 4 mM barium. t_1 and t_2 increased with the duration of the intracellular action potentials.

An explanation is offered for the initial positive inotropic and the subsequent negative inotropic and negative klinotropic effects.

Studies on endocrine and lymphatic organs of congenitally athymic nude mice

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The histologic structure of the adrenal cortex, the thyroid, parathyroid and pituitary glands did not show significant alterations in congenitally athymic (recessive mutant nu/nu) mice when compared with litter-mate controls. This is in agreement with the authors' previous finding that in Wistar rats neonatal thymectomy did not influence the function of adrenal and thyroid glands (Acta med. Acad. Sci. hung. *21*, 297, 1965).

At the site of the thymus an epithelial rudiment was only found with crypts lined by cuboid or flattened epithelium containing metachromatic masses, but without lymphoid elements. In lymph nodes and spleen a remarkable lack of small lymphocytes was observed in the thymus-dependent areas with a high proportion of reticular cells and megakaryocytes in the spleen.

As a functional consequence of the dysgenesis of the thymus and the lack of thymus-derived cells, delayed-type hypersensitivity to oxazolone was found to be considerably lower than in the litter-mate controls.

The use of congenitally athymic (nu/nu) mice is considered to be a new genetic model for studies on thymus deficiency and its restoration.

In vitro metabolism of (4—^{14}C) 4-androstene-3,17-dione and (4—^{14}C) testosterone in healthy and pathological human skin

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Abdominal skin slices from healthy males and females and from patients with various endocrine disturbances were incubated with (4—^{14}C) 4-androstene-3,17-dione. The following metabolites were indentified: 5α-androstane-3,17-dione, androsterone, epiandrosterone, testosterone and 5α-dihydrotestosterone. Formation of 17-ketosteroids and testosterone was enhanced in the incubated abdominal skin slices of an agonadal patient. The abdominal skin slices of a patient with testicular feminization synthetized similar amounts of testosterone and 17-ketosteroids as did the normal female skin.

Abdominal skin slices of healthy males and females synthetized from (4—^{14}C) testosterone 4-androstene-3,17-dione, 5α-androstane-3,17-dione, epiandrosterone and adrosterone in the greatest amounts. In addition to these 17-ketosteroids, 5α-dihydrotestosterone was formed. In the agonadal patient 5α-dihydrotestosterone was synthetized in lower amounts, but the bulk of the substrate was converted to 17-ketosteroids. The abdominal skin slices of the patient with testicular feminization synthetized the same amount of 17-ketosteroids, but less 5α-dihydrotestosterone, than did those of healthy subjects.

Age-dependent changes in the relationship between heat production and body temperature after hypoxia and hypercapnia in the guinea pig

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Oxygen consumption and colon temperature of new-born and adult guinea pigs were measured in cold environment during and after hypoxia (8% O_2) and hypercapnia (6% CO_2). In the first 15 min after hypoxia and hypercapnia the ratio between the increase in body temperature and oxygen consumption (VO_2) was in all age-groups higher after 8% O_2 than after 6% CO_2. The ratio between the increase in body temperature and the increase in VO_2, was however higher after hypercapnia than after hypoxia. This was due to the fact that, in response to hypercapnia, the decrease in oxygen consumption was considerably less. Therefore, after the termination of hypercapnia, the increase was also smaller than during and after the hypoxic period. The post-hypoxic and post-hypercapnic increases in body temperature, when expressed in per cents of the changes during hypoxia and hypercapnia, were practically identical but decreased with age.

Significance of lymphatic and interstitial fluid pressure during oedema development

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Pancreatic oedema was induced in dogs by infusing physiological saline by means of a peristaltic pump into the superior pancreatico duodenal artery through one of its collateral branches, without interfering with blood flow in the artery. Lymph flow was measured in pancreoduodenal lymphatics, and intralymphatic pressure in one branch of a pancreoduodenal lymph vessel dividing in reversed Y-form. Interstitial pressure was determined by means of a perforated capsule implanted into the pancreatic tissue two weeks before saline loading.

A saline load applied at a rate of 16 ml/min for 60 min caused pancreatic oedema and increased lymph flow from the pancreatico, duodenal lymphatic and lymphatic pressure. Interstitial pressure in the pancreas was also increased. The pancreatic oedema became visible by the 15—20 min of infusion, its growth ran parallel with the rise of interstitial pressure; oedema formation preceded the peak values for lymph flow and lymphatic pressure. Increase of lymph flow during oedema formation appears to be a consequence of the increase in interstitial fluid pressure.

Altered renal response to vasopressin after indomethacin pretreatment in hydrated waking dogs

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In vitro studies have demonstrated that prostaglandins are capable of antagonizing the hydrosmotic effect of vasopressin. The present experiments aimed at testing the possible role of the above findings in intact animals.

8-lysine-vasopressin (2 mU/kg + 10 mU/kg/h intravenously) was given during water diuresis. The synthesis of prostaglandins was blocked by 2 mg/kg of indomethacin.

Indomethacin greatly enhanced the antidiuretic, but abolished the saluretic, action of vasopressin. The results suggest that intrarenal prostaglandins play an important role in modulating renal responses to vasopressin.

Behaviour of platelets in rat anaphylaxis

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The platelet count decreases precipitously in the first and second minute of severe anaphylaxis in the rat, to remain subsequently at the low level.

Experiments were carried out in order to clarify where the majority of platelets disappear during the first two minutes and why the other part of the platelet population remains in the circulation. ^{51}Cr-labelled platelets were used to follow their route. The remaining platelets were tested by various methods (bleeding time, clot retraction, platelet adhesion, platelet aggregation, platelet release reaction) in order to investigate their haemostatic ability. Finally, an attempt was made to reproduce the anaphylactic events under conditions *in vitro*.

The results emphasize the importance of platelets in the development of altered haemostatic mechanism in rat anaphylaxis.

Acrylamide gel electrophoresis of thrombins of various species

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With the aid of a Renal apparatus and small pore-size acrylamide gel, the diagnostic preparation Tophostasics (Hofmann-La Roche) was compared with human, chicken, goose, duck, sheep, bovine, rat and guinea-pig thrombins precipitated with acetic acid and purified with acetone. The marketed product as well as the different thrombins contained contaminating proteins in large quantities. A new method was developed for cutting gel columns into slices allowing to localize the thrombin efficiency. Coagulation activity was found in the slowly migrating protein fraction.

Relationship between RBF arterial and renal vein plasma renin activity and renin secretion

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In mongrel dogs under pentobarbital anaesthesis the relationship between blood pressure, renal blood flow, arterial and renal vein PRA and renin secretion rate was investigated in normotensive-isonatraemic, in sodium depleted, in sodium-loaded group. A close relationship between arterial PRA and renin secretion occurred to bleeding, suprarenal aortic compression and constriction of the renal artery.

A close correlation was found between arterial and renal vein PRA under every condition. Arterial PRA significantly differed from the values found in normotensive, control animals. The secretion rate of renin did not exhibit any significant difference from that of the normotensive controls, with the exception of the sodium-loaded group. A close relationship between arterial PRA and renin secretion occurred only in the normotensive-isonatraemic group.

It is suggested that arterial PRA the best index of net renin production.

Inhibition of macrophage migration by duct lymphocytes in adrenalectomized, non-sensitized rats

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MIF production of duct lymphocytes was studied in the presence of specific antigen in CFY male rats with BSA. In vitro MIF production was determined by measuring the migration of guinea-pig alveolar macrophages.

MIF production was inhibited by the lymphocytes of sensitized and bilaterally adrenalectomized rats. Their lymphocytes exerted a 30—40% inhibitory effect even in the absence of antigen.

In another series of experiments the effect of duct lymphocytes was investigated on macrophage migration in non-sensitized, adrenalectomized rats. A spontaneous MIF-like effect was produced only by the lymphocytes of adrenalectomized animals.

Experiments were performed on untreated further on adrenalectomized ACTH-treated as well as on hypophysectomized and adrenalectomized rats to clarify the role of the pituitary in the phenomena observed.

Combined effect of physical training models and nutritional factors in the rat

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The effect of two training methods, endurance training and interval training, as well as of a special diet was studied in untrained rats and in animals previously subjected to exhaustive swimming exercises daily for three weeks. Swimming performance and various biochemical indices (plasma steroid level, serum LDH activity, blood sugar level and liver glycogen content) were measured. Endurance training consisted of 60 min swimming daily with 2 g/100 g of additional weight. Daily swimming exercises with 4 g/100 g of additional weight for 5×10 min with one min intervals constituted the interval training.

The experimental period lasted 8 days; performance was checked and biochemical estimations were carried out on the 9th day. The special diet was a 6-day period of protein-rich and carbohydrate-poor regiment followed by a diet rich in cabohydrate and poor in protein for 2 days. Control animals were given normal compressed food.

The 8-day training significantly improved performance in every previously untreated animal, with the greatest improvement in the endurance-trained group consuming the special diet. An even more marked improvement was found in animals subjected to previous training. Here the best results were achieved in the group subjected to interval training and given the special diet. Biochemical studies revealed several changes reflecting the beneficial effect of physical training even in previously untreated animals that only swam on the 8 training model days.

Effect of azidomorphines on respiration

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The effect of azidomorphine (AM), 14-OH-azidomorphine (OAM), azidocodeine (AC), 14-OH-azidocodeine (OCA), morphine, codeine, hydrocodone and oxycodone was investigated on respiratory frequency, depth and minute volume in cats anaesthetized with 30 mg/kg of pentobarbital. The drugs were given intravenously in a cumulative manner. Azidomorphines, except OCA, in cough-depressing doses (AtD100) did not influence respiration. In contrast, the non-azido derivaties significantly depressed at least one of the respiratory parameters.

The time course of the respiratory response to a single intravenous injection of AtD100 was also analysed. The half time of the frequency-depressant effect of morphine was longer than that of azidomorphine. The cough reflex remained depressed after the respiratory parameters had returned to the control level.

In unaesthetized rats the suppression of respiratory frequency was significant only with the AtD50 of OAC among the azidomorphines after oral administration.

Interaction between adrenoreceptors and cAMP in the regulation of renal function and renin secretion

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The effect of dibutyryl-cAMP on renal function and renin secretion (RS) has been investigated in anaesthetized dogs under normotensive, isonatraemic conditions as well as in animals pretreated with selective alpha- and beta-antagonists. The antagonists and dibutyryl-cAMP were infused into the left renal artery for 20 min each.

During infusion of 1 mg/kg of dibutyryl-cAMP, RBF and RS significantly increased in the first 10 min. During the second 10-min of the infusion these parameters continued to increase and an additional significant elevation was found in the arterial PRA.

After pretreatment with phenoxybenzamine or propranolol at a rate of 10 μg/kg/min, the infusion of 1 mg/kg of dibutyryl-cAMP failed to change the renal haemodynamic parameters but significantly increased RS.

It is concluded that dibutyryl-cAMP has a direct facilitating effect on RS that is independent of the adrenoreceptors.

Role of serotonin in mediation of the behavioural effect of adrenocortical hormones

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Lesion of the mesencephalic n. raphe dorsalis resulted in a decrease of cerebral serotonin (5-HT) content in rats. Acquisition of a one-way active avoidance reflex was facilitated while its extinction was inhibited in raphe-lesioned animals. This supports the authors' previous finding that cerebral 5-HT is involved in the regulation of avoidance behaviour.

DL-parachlorophenylalanine treatment (300 mg/kg b.w.) that decreases cerebral 5-HT content, prevented both the elevation in brain 5-HT level induced by low doses of corticosterone and the behavioural effect of the hormone. Nialamide pretreatment (125 mg/kg), on the other hand, which elevates cerebral 5-HT content, counteracted the decrease of 5-HT concentration induced by high doses of corticosterone and abolished the behavioural effect.

The results support the view that the cerebral serotoninergic mechanism participates in the mediation of the behavioural action of adrenocortical steroids.

Analysis of pulse-synchronous and respiration-synchronous pressure changes in the cest of anaesthetized cats

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The cardirespiratory effect of anticholinesterases and the highly complicated mutual interference of pulmonary circulation and ventilatory mechanics have been analysed previously. The present paper deals with the superposition of the pressures measured in the two systems. The pulmonary arterial (P_{ap}) and oesophageal (P_{oe}) pressure signs and the pneumotachogram (P_t) have been studied by Fourier-analysis.

It was demonstrated that the seemingly pulse-synchronous of P_{oe} actually followed the variations of P_{ap} and that flow undulations of the same origin were observed on the P_t. The possibility of separation of the respiration and pulse synchronous events has been studied in case of (1) variations of the circulatory blood volume; (2) application of external stenosis; (3) after drug administration.

The respiration-synchronous undulations were always superposed upon the P_{ap}, but the pulse-synchronous superposition upon the phasic signs of the ventilatory mechanics depended on several factors.

Characterictics of Kinase I

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When a vasoactive kinin is incubated with blood or with tissue homogenate, it is rapidly cleaved to smaller peptides. Plasma of man and animals contains a carboxypeptidase (E.C.3.4.12.7) that cleaves basic C-terminal amino acids, including Arg^9 of bradykinin.

The purified plasma enzyme has a molecular weight of 280,000. It gradually dissociated to smaller subunits, to 90,000 and 45,000 molecular weight proteins, called "heavy" and "light" chains.

The dissociated enzyme lost its activity very easily. The native high molecular weight enzyme is more stable before dissociation.

The molecular weight of the liver enzyme was estimated at 45,000, the same as that of the light chain of the plasma enzyme.

Carboxypeptidase N differs from B in its distribution in the body, and because its molecular weight, subunit structure, substrate specificity and sensitivity to inhibitors are different.

Relationship between hypnotic susceptibility and cortical alpha-activity

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In the waking state a relationship was found between hypnotic susceptibility and the modification of cortical alpha-activity. In the present experiments the characteristics of alpha-activity were examined in waking and in hypnotized university students of high and low susceptibility, with 9 subjects in each group. No difference was found in the average frequency or in the amplitude of alpha-activity as a function of susceptibility to hypnosis in either the waking state or during hypnosis. Its duration, however, showed a relationship characteristic of the degree of hypnotizability.

a) In the waking state alpha-activity persisted longer in the highly susceptible subjects than in those of low susceptibility;

b) in hypnosis, there was no change in the duration of alpha-activity in the highly susceptible subjects;

c) there was a significant decrease in the duration of alpha-activity in subjects of low susceptibility, as a result of hypnosis.

The differences seem to indicate that susceptibility to hypnosis is related to the synchronization activity of the central nervous system.

Antagonism in vascular action between angiotensin II and its analogues

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Several analogues of angiotensin II (AII) have been shown to antagonize the vascular action of AII. The antagonistic potency is dependent on amino acid alterations at different positions along the peptide chain. In the present investigations newly synthetized analogues of AII have been tested for their ability to reduce the vascular effect of AII and for the specificity of their action.

The experiments were performed on anaesthetized cats and rats as well as on isolated organs. The substances were applied either in single injection or by continous infusion. Hypertensin (CIBA) was used to investigate the antagonism. The effect of the analogues on blood pressure was varying. Some of them produced hypertension while others hypotension. The blood pressure elevating effect was less pronounced than that of AII applied in identical doses. The heart rate was not altered. All substances exhibited antagonistic properties. Specificity of the action and structure—effect relationships are discussed.

Interstitial fluid pressure under physiological and pathological conditions

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According to *Guyton,* pressure of the interstitial fluid is equal to the pressure in implanted perforated capsules after a period of equilibrium and regression of inflammation.

Interstitial fluid pressure was measured according to *Guyton* by implanting a capsule into the subcutaneous tissue of the rat. The pressure was between —8 and —5 mm Hg, 4—6 weeks after implantation; it proved to be a function of time after implantation. After intravenous injection of 20% polyvinylpyrrolydone solution, the pressure decreased from —8 to —24 mm Hg. During haemorrhagic shock (2.5-hour hypovolaemia) the negative intersitial fluid pressure decreased and did not return to the control value 10 min after blood replacement. The degree of shock was controlled by monitoring glucose, inorganic phosphorus and FFA values in the plasma. The total protein concentration of the capsule fluid was also measured, assuming that it played a role in the pressure changes.

Effect of hypokinesis on ultrastructure of the rabbit skeletal muscle

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The soleus and gasctrocnemius muscles of adult rabbit were immobilized for 2, 4 and 6 weeks by plaster splints. Control specimens were obtained from the contralateral hind limbs and from non-immobilized rabbits of comparable body weight. Semi-thin and ultra-thin sections of the muscles revealed that hypokinesis led to considerable structural damages. First, a gradual and marked increase was observed in the number of fat cells and connective tissue elements, associated with a loss of muscle fibres. In the degenerated fibres, the myofibrillar system was disorganized; at some places the Z-lines disappeared and the contractile filaments of the myofibrils became loose and disoriented. Many myofibrils were split up. A further characteristic feature of the degenerative change was an undulation of myofibrils and also of the peripheral regions of muscle fibres. Numerous vesicles could be seen in the maintained membranous system, especially in regions close to the sarcolemma.

Oestrogen responsiveness of the hypothalamus during oestrus and after ovarectomy

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The experiments were performed on sexually mature female rats. Prior to the investigations vaginal smears were taken from the animals at 9 a.m. for 14 days and checked after staining with methylene blue. Only animals showing a regular cycle were used. Ovarectomy was performed 48 hours before measurements which were carried out at 9 a.m. At this time, the animals received 0.1 μg of ^{3}H-oestradiol subcutaneously and were decapitated one hour later. Among the cerebral regions investigated striking changes were found in the preoptic-anterior hypothalamic area (POA—AH) and the eminentia medina-basal hypothalamus (ME—BH). Changes were marked in the radioactivity bound to the nuclear fraction and the nuclear receptor. The amount of ^{3}H-oestradiol bound to nuclear receptor was considerably increased in both regions when compared with intact animals at any stage of the cycle. The lowest values during the cycle were found during prooestrus in both regions, the highest radioactivity in POA—AH during oestrus and in ME—BH during metaoestrus.

Microcirculatory structure and function in the adjuvant-induced polyarthritis of the rat

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Freund's adjuvant-induced polyarthritis of the rat is now the closest experimental approximation to human rheumatoid arthritis. In disorders of connective tissue the abnormalities of the microvasculature are prominent. Microcirculatory function has not yet been investigated in this model. Previous studies revealed increased platelet aggregation in arthritic rats. In the present study thrombus formation was investigated by the laser technique. In arthritic rats thrombus formation in response to a given stimulus was significantly increased. In later stages of the inflammation leukocytic sticking and emigration were the prominent features. The number of rolling and sticking leukocytes was greatly increased in the mesenteric venules of arthritic rats and their skin exhibited striking morphological changes with stasis of erythrocytes. The role of these events in the pathogenesis of the disease is discussed.

Effect of phenobarbital pretreatment on the hepatic transport of bromsulphophthalein (BSP) in the rat

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Hepatic uptake, conjugation and biliary excretion of BSP was investigated in phenobarbital-treated (50 mg/kg day intraperitoneally for 4 days) male Wistar rats of 170 g body weight. Under intraperitoneal urethane (1.2 g/kg) anaesthesia the left carotid artery and the common duct were cannulated and the bile was collected. Concentration of BSP in plasma, liver and bile was determined at various intervals after intrafemoral administration of ^{35}S—BSP.

Increased disappearance of BSP from plasma and a simultaneous increase in hepatic BSP uptake were found in rats treated with phenobarbital. The increased hepatic removal of BSP was attributed to the increased liver weight. Phenobarbital treatment also enhanced the biliary excretion of BSP, which was due to the enhanced bile flow and increased conjugation of BSP with glutathione. The importance of accelerated conjugation in biliary excretion of BSP was supported by the finding that, when applied in equimolar doses, more BSP-GSH was excreted than BSP.

Guanidinosuccinic acid (GSA) biosynthesis

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Recent studies from this laboratory have shown that urea production is suppressed by elevated urea concentrations both in vivo and in vitro. The rate of GSA synthesis could be increased by loading with urea in vivo.

The present experiments had the aim to reveale the molecular mechanism of the effect of urea as well as the biochemical pathways involved in GSA synthesis.

The results were as follows.

1. Urea is a potent competitive inhibitor of the arginino-succinate lyase (ASLase) enzyme.

2. Inhibition by urea of ASLase activity is responsible for the diminished rate of ureogenesis regularly seen in the presence of elevated urea concentration.

3. Due to the inhibition of ASLase activity the equilibrium concentration of argininosuccinate (AS) is increased.

4. Transient accumulation of AS leads to the activation of an unknown enzyme catalyzing AS transformation according to the equation,

$$AS + NH_3 \rightarrow \text{ornithine} + GSA$$

5. Accordingly, GSA biosynthesis is a process of ammonolysis.

6. Urea should be regarded as a substance of indirect toxicity in uraemia since, due to its ASLase-inhibitory ability, it leads to increased production of a GSA, a known uraemic toxin.

Effect of p-bromomethamphetamine on the mouse-killing behaviour of the rat

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Para-bromomethamphetamine (V-111) strongly inhibits the neuronal reuptake of serotonin (5-HT) in cerebral synaptosomal preparations. In acute experiments it increases, while during chronic treatment it decreases, the serotonergic tone of the brain (*Knoll, J.* et al.: Acta physiol. Acad. Sci. hung. *37,* 151, 1970). V-111 impairs some learning performances in the former case but improves them in the latter one (*Knoll, B.* et al.: Proc. int. Union physiol. Sci. p. 217, 1974). Mouse-killing (muricide) behaviour is a type of the rat's agressive behaviour (*Karli, P.:* Behavior *10,* 81, 1956); it can be facilitated by p-chlorophenylalanine (pCPA) or by midbrain raphe lesion (*Grant, L.* et al.: Pharmacol. Biochem, Behav. *1,* 77, 1973). V-111, due to its selective influence on the serotonergic regulation in the brain, inhibits muricide behaviour after acute treatment, and while its chronic administration induces aggressiveness in non-agressive animals.

Effect of HCG on survival of fetal zone cells in human embryonic adrenal tissue cultures

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In tissue cultures of human embryonic adrenals, after the second trimester of pregnancy only the cells from the subcapsular or definitive zone survive and proliferate. After administration of ACTH to the culture medium these cells produce substantial amounts of corticoids. The major part of the embryonic adrenals, the so-called fetal zone does not survive either in the control or in the ACTH-treated cultures. If human chorionic gonadotrophin (HCG) is added to the culture on explantation and further 15 I.U./ml HCG are administered at each washing, the fetal zone cells survive. The rich smooth-surfaced endoplasmic reticulum, the well-developed Golgi apparatus, the round or oval mitochondria with few vesicles are characteristic of the fine structure of fetal zone cells. Hormone production by these cultures approximates the production by control cultures. Earlier data in the literature suggest that HCG might play a role in the survival of the fetal zone in vivo. This hypothesis could not be confirmed. The phenomenon observed in tissue culture suggests the key role of HCG in survival of fetal zone cells in embryonic adrenal cultures after the second trimester of pregnancy.

ACh accumulation proximal to nerve ligature

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It is now well established that ACh is synthetized in the cholinergic nerve terminals, and all the necessary precursors and enzymes have been found there. However, ACh, its precursors and the enzymes necessary for synthesis as well as the metabolites are present also in the axon. It has been shown (*Hebb* and *Silver, Kása* et al., *Lubinska* and *Tucek*) that ChAc and ChE accumulate proximally to the nerve ligature; the enzymes are assumed to be transported by axonal flow.

The intraaxonal flow of ACh has been studied in the preganglionic trunk of the cervical sympathetic nerve of the cat. Ligature of the preganglionic nerve for 24 hours did not cause any significant increase in ACh content proximal to the ligature (95.6 ± 32.1 nmol/g) when compared with the distal part (58.7 ± 13.5 nmol/g). After 120 hours, however, the ACh content was 4.6-times higher above than below the ligature (164.1 ± 60.0 and 35.3 ± 3.9 nmol/g) respectively. The ACh content of the superior cervical ganglion did not differ on the ligated and non-ligated (control) side 24 hours after ligature. After a 120-hour ligature, it was however, significantly lower (18.6 ± 5.8 nmol/g) in the ligated than in the control ganglion (42.9 ± 9.4 nmol/g). When a second ligature was applied 10—15 mm below the first one, two peaks in ACh accumulation were observed; both of them were situated promixal to the ligature. The refore, an intraaxonal flow of ACh along the preganglionic nerve of the sympathetic trunk is supposed. In addition, the accumulation of ACh seemed to be a process independent of the continuity with the perikaryon.

Effect on sugar absorption of uraemia caused by renal obstruction

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Kidneys of rats and rabbits were damaged by urethral compression for three days, and the active transport of glucose from the intestines was examined.

Under uraemic conditions brought about by urethral compression the blood levels of non-protein nitrogen, potassium, creatinine and glucose were increased and the sodium and chloride contents decreased. Glucose transport from the intestines also diminished.

Urethral compression was discontinued after three days; six days later the active transport of glucose was nearly normal.

After elimination of the renal damage, renal function approximated the normal state within a short period of time.

Effect of direct cortical stimulation on the redox state and blood content of the cerebral cortex during hypotension and hypoxaemia

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Electrical stimulation of the cerebral cortex evokes oxidation of NADH and an increase in blood content. These effects were used to indicate the redox and vascular states of the cortex during hypoxaemia and hypotension.

Fields of 100 μ in diameter were studied in the anterior suprasylvian gyrus of cats. The redox state and the vascular state were measured by surface microfluorometry and UV reflectometry, respectively. The bilateral ECoG was recorded simultaneously in fronto-parietal leads.

1. At normal blood pressure level a marked vasodilation occurred while the changes in the redox state of the tissue pyridine nucleotides varied considerably.

2. A decrease of arterial blood pressure to 60 mm Hg diminished the vascular response, while at 40 mm Hg no detectable vasodilation was present any more.

Experimental model for spatial localization of intracerebral foci

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On the basis of the principles of vector EEG a stereo-electroencephalographic method has been developed that involves a computer suitable for the determination of the site of intracerebral foci.

In 16 cats (12 acute and 4 chronic preparations) the intracerebral focus was stimulated by an electrode that stimulated various specific and non-specific areas of the brain. The evoked potentials elicited in this way were recorded monopolarly in the three spatial dimensions on identical points of the hemispheres. The phase difference of the recorded waves and the velocity of the excitation process were determined with a computer from the cross-correlation and averaged curves. On the basis of the above data and knowing the place of the registration points the site of the stimulating electrodes was estimated by means of a computer program. The results corresponded well to the actual position of the electrodes.

This simple method might prove suitable for routine investigations.

Evidence for specific GABA uptake by certain nerve terminals of the external plexiform layer of the rat olfactory bulb

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GABA has been found to inhibit certain synapses of the olfactory bulb, but identification of the neural structures that take up GABA in this brain region is lacking.

(^{3}H)-GABA was injected stereotactically into the ventricle of the olfactory bulb. After perfusion with fixative, the bulbs were removed and prepared for light and electron microscopic radioautography. Labelling of identified tissue components was evaluated statistically on electron microscopic pictures.

GABA-uptake was high in certain nerve terminals and glial processes, while (^{3}H)-glycine or (^{3}H)-leucine did not show any specific accumulation.

The results provide support for the assumed inhibitory nature of the granule cell terminals.

Effect of phenoxybenzamine on cerebral blood flow during haemorrhagic shock

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The experiments were performed on baboons anaesthetized with phencyclidine and premedicated with 5 mg/kg of phenoxybenzamine (PBZ). Hypovolaemic shock was produced according to a modified Wigger procedure. Cerebral blood flow (CBF) was measured by ^{133}Xe-clearence. Blood gas tensions as well as glucose, lactate, and pyruvate concentrations were determined in arterial and cerebral venous blood samples.

Before haemorrhage, CBF was not altered by PBZ when compared with the non-treated control. CPF did not decrease during bleeding; after reinfusion, it considerably exceeded the control value. Cerebral oxygen consumption was increased in shock. Uptake or release of lactic acid and pyruvic acid remained unaltered while glucose consumption seemed to be affected by PBZ both before and during shock.

Effect of ureteral occlusion on the intrarenal distribution of renal vascular resistance

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Total renal blood flow increased or remained unchanged during ureteral occlusion in spite of the augmentation of postcapillary vascular resistance. It is generally accepted that the increase of the postcapillary resistance is compensated or even overcompensated by the decrease of the precepillary one. Results obtained by the Rb-technique (Rb-RBF) however indicated other possibilities of compensation. Ureteral obstruction in the isolated (denervated) kidney as well as in the in situ (innervated) kidney combined with inhibition of alpha-receptors or carotid occlusion resulted in a parallel and nearly equal reduction of Rb-RBF and TRBF. Thus, augmentation of the postcapillary resistance elicited by ureteral occlusion was not compensated under these conditions. Data for changes in the cortical and medullary circulation indicate that (1) the compensation needs functionally intact sympathetic vasoconstrictor mechanisms not influenced by other extrarenal factors; and (2) compensatory reduction of the vascular resistance is brought about by the medullary vessels.

The antitussive effect of azidomorphines

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It was reported earlier (*Knoll* et al. J. Pharm. Pharmac. *25*, 929,1973) that hydrogenation to remove the double bond between C7 and C8 of morphine structure and substitution of an azido group in position C6 strongly increased the analgesic potency. Methyl or ethyl ether substitution in position C3 increased the lipid solubility. Of the 3-ether-azidomorphine derivatives, the pharmacology of azidoethylmorphine (AEM), azidocodeine (AC) and 14-OH-azidocodeine (OAC) was analysed and compared with codeine (C) after oral administration in the rat.

1. The oral antitussive activity of AEM proved 60-times higher than that of. C. 2. The ratio of LD_{50} to antitussive ED_{50} of AEM was 54-times more advantageous than that of C. 3. The ratio of analgesic ED_{50} to antitussive ED_{50} of 3-ether-azidomorphines revealed high antitussive speficifity; the antitussive receptors were more sensitive to 3-ether-azidomorphines than the analgesic ones. 4. In antitussive doses AEM and AC caused no respiratory depression. CAC and C depressed the respiratory rate. 5. The ratio of the dose reducing respiratory rate by 75% to the antitussive ED_{50} was 14.3 for AEM and only 1.4 for C. 6. AEM and AC rapidly penetrate into brain tissue; this fact may explain their high antitussive potency.

Cation efflux from the rat's ventricular myocardium

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Myocardium strips were isolated from the rat's right ventricular muscle 16—20 hours after the intraperitoneal injection of ^{24}Na, ^{42}K, or ^{86}Rb. The preparation was perfused with tracer-free Ringer solution of 34 °C. Activity of the strip was determined at intervals by using a well-type counter. After the necessary corrections the values for radioactivity were plotted against time semilogarithmically and the parameters of exponential functions calculated by the least square rule.

Three different compartments of K and Na, and two of Rb were found on basis of equations of the fitting functions. From the equations the size of the ion fractions and cation fluxes was estimated by supposing both parallel and series models of ion compartments.

A new, non-depolarizing neuromuscular blocking agent

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Among bisquaternary steroid salts, compound RGH-1106 proved to be a most effective neuromuscular blocking agent.

Its activity was studied in anaesthetized and waking animals as well as in nerve-muscle preparations.

In all species examined the duration of action was 15—25 min at the dose producing 50% inhibition (2—4 mcg/kg). The response of the muscle to direct stimulation was normal during the block. The neuromuscular blockade was reversed by anticholinesterases or by tetanic stimulation of the nerve.

The mechanism of action was of the competitive type, because the compound produced flaccid paralysis in the conscious chick. The blocking action was additive in the case of drugs with a similar type of action (d-tubocurarine) and it inhibited the twitch caused by intraarterial injection of acetylcholine. The haemodynamic parameters and bronchial resistance were not altered by doses larger than those producing complete neuromuscular block.

Effect of dexamethasone on the subcellular phospholipid composition of tonic and tetanic muscles

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The phospholipid composition of the subcellular fractions of the tonic slow soleus muscle and the tetanic, fast semimembranosus muscle of the rabbit was examined in the course of myopathy induced by dexamethasone. Administration of dexamethasone for two weeks did not cause any significant change in the quantity of phosphatides in any of the subcellular fractions of the muscles examined. The amount of lipid aldehyde in phosphatides decreased in both the sarcoplasmic and the sarcolemmal fractions of the two kinds of muscle. The decrease was more pronounced in the sarcoplasmic than in the sarcolemmal fraction, and more marked in the tetanic than in the tonic muscle.

Polysensory interactions in the orbitofrontal cortex

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Bilateral interactions in the nucleus tractus solitarii, evoked by stimulation of the vagal nerves, can be described mathematically. The question was analysed systematically in chloralose anaesthetized cats in the orbitofrontal cortex, regarded as a polysensory area. Analysis of the separately and simultaneously evoked potentials induced by various sensory stimuli showed that the character and the degree of spatial of the size interactions can be expressed quantitatively as a function of the separately evoked responses.

Effect of 2,4-dichlorophenoxyacetate (2,4-D) on LDH activity of the rat muscle and cell culture

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According to data in the literature, injection of 2,4-D gives rise to myopathy in rats due to a general disturbance of the intermediary metabolism. These changes comprise alterations in carbohydrate metabolism. In order to study the question, rats were made myotonic by intraperitoneal injection of 2,4-D. LDH activity and isoenzyme pattern in the muscles were examined.

In the acute state, the H-related isoenzymes increased in heart tissue while the M-related ones decreased or disappeared, as compared with the control. Slight shifts in the isoenzymes were found in the red and white muscles.

Chronic treatment evoked no essential change in the heart, when compared with the results obtained in acute experiments. LDH-4 and LDH-5 underwent a significant change in the red muscle, while only LDH-5 exhibited an essential change in the white one.

Though the studies revealed changes in the LDH isoenzyme pattern, it is assumed that these shifts had an indirect role in the myopathy induced by 2,4-D.

Transcapillary fluid transport after nephrectomy

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Changes in plasma volume, haematocrit value and plasma protein concentration have been examined in hypervolaemic anaesthetized dogs after ligature of the renal hilum, and in control animals without nephrectomy. Hypervolaemia was induced by intravenous infusion of physiological saline. Ten min after fast infusion (10 ml/kg b.w. of physiological saline of body temperature given in three minutes) the plasma volume increased by 8 ml/kg b.w., as compared with 1.7 ml/kg found in the controls.

The haematocrit value did not change in the controls and decreased in the nephrectomized group in proportion to the increase of the plasma volume.

Plasma protein concentration in control animals decreased more markedly than it was expected from the changes in plasma volume. After nephrectomy, it fell corresponding to the dilution.

After slow infusion (0.25 ml/kg b.w. of physiological saline) the same changes were found in both groups.

It is concluded that transcapillary fluid transport considerably decreases after nephrectomy.

Three-dimensional analysis of arterial elasticity

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The theory of incremental deformation mechanics was applied for studying non-linear anisotropic elastic properties of the arterial wall. Cylindrical human and canine arterial segments from different regions were subjected to quasi-static

large deformation tests performed at different initial axial stretches in vitro. Intraluminal pressure was changed slowly (200 mm Hg/min; 0—250 mm Hg); axial extension force and outer diameter of the artery were monitored continuously. Three-dimensional stresses, strains and incremental elastic modules were computed considering the artery as a homogeneous incompressible orthotropic cylinder.

It was found that the coupling between axial and tangential deformations was non-linear; its strength and sign depended on the intraluminal pressure, the initial axial length and the type of artery. Non-linear mechanical properties were characterized by nine incremental elastic modules.

Electrophysiological characteristics of the antiarrhythmic effects of amiodarone on the ventricular musculature and Purkinje fibre of the dog heart

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In previous experiments a prolongatio of the atrial action potential was demonstrated to occur in hypothyreosis, independently of the pulse rate. Hyperthyreosis acted in the opposite way. Atrial arrhythmia occurs rarely in hypothyreosis and often in hyperthyreosis, a finding to be explained by the changes mentioned above. Hypothyreosis, lengthening of atrial action potentials and antiarrhythmic effect can also be achieved by prolonged administration of amiodarone a benzofurane derivative. The drug decreases the frequency of atrial and ventricular arrhythmia.

In order to investigate the mechanism of the ventricular antiarrhythmic action, dogs were treated with a high intraperitoneal dose of amiodarone (15 mg/kg/day) for 3 weeks. Intracellular action potentials were recorded from various parts of preparations containing the Tawara bundle, Purkinje fibres and ventricular musculature of the right heart. Amiodarone treatment caused a much more marked prolongation of the action potential in the working muscle than in the Purkinje fibres or the Tawara bundle, indicative of a decreased inhomogeneity of ventricular electricity. Amiodarone also diminished the adrenaline-induced augmentation in the automacy of Purkinje fibres. The alterations observed may explain the antiarrhythmic action of amiodarone, or that of the hypothyreosis caused by the drug.

Effect of Triton WR on experimental inflammation

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Previous investigations demonstrated Triton WR 1339 to have a stimulatory effect on certain components of the inflammatory reaction (i.e. phagocytosis). In the present experiments the effect of Triton WR 1339 was examined on local and systemic reactions to inflamming, Selye's modified granuloma pouch technique.

The animals were divided into six groups: 1) control; 2) granuloma pouch; 3) granuloma pouch + 1 ml Triton solution (250 mg/ml) administered intravenously every other day; 4) granuloma pouch + 1 ml Triton solution (125 mg/ml) administered intravenously every other day; 5) granuloma pouch + 1 ml Triton solution (250 mg/ml) injected into the pouch itself; 6) granuloma pouch + 1 ml Triton solution (125 mg/ml) injected into the granuloma pouch itself.

The animals were killed by decapitation on the 10th day of the experiments. Leukocyte count, protein content and concentration of the acute phase proteins (haptoglobin, coeruloplasmin, alpha$_2$-MG, seromucoid) were determined in the serum and the inflammatory exudate of the granuloma pouch.

Triton had no influence on the serum level of acute phase proteins either after intravenous or local application. Leukocyte count diminished after intravenous but not after local Triton treatment. High doses of Triton decreased the volume of exudate by both ways of administration. The protein content and acute phase protein level of the exudate did not change, except the alpha$_2$-MG fraction; this diminished after both types of Triton treatment.

Bile constituents in plasma and lymph after occlusion of the common duct

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In the first hours following common duct and cystic duct ligature in dogs, bilirubin and bile acids regurgitating from the biliary tract are mainly transported by the lymphatics. Both substances reached the maximum lymphatic concentration between the 4th and 6th hour and their concentration in lymph remained above the plasma level during the 24 hour observation period. The plasma concentration of bilirubin and bile acids rose more slowly but the rise was continuous. The amount of both substances transported by the lymphatics increased until the 6th and 8th hour, respectively, and exceeded the amount transported by the veins as early as by the second hour. It is concluded that during occlusion, when pressure in the biliary tract reaches the secretory pressure of bile, bilirubin and bile acids escape from the small biliary channels and will consequently be transported into the blood stream by the lymph vessels.

Changes of adenyl cyclase activity after capsaicin desensitization in the rat brain

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Desensitization of the hypothalamic thermodetectors by capsaicin induced an irreversible impairment in the thermoregulation of rats. In order to elucidate the action of capsaicin at the molecular level, its effect on adenyl cyclase activity was investigated in the preoptic area of the hypothalamus, the cerebral cortex and cerebellum of the rat brain. Adenyl cyclase activity was significantly increased in the preoptic area seven days following capsaicin desenzitization.

Capsaicin (10^{-5}M) increased the adenyl cyclase activity of untreated brain regions in vitro. The enhancement of adenyl cyclase activity after desenzitization was inhibited by addition of capsaicin in the preoptic area in vitro, whereas the activating effect remained unaltered in other regions of the brain. Considering that capsaicin acts selectively on adenyl cyclase activity of the preoptic area it is assumed that the pharmacological effect of capsaicin is mediated through the cyclic-AMP system.

Analysis of muscular fatigue

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It has been shown that the fatigue of isolated muscle represents a complex phenomenon. Previous biophysical, electrophysiological and mathematical studies have lent support for the assumption that the excitability of muscle before stimulation and the changes in excitability during continuous stimulation play an important role in fatigue.

In the present experiments the changes in stimulation threshold were investigated during fatigue under various experimental conditions. A close relationship was demonstrated between the effect of local anaesthetics and antihistamines on muscle fatigue and excitability.

Alterations of cerebellar visceral input during the sleep—wakefulness cycle

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The evoked potentials elicited by stimulation of large (Aβ) and small fibres of the splanchnic nerve were studied during the sleep—wakefulness cycle. The responses were recorded from the anterior cerebellar lobe as well as the fastigial and ventro-lateral thalamic nuclei.

During slow-wave sleep a marked facilitation of the evoked responses was found after both weak and strong stimuli. Weak stimuli failed to activate the splanchnico-abdominal reflex.

Effect of early alpha-methyldopa treatment on behaviour of the rat

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A method was elaborated for studying the development of paradoxical sleep (PS) in the new-born rat. The animals were separated from their mother, fed artificially through an immolated gastric tube and recorded continuously during the first three postnatal weeks.

Daily administration of 500 mg/kg of d,l-alpha-methyldopa (MDOPA) resulted in lasting and marked (75%) deprivation of PS (*Carlier, E.* et al., Psychopharmacologia *37,* 205—215, 1974). In order to test the hypothetical role of PS in the maturation of the central nervous system, baby rats not isolated from their mother were given 250 mg/kg of l-alpha-methyldopa daily during the first three postnatal weeks. At the age of five weeks the open-field test revealed increased locomotor activity. In shuttle-box conditioning started on the 41st day of life the treated animals showed a higher rate of acquisition but the control animals reached the same level of performance.

Noradrenaline, dopamine and serotonin content of the brain was not affected by the treatment.

Physical factors influencing the effect of some spasmogens

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The pharmacological activity of pentetrazole and some pyridine-tetrahydroisoquinolines has been measured in mice *in vivo* and on fundus strips of the rat stomach *in vitro*. The activities were compared with those of solutions treated in changing magnetic and static electric fields as well with supersonic waves.

Treatment of the solutions in changing magnetic and static electric field decreased spasmogenic activity. The static electric field altered the intrinsic activity. Supersonic waves abolished nearly all the activity measured *in vitro* but did not influence the effect *in vivo*.

Aggregation and contact-inhibition of platelets

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The negative charge of platelets was studied by means of electrophoresis. The nature of the anions carrying the charge was examined by electrophoresis or chromatography of three different thrombocyte extracts.

Part of the negative charges causing contact-inhibition proved to be due to creatine phosphate that was determined, through transformation to creatinine, with alkaline picronitrate. Another part was due to the sialic acid terminals of the gangliosides and sialoglycoproteins. These latter were measured by thiobarbituric acid assay.

Phosphate is split of from creative phosphate by ADP or adrenaline, while sialic acid terminals are blocked by serotonin or collagen, both reducing contact-inhibition and inducing aggregation of thrombocytes. The aggregation was studied by turbidimetry.

A synergism was found between the activity of ADP or adrenaline and that of collagen and serotonin, respectively, with antagonism between the members of the two groups. The receptors of the two groups of aggregation-inducing agent could be protected by different drugs: pyridine-carbamate and ethyl-apovincamate, respectively.

Drug effects under hypoxic conditions

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Animals kept under hypoxic conditions are known to develop tolerance to hypoxia. The question arose whether the seemingly beneficial effect of some drugs would be due to their causing hypoxia. The present experiments showed that the protective effect developed also under acute conditions. Groups of mice of 5 animals each were placed into a vessel containing O_2 and N_2 in various ratios, and their average survival was measured. Keeping the animals under slightly hypoxia conditions for 30—60 min afforded protection to lethal hypoxia. Noradrenaline when applied in intravenous doses from 10 to 100 μg/kg acted in a way similar to hypoxia. Immediately after the injection, it acted as under additional hypoxia, and lethality increased. In 5, 10 and 30 min a gradual tolerance have developed.

Propranolol (10 mg/kg) produced a uniform protective effect, instead of the gradually developing tolerance for 0—30 min after the injection. The method is suitable to distinguish between drugs acting by developing tolerance to hypoxia and those causing a diminution of the O_2 demand.

Effect of serotonin and 5-hydroxytryptophan on the hypothalamo-pituitary adrenal system in vitro

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The action of serotonin and 5-hydroxytryptophan has been studied on the hypothalamo-pituitary adrenal system *in vitro*. Serotonin inhibits the hypothalamo-pituitary-adrenal system at the hypothalamic level, by inhibiting the synthesis and/or release of corticotropin releasing factor. The precursor of serotonin, 5-hydroxytryptophan, stimulates corticosterone production by the rat adrenal in a dose-related manner, but inhibits the ACTH release elicited by hypothalamic tissue. It was not capable of blocking the increase in ACTH secretion caused by hypothalamic extract containing corticotropin releasing factor.

The results show that the action of serotonin and 5-hydroxytryptophan are similar, both substances inhibiting the hypothalamo-pituitary-adrenal system at the hypothalamic level.

Bronchodilator activity of anticholinergic drugs

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Sympathomimetics are thought to be more effective than anticholinergics in relieving bronchospasm in humans. In patients with chronic bronchitis, however, the bronchodilator effect of atropine and related drugs is comparable with that of beta-sympathomimetics, according to recent data in the literature.

Study of the pharmacological properties of new compounds revealed a substance (RG-1988) to be a potent cholinolytic agent. Its activity was investigated in connection with several autonomic functions and compared with that of reference substances. The cholinolytic activity proved to be of similar intensity as that of atropine in several tests, but the effect on the CNS was negligible.

In the present study RG-1988 was compared with atropine as regards the peripheral, central and bronchodilator activities.

Measurement of ACTH release in hypophyseal cultures by radioimmune assay (RIA)

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ACTH release was studied in hypophyseal monolayer and organ cultures. The ACTH content in the medium was determined by RIA, and a quick method was developed for the extraction of the hormone with QUSO. The amount of ACTH was related to the culture and/or to the protein content of the cells.

Determining the amount of ACTH released after 6 and 24 hours in the monolayer and organ cultures respectively, conclusions could be drawn as to the dynamics of ACTH release *in vitro*.

In further investigations performed under identical experimental conditions it was studied whether dexamethasone (0.5—1—2 μg/ml medium) would influence ACTH release in non-stimulated hypophyseal cultures. The results obtained contradict the hypothesis that corticoids would exert a feedback action on the pituitary gland.

Microelectrophysiological analysis of the cardiac effect of prostaglandins

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It was shown previously that low concentrations of PGE_1, PGE_2 (5 and 20 ng/ml) and PGF_{2alfa} (0.5 and 10 μg/ml) exerted a dose-related biphasic action on the maximum depolarization rate (V_{max}) of the transmembrane action potential of different mammalian heart preparations (*Kecskeméti* et al., Europ. J. Pharmacol. *24*, 289, 1973; Polish J. Pharmacol. *26*, 171, 1974). These changes were unrelated to the alterations in resting potential (RP).

In the present work the effect of PGE_1 and PGE_2 at higher concentrations (0.1 and 0.5 μg/ml) was analysed on the transmembrance action potential of the cat and guinea-pig left auricle. These concentrations of PGE_1 and PGE_2 depressed not only V_{max} but caused a 14 to 20% depression of RP. This depolarizing effect remained unchanged in Na-free Tyrode solution but failed to appear in the presence of 0.075 μg/ml of carbachol. Thus, PGEs at concentrations of 0.1 μg/ml and higher are not likely to affect the resting Na-permeability of the cardiac membrane, but they may probably evoke some change in the resting K-permeability. In none of the studied concentrations did the prostaglandins affect repolarization of the transmembrane action potential.

Effect of biogenic amines on the blood pressure of germ-free rats

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Blood pressure of abacterial white Wistar rats was recorded under the influence of drugs stimulating and inhibiting the sympathetic and parasympathetic nervous system.

The hypertensive effect of adrenaline and noradrenaline decreased and the hypotensive effect of isoprenaline significantly increased in the germ-free animals as compared with the controls. The sensitivity of germ-free animals was significantly higher than of the controls to acetylcholine, histamine, serotonin and bradykinin.

All the competitive antagonists of catecholamines and biogenic amines were effective. An exception was propranolol that could not prevent the hypotensive effect of isoprenaline in germ-free animals.

Isolation and purification of enzyme proteins of the fragmented sarcoplasmic reticulum (FSR) prepared from fish muscle

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Acetylcholinesterase was quantitatively extracted from the lyophilized FSR fraction of fish muscle at a Triton X-100 : protein = 0.3 : 1 ratio. Solubility and changes in the properties of Ca^{2+}, Mg^{2+}-dependent ATPase remaining in the pellet were studied under various conditions of treatment.

It was found that, in contrast to the data of Miagala and Hasselbach the enzymic character of ATPase was lost during lipid extraction with Triton X-100 in the presence of Ca^{2+}. The enzyme activity of Ca^{2+}, Mg^{2+}-dependent ATPase was influenced by the strength and character of the phospholipid-protein interaction and by the experimental conditions applied.

The effect of different buffers and ionic strengths on the properties of the protein was also studied.

Function of the unaffected kidney in renal hypertensive rats after removal of the ischaemic kidney

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It has been shown earlier that unilateral ligature of the renal artery is followed by lasting hypertension accompanied by significant changes in renal function. Early removal of the ischaemic kidney was followed by restoration of the disturbed renal function. If, however, the ischaemic kidney was removed after, the renal distribance persisted.

The changes in the function of the unaffected kidney are attributed to the presence of the ischaemic kidney, or rather to the time elapsed between the ligature of the renal artery and removal of the affected kidney.

Lactic dehydrogenase isoenzymes in haemorrhagic shock

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Lactic dehydrogenase (LDH) activity increases under hypoxic conditions, a change also demonstrable in the circulating blood. Plasma LDH activity considerably increases in haemorrhagic shock, especially after reinfusion. The LDH isoenzymes have a relative organ specificity; their changes in shock have not yet been studied. Using spectrofluorometric and gel electrophoretic methods, total LDH activity and isoenzyme fractions in the plasma were determined during haemorrhagic shock in the dog. Total LDH activity was considerably increased. With time, LDH-1 decreased, LDH-4 and LDH-5 increased, while LDH-2 and LDH-3 exhibited little change. The relative concentration of H subunits diminished, that of M was elevated. In absolute amount, both H and M increased progressively during shock and H was always higher than M. This suggests that in shock the severe hypoxic damage to the liver and the skeletal muscles is accompanied by progressive heart injury, especially at the later stages.

Morphological and functional characteristics of neurones identified by $CoCl_2$ staining in CNS of Lymnaea stagnalis

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Part of the neurones in the central nervous system of *Lymnaea stagnalis* was stained by intracellular injection of $CoCl_2$ after previous identification on the basis of their activity pattern. Morphology and direction of axonal pathways of these neurones were investigated. The sensivitivity of these cells to iontophoretically applied serotonin and dopamine was also examined.

On the basis of the electrophysiological parameters of the identified giant neurones, the specific features of their chemical sensitivity and their axonal connection, it has been concluded that most of them may play an important integrative role in the interganglionic relations, and their connections with the periphery might be indirect.

Site of action of 5-HT on the membrane of the Helix pomatia ventricle

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The membrane effects of 5-HT were investigated on muscle cells of the isolated Helix heart. Low concentrations (10^{-9}—10^{-7} M) of 5-HT depolarized, whereas high concentrations (10^{-5}—10^{-4} M) hyperpolarized the muscle cells. The turning-point from depolarization to hyperpolarization was around 10^{-6} M. 5-HT affected the amplitude of the electrotonic potentials (ETP) biophasically, decreasing it at high concentrations while increasing it at low concentrations.

The ion-dependence of the 5-HT effect was studied on spontaneous action potentials (AP), ETPs and evoked APs. It was found that Cl-ions were responsible for the positive after-potentials appearing following the application of 5-Ht. Sodium and calcium ions took part in the formation of the ascending phase and the plateau phase of AP.

It is suggested that the hyperpolarizing effect of 5-HT is due to an increased Cl-conductance, whereas the depolarizing effect is associated with an increase in Na-and Ca-conductance. Bromolysergide and methysergide completely blocked the membrane effect of 5-HT. Thus, at least two different sites of action of 5-HT can be assumed to be located on the surface membrane of the snail heart.

Anti-inflammatory activity of adrenaline and alloxan diabetes against the dextran-induced anaphylactoid reaction

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The anaphylactoid reaction induced by dextran is known to be inhibited by adrenaline and alloxan diabetes. The aim of the present experiments was to study the effect of propranolol, insulin and the insulin-released anaphylactoid inflammation promoting factor (AIPF) against the anti-inflammatory action of adrenaline and diabetes. In normal rats, propranolol, insulin and AIPF were all effective against the anti-inflammatory activity of adrenaline. Blood sugar level and the concentration of free fatty acids in the serum were not reduced by AIPF. The inhibition of the inflammatory response brought about by diabetes was abolished by insulin, but not by propranolol or AIPF.

It is suggested that insulin antagonizes the effect of adrenaline by inducing AIPF and prevents the anti-inflammatory effect of diabetes by relieving the metabolic disturbance.

Muscle contraction eliciting effect of caffeine

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Caffeine elicits contraction by acting on different structural elements of the muscle. In previous studies two effects of the drug could be separated. A fast effect exerted on surface membrane elements of muscle fibres (i.e. sarcolemma and T-tubules) and a slow one acting on intracellular elements (sarcoplasmic reticulum) after potassium contraction. The present experiments revealed a different sensitivity of the two phases to caffeine depolarization and potassium depolarization. The amplitude of both phases became maximal within a narrow range of caffeine concentration. On fibre preparations of the frog semitendinosus muscle this range was 3 to 6 mM of caffeine for the slow phase and 5 to 10 mM for the fast phase.

The speed of contraction for the individual phases increased above the threshold concentration of caffeine. The first phase could be masked by simultaneous application of potassium at high concentrations, while the second one was not sensitive to potassium depolarization.

Schild analysis of the antihistamine effect of p-bromomethylamphetamine (V-111)

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Isolated guinea-pig ileum has been used in Krebs solution (95% O_2 + 5% CO_2) at 37 °C for studying antihistaminic effect. The contractions in response to different concentrations of histamine were recorded isometrically.

The log-dose-response curves in the presence of V-111 ran parallel with the curves recorded in the absence of the antagonist, but the maximum response was reduced. V-111 in concentrations from 6.5×10^{-6} to 1.6×10^{-4} M shifted to the right the dose-response curves of histamine, resulting in dose ratios (DR) of 3.5—583.3. The logarithm of (x—1), where x equals histamine dose ratio, was plotted against the negative logarithm of the antagonist used.

These values show that the pA_2 for V-111 was 5.48. The value for pA_2—pA_{10} was 0.53, indicating that V-111 is a non-competitive antagonist of histamine.

In contrast to V-111, mepyramine proved to be a competitive antagonist of histamine. The heights of the maximum responses to histamine were not reduced by the drug. The calculated regression line intercepted the abscissa at a pA_2 value of 8.83. The value for pA_2—pA_{10} was 1.01.

Close contacts of dendrites without synaptic differentiation as probable sites of impulse transmission

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The cobaltous chloride staining technique developed for the insect nervous system (*Pitman* et al., Science *176,* 412, 1972) has been adapted to the frog (*Székely* and *Gallyas,* Acta biol. Acad. Sci. hung., in press) and further developed for use in electronmicroscopic investigations. Motoneurones in the lumbar swelling of the frog's spinal cord were filled with $CoCl_2$ by ventral root iontophoresis. Co^{2+} ions were precipitated with sulphides, and the CoS precipitate was intensified with physical developers on ultrathin sections for electron microscopy. In the ventral part of the spinal grey matter dendrites, or a dendrite and a perikaryon, or perikarya, all labelled with cobalt, could be observed in close contact with each other. Sometimes a narrower cleft and thicker membranes, resembling gap junctions, could be seen in the region of contact. The findings are interpreted in the sense that these close contacts between labelled neuronal elements represent the structural substrate for the recurrent facilitation of motoneurones shown in the frog's spinal cord.

Effect of haemorrhagic shock on the myocardium

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Rat hearts were isolated from three groups of animals (I.: Control, II.: anaesthetized with 5 mg/100 g pentobarbital; III.: 2.5-hour haemorrhage and perfused according to Langendorff). In the control period of perfusion (15—20th min) the coronary resistance of the hearts was significantly higher and their mechanical performance lower in the shocked animals than in the first and second group. Oxygen consumption did not differ significantly. This means that the efficiency was less in the shocked than in the non-shocked groups. Elevation of heart rate or perfusion pressure increased the performance of the hearts of the animals in shock, but the difference from the first two groups persisted. Addition of calcium in three times higher concentration (3.9 mM) restored the performance of the heart in shock and abolished the difference versus the control hearts. Pretreatment with pentobarbital rendered the isolated hearts insensitive to the calcium effect. Electronmicroscopic studies revealed seriously demaged mitochondria and disappearance of calcium from them at 2.5-hours following the haemorrhagic shock.

Effect of cellulin-A and changes in ionic milieu on the transmembrane currents of the sino-auricular fibre of the frog

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The effect of cellulin-A in a low-sodium milieu and the presence of $MnCl_2$ was studied by using a voltage clamp technique based on the double sucrose gap method. A 20% decrease in the sodium content of Ringers solution resulted in a 60% depression of the maximum inward current, and the equilibrium of inward and outward currents set in at a lower clamp potential. Upon the effect of 1 U/ml cellulin-A the maximum inward current in the low-sodium milieu exceeded even the control (normal sodium) values. In addition, the direction of the membrane current was reversed at a higher clamp potential level.

The present findings agree well with previous data proving the facilitatory effect of cellulin-A on both components of inward membrane current.

Addition of 3.5 mM of $MnCl_2$ reduced the maximum amplitude of the inward current by about 50% and abolished the slow component. Under these conditions cellulin-A enhanced the maximum inward current by 30 to 40% as compared with the normal values; but failed to restore the slow component of the membrane current blocked by Mn^{2+} ions.

Relationship between changes in cerebral serotonin content and avoidance behaviour induced by pituitary-adrenocortical hormones

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Eventual interrelationships between changes in hypothalamic and mesencephalic serotonin (5-HT) content and in avoidance behaviour have been investigated after ACTH and corticosterone treatment. ACTH treatment of normal male rats resulted in an elevation of hypothalamic and mesencephalic 5-HT content. In adrenalectomized animals, however, ACTH failed to influence cerebral 5-HT content. Corticosterone treatment exerted a dose-related dual effect on 5-HT content of the brain; low doses (1.0 and 2.0 mg/kg b.w.) of the hormone increased, while large doses (10.0 mg/kg b.w.) decreased it. Extinction of an active avoidance reflex as well as passive avoidance behaviour (fear versus thirst conflict situation) were also influenced by corticosterone treatment in a dose-related manner.

The data suggest that corticosteroids exert their behavioural effect, at least in part, via a serotoninergic mechanism, while ACTH acts through a different way.

Improved technique of producing endothelial injury by laser beam without direct damage to blood cells

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Ultrastructural study of microcirculatory thrombus induced by He-Ne laser and application of the energy-adsorbing dye Evans blue demonstrated severe endothelial damages covered with disintegrated platelets. In order to exclude the direct effect of laser beam on platelets and red corpuscles, the technique of Baez was used for perfusion of intact mesenterial microvasculature of the living rat in situ. During laser irradiation arteries deriving from the ileocolic artery were filled with albumin solution containing Evans blue. Thereafter, the blood circulation was restored and thrombus formation was investigated at the site of irradiation.

According to the ultrastructural findings thrombus formation is based on the interaction between the exposed subendothelium and intact platelets. It is an additional advantage of the technique used that no Evans blue enters the systemic circulation.

Potassium and rubidium exchange in skeletal muscles

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The effect of cardiac glycosides on K influx in muscle cells depends on the intracellular Na concentration. On the other hand, *Varga* and *Horowicz* found that K influx was inhibited by physostigmine. In the present study the effect of substitution of choline for external sodium was investigated on the ^{42}K—and ^{56}Rb-influx and on the inhibitory action of strophanthin and physostigmine. There was a marked net loss of $[Na]_i$ and no change in fibre K in muscles bathed in choline-Ringer for 2 hours. K influx of the muscle was 6.6. μM/g/hr in normal Ringer containing 5 mm of [K]; and it decreased to 5.2 μM/g/hr in the absence of $[Na]_o$. Application of 10^{-4} M strophanthin caused a reduction by about 20%, i.e. to 5.3 μM/g/hr in the presence of $[Na]_o$, but K influx was not affected in choline-Ringer.

The observations indicate that, at low $[Na]_i$, the reduced K influx is completely insensitive to strophanthin. Physostigmine (10^{-3}M) inhibited K influx in the presence as well as the absence of external Na by about 60—70% (2.6 μM/g/hr and 1.5 μM/g/hr, respectively).

Rb influx was also significantly less in muscle bathed in choline-Ringer with 5 mM $[Rb]_o$ (0.7 μM/g/hr) than in muscle incubated in normal Ringer (2.4 μM/g/hr). In choline-Ringer, physostigmine did not reduce Rb influx.

It is concluded that unidirectional K influx in skeletal muscle is the sum of different components. One of the components is a physostigmine-sensitive, presumably K:K exchange process. Another component is a strophanthin-sensitive Na : K transport. Rb can mainly use the latter transport component.

Renal parameters in mild saline diuresis

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Parameters of the left kidney of non-hydrated and mildly hydrated (intravenous infusion of isotonic saline in an amount of 1—2% of the body weight) were studied and compared in anaesthetized dogs.

In two series of experiment no difference was found in mean arterial pressure, renal vascular resistance (R_{kidney}) and directly measured renal blood flow (RBF_{dir}) between the two groups.

C_{inulin} was 72 ± 17 ml/min/100 g in the first group 74 ± 23 ml/min/100 g in the second one; urine flow amounted to 0.77 ± 0.55 ml/min/100 g and to 1.97 ± 1.14 ml/min/100 g, respectively. The differences in sodium excretion were of the same magnitude as in the case of urine flow.

Plasma protein concentration in the two groups differed significantly; it was 5.31 ± 0.73 g/100 ml in the non-hydrated group, and 4.76 ± 0.41 g/100 ml in the mildly hydrated one.

As the experiments were carried out under practically physiological conditions of hydration, it is assumed that the increase of urine and sodium excretion was not due to changes in glomerular filtration rate but to those in the tubular reabsorptive processes; these may be attributed to alterations in plasma protein concentration.

Hyperlipaemia induced by Ehrlich ascites tumour

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After transplantation of ascites tumour cells, hyperlipaemia develops in experimental animals. The hyperlipaemia is due to endogenous fat mobilization and occurs in five stages. Stage I. Latency period with no quantitative changes, or a slight decrease, in serum lipid content; Stage II. Progressive hyperlipaemia with a rapid increase of serum lipids; Stage III. A quick, transitory decrease in the serum lipid

level; Stage IV. Second period of progressive hyperlipaemia with a 5 to 8-fold prolonged elevation of the serum lipid content. Stage V. Marked fall of the serum lipid level immediately before death, an event indicative of the exhaustion of the fat stores. The respective stages of fat mobilization are also characterized by changes in the distribution of serum lipoproteins. Parallel with the development of the hyperlipaemia, alterations also occurred in the composition of the liver, mesenteric tissue and ascites fluid cells. It is concluded that the serum lipid content is a sensitive marker of the tumour-host relationship in connection with the actual state of fat depots, and offers reliable information on the mechanism of fat mobilization.

Postsplenectomy thrombocytosis as determined by thrombocyte size in the mouse

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Splenectomy usually elicits thrombocytosis. The platelet count gradually increases after splenectomy in the mouse and exceeds the initial level by about 50% in the second week. The megathrombocyte count can be used to assess the rate of thrombocytopoiesis just as the reticulocyte count can be used for the characterization of erythropoiesis. The diameter of the thrombocytes decreased by the 7th day. In accordance with previous studies of ^{75}Se-methionine incorporation into bone marrow, determination of platelet size failed to reveal an enhanced thrombocytopoiesis after splenectomy. It is assumed that the loss of the storing function of the spleen is responsible for the thrombocytosis.

Effect on ventilation of enlarged dead space

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The effect of dead space enlargement by 500 and 1000 ml tubes was measured on the ventilation and blood acid—base parameters by pneumotachographia, quick oxygen and CO_2 analysis and Astrup techniques.

In 10 subjects in individually randomized self-control experiments minute ventilation increased from 12.8 to 19.8 and 27.7 l. Mean oxygen concentration in front of the mouth was 2.02, 2.78 and 3.60 vol per cent less than in the room-air at the free end of the tubes. Oxygen consumption increased significantly. Acid-base parameters of fingertip blood also underwent changes during the 6—7 min of the experiments.

Enlargement of dead space by 500 and 1000 ml caused a significant increase of the ventilatory work also in healthy, adult sportsmen.

K^+-transport of mitochondria isolated from ischaemic brain

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Ion-selective electrodes are now increasingly used instead of glass electrodes for measuring K^+ concentration. The selectivity of liquid ion-exchanger (Corning)-PVC membrane as well as that of valinomycin-silicon rubber membrane electrodes were studied in the presence of different disturbing ions (Na^+, PO_4^{3-}, tris, protein). The pH sensitivity of the electrodes was also studied. Their function satisfied the Nernst equation. The valinomycin-silicon rubber membrane electrode proved suitable for experiments as judged on the basis of selectivity.

Brain mitochondria were isolated from the cerebral cortex of control rats as well as animals subjected to ischaemia for one or four hours and the K^+-uptake was measured. There was no significant difference between the control and the one-hour group but 4 hours anoxia decreased the K^+-uptake. The DNP-induced K^+ efflux increased progressively with the duration of anoxia.

Pathomechanism of adrenal necrosis produced by basic polyglutamic acid derivatives

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Basic polyglutamic acid derivatives induce adrenal haemorrhage and necrosis in rats after intravenous injection (*Kovács* et al., 1960; *Jancsó;* 1961; *Kótai,* 1967; *Lázár* et al., 1972). Spironolactone gives significant protection against these compounds whereas pregnenolone-16-α-carbonitrile, ethyloestrenol, triamcinolone and hypophysectomy are ineffective in this respect.

The basic polyglutamic acid derivatives were found to decrease blood fibrinogen content and platelet count, and to induce secondary fibrinolysis. Their administration resulted in Indian ink retention in the kidney and lungs, a useful parameter of initial fibrinoid formation during the generalized Shwartzman reaction. Thus, intravascular coagulation and secondary fibrinolysis seem to be important factors in the pathomechanism of adrenal apoplexy induced by basic polyglutamic acid derivatives.

Neurotoxic effect of the cholinesterase inhibitor fenitrothion in chicken and mammals

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The effect of subacute fenitrothion (0,0-dimethyl-0-(p-nitro-m-tolyl) phosphorothioate poisoning was investigated on the conditioned avoidance reflex of rats and the conduction velocity of the sciatic nerve of rabbits. The toxic manifestations were studied in chicken.

1. A daily subcutaneous dose of 10 mg/kg caused ataxia, tremor, palsy and death of the chicken by the third weak, without any cholinergic signs.

2. In the rat, oral administration of 10 mg/kg daily diminished the number of positive conditioned responses and slowed down the rate of extinction. The initial cholinergic signs decreased and finally disappeared in the course of treatment.

3. In rabbits, oral treatment with 10 mg/kg daily decreased the conduction velocity of the sciatic nerve, without any other toxic sign. Administration of 25 mg/kg daily increased conduction velocity, lengthened the duration of the evoked muscle potentials and produced marked cholinergic manifestations.

The findings indicate that fenitrothion, like some other organophosphorus compounds, has a delayed neurotoxic effect that does not seem to be in connection with the cholinergic signs.

Effect of bilateral striatal lesions on food uptake of the rat

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Chemical or electrolytic lesions of the nigrostriatal dopaminergic system at hypothalamic level result in aphagia and adipsia, with a marked decease in striatal catecholamine content. In the present experiments the effect of bilateral electrolytic lesions of the caudate nucleus and putamen was investigated on food uptake. Body weight, food and water consumption of the operated animals did not differ significantly from those of the controls.

The results suggest that the nigrostriatal pathways participate in the organization of food uptake at the hypothalamic and pallidal levels only; or, the lateral hypothalamic aphagic and adipsic syndromes may be due to a common lesion of the nigrostriatal and mesolimbic systems.

Calcium transport in surviving uterine tissue

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It was shown earlier that a potential difference existed between the two sides of the hen's uterus, the mucosa being always negative against the serosa. A role of the avian uterus is to transport Ca ions from the blood into the uterine lumen, to the surface of the egg to be formed. In the present experiments hen uteri at different stages of sexual activity were examined with ^{45}Ca in vitro. It was observed that

1. the direction of Ca-transport from serosa to the mucosa, i.e. the Ca influx is greater than the Ca efflux;

2. this transport always takes place against a concentration gradient, but in direction of the electric gradient;

3. the efficiency of Ca influx depends on the functional state of uterine tissue;

4. the transport is influenced by the energy (i.e. glucose) content of the incubation medium.

The technique outlined above seems to be suitable for investigating ion-transport processes.

A new theory of atherogenesis in the aorta and the coronaries: The role of lipids generated in myocardial hypoxia

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It has been shown that emulgeated lipids injected into the myocardium of dogs reach the aorta and the major coronary arteries via intracardiac lymphatic pathways. This observation raised the question of whether the lipids generated in hypoxic myocardial areas might be the source of lipid deposits in the aorta and the coronaries.

The upper or lower part of the descendens ramus of the left coronary was ligated in rats. The animals were killed 2—3 weeks later. The histological sections from the myocardium and aorta were stained with oil red 0. In the region of infarction distal to the ligature a great amount of myocardial lipids could be observed, especially in the marginal zone. Lipid accumulation also occurred proximal to the ligature, mainly in the lymphatic spaces around the vessels and also in the myocardial fibres. After two weeks the connective tissue around the major coronary vessels and aorta became rich in lipids and lipid deposits occurred also in the adventitia of these vessels. Three weeks after the ligation the adventitia and the media contained large lipid deposits. In the intima, endothelial phagocytosis of fats was observed.

Effect of acute hypophysectomy on natriuresis during isotonic saline expansion in rats and dogs

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Acute hypophysectomy (AH) performed prior to an isotonic saline infusion decreased the ability of hypophysectomized (H) rats and dogs to respond with natriuresis. In rats (isotonic saline, 6% of body weight), the sham-operated group excreted 6.28 ± 0.59 (S.E.) μEq/min of sodium *vs.* 1.06 ± 0.25 μEq found in H rats ($p < 0.001$). No difference was found in urine flow (50.64 ± 4.61 μl/min in normal rats *vs.* 64.82 ± 6.15 μl/min in H rats. Thus, the diminished solute excretion in H rats resulted in a positive C_{H20}. Similar results were obtained in H dogs infused with 10% b.w. of isotonic saline. Urine flow in the normal and the H groups increased (14.23 ± 1.39 ml/min in normal *vs.* 11.03 ± 1.92 in H dogs). The natriuretic effect of expansion was less pronounced in H dogs (1.59 ± 0.22 mEq/min in normal *vs.* 0.75 ± 0.18 in H dogs, $p < 0.05$) due to the decreased urinary sodium concentration. The impairment of sodium rejection in the distal nephron is suggested to be responsible for the diminished natriuresis during isotonic saline infusion in H rats and dogs, in agreement with the concept of the humoral mechanisms of extracellular fluid volume regulation.

Pathomechanism of pulmonary oedema in acute anticholinesterase poisoning

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Lethal or close-to-lethal doses of mevinphos were administered to rats and the appearance of pulmonary oedema (PO) was observed.

1. In the case of a single mevinphos treatment the animals died without any sign of PO.

2. In mevinphos treatment was preceded by increasing dose of an anticholinergic drug, PO appeared after low doses of the anticholinergic agent, but its incidence decreased at higher dose levels. The highest doses of the anticholinergic agent failed to cause PO.

3. PO evoked by the combined administration of anticholinesterase and low doses of an anticholinergic drug could be prevented by pretreatment with ganglion-blocking (hexamethonium) and alpha-lytic (phentolamine) agents or by administration of cocaine. Lethality, however, could only be decreased by the alpha-lytic drug. Thus, at low doses of anticholinergic drugs elicit additional undesired effects in the course of acute anticholinesterase poisoning.

Diuretic effect of vasopressin in waking hydropenic dogs

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The mechanism of the diuretic effect of exogenous vasopressin in hydropenic dogs was investigated in waking animals with indwelling catheters and flow transducers. Hydropenia was brought about by 24-hour water deprivation.

8-lysine vasopressin given intravenously in a dose of 4 mU/kg+20 mU/kg/h significantly increased urine output and renal blood flow. The effects were assumed to be brought about secondarily, by prostaglandins. Thus, 2 mg/kg of indomethacin was given in order to inhibit prostaglandin synthesis. After the administration of indomethacin, vasopressin failed to increase urine output or renal blood flow.

Effect of para-substituted amphetamine derivatives on tryptophan hydroxylase activity

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p-Bromomethylamphetamine (V-111) and p-fluoroamphetamine inhibit rat brain tryptophan hydroxylase (TH) both in vitro and in vivo. The stereoisomers of V-111 have equal potency in inhibiting the enzyme, and the same is the case with the stereoisomers of p-cholorophenylalanine. p-Bromo- and p-fluoro-phenylalanine have no effect of HT, and only p-iodophenylalanine inhibits the enzyme to a slight extent. Compounds which inhibit TH, decrease the level of serotonin (5-HT) in the central nervous system.

The time-course of the decrease of cerebral 5-HT content due to V-111 treatment (15 mg/kg) does not run parallel with the inhibition of TH. Serotonin content starts to decrease from the 3rd—4th hour after injection and remains diminished for about three weeks. In the course of an 8-day treatment with 15 mg/kg daily, TH activity gradually decreased to 35% by the 4th day but fully recovered by the 8th day in spite of continuous treatment. Turnover studies performed on the 8th day also revealed a decreased 5-HT content with a normal turnover rate.

The experiments suggest that V-111 treatment damages to the storage mechanism of 5-HT, and the synthetized amine will be metabolized by MAO.

Absorption and transport from the biliary tract after occlusion of the common duct

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$Na^{125}J$, and ^{131}J-labelled albumin were infused in dogs through the common duct at pressures from 20 to 25, and 40 mm Hg respectively. At 40 mm Hg, the amounts of iodide and labelled albumin in the plasma were nearly identical, after correction for the secondary loss from the circulation. At 20—25 mm Hg, more iodide than labelled albumin was found in the blood. In thoracic duct lymph the same fraction of the infused amount of albumin was recovered at both pressures. The concentration of albumin was substantially higher in the lymph than in the plasma in both types of experiment. It is concluded that, at increased pressure, fluid leaks first from the small biliary ducts into Mall's spaces. As a consequence of water absorption and diffusion of small molecules and ions into the blood capillaries, the concentration of protein or protein-bound molecules increases in this part of the hepatic interstitial fluid. This is reflected in their high concentration in the lymph. If bile pressure rises further, fluid also leaks into Disse's spaces. This leads to a non-selective inflow of solvent and solute into the sinusoids and to the almost complete disappearance of the difference in the venous transport of ions and colloids.

Hypothalamic blood flow in haemorrhagic hypotension

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Previous investigations demonstrated an augmentation of the tissular CO_2 content of the hypothalamus in the course of acid—base disturbances due to haemorrhage. Correction of extracellular acidosis by means of bicarbonate infusion prevented the development of tissular hypercapnia.

In the present study the contribution of local blood flow to the beneficial effect of bicarbonate infusion was examined in haemorrhagic hypotension.

In untreated animals hypothalamic blood flow (HBF) diminished after bleeding to 56% of the initial level by the end of the period of hypotension. Similar results were obtained in another group treated with physiological saline infusion. Administration of sodium bicarbonate throughout the entire hypotensive period moderated the decrease in local blood flow. There was no significant change in

HBF during the first bleeding phase, whereas a 35% decrease occurred by the end of the second bleeding period. With the development of tissular hypercapnia the oxygen supply of the hypothalamus decreased in the control group. Thus, when acidosis was prevented by bicarbonate, the arterial oxygen transport was sufficient to ensure normal oxigen supply to the tissues.

Interaction of organophosphate pesticides and bencyclane with the acetylcholinesterase of human erythrocyte ghosts

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The data in the literature are contradictory as regards the interactions of organophosphate pesticides with reversible anticholinesterase drugs. Kinetic analyses were performed to study the simultaneous effect of irreversible and reversible of acetylcholinesterase (AChE) inhibitors of human erythrocyte ghost. The organophosphates paroxypropione and dichlorves were used as irreversible enzyme inhibitors, and bencyclane served as a reversible one. The antiChE activity of bencyclane has already been shown (*Cseh* et al., 1974). Both bencyclane + dichlorves and bencyclane + paroxypropione exert a dual effect on erythrocyte AChE, depending on the pH and the concentration of the drugs. Enzyme-inhibitory concentrations (10^{-4} M) of bencyclane reduce the intensity as well as the rate of inhibition of AChE by both dichlorves (10^{-7} M) and paroxypropirone (10^{-9} M). Concentrations of bencyclane (10^{-6} to 10^{-5} M) which do not influence AChE activity by themselves resulted in a marked potentiation of both the intensity and rate of AChE inhibition by dichlorves. The inhibitory action of paroxypropione was not potentiate by bencyclane. The pH-dependence of the bencyclane effect and its independence of sequence and time of the reactions indicate that bencyclane influences the phosphorylation of the esteric site through a cooperative effect upon the anionic site of the enzyme.

Serum polypeptide spectrum in the course of toxic states

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According to previous data, four polypeptide fractions of basic character can be separated from sera of healthy individuals and of those with chronic uraemia by Dowex chromatography. The serum level of these peptides rose under uraemic conditions. Two of the four Dowex fractions displayed pour diffusibility and proved moderately or highly toxic.

In order to decide whether the composition of the peptides had changed, further separation was attempted by gel filtration of the toxic uraemic fractions. For this purpose appropriate Dowex fractions prepared from sera of healthy subjects and of uraemic patients were gel-filtered on Sephadex G-25 column. Elution pattern as well as toxicity (LD_{min}) of the individual fractions were compared.

The investigations revealed the existence of some new compounds with potential toxicity in uraemia. They have a molecular weight of about 1800—2500 daltons and thus belong to the group of middle molecular substances (MMS). Chemically, they are polypeptides containing arginine in fairly large amount. The pathological significance of these peptides might lie in their high toxicity and poor or non-diffusibility.

Examination of the stability of evoked vertex potential by means of amplitude histograms

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Objective audiometric experiments were performed to detect averaged evoked responses to pure tone stimulation, to develop such optimal methods of stimulation and detection allowing to measure the hearing threshold as exactly as possible. The effect of the physical parameters of the evoking stimuli and different motivational states on the amplitude and wave-shape of the evoked response was shown previously.

In the present paper the problems of the averaging method are discussed. It is shown that by means of the amplitude histogram method, the effect of artifacts on detection of the hearing level can be eliminated. Moreover, the stability and the interrelations of the components of evoked responses can be studied.

Sex steroid binding properties of human plasma at perinatal age

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Fifty-times diluted plasma samples of immature, premature and full-term babies were studied for sex steroid binding ability.

Receptor of high affinity for either oestradiol or testosterone could not be detected in samples obtained before the 30th week. Significant ^{3}H-oestradiol and testosterone binding was observed in the plasma samples from the 32nd week on.

Maternal SBP capacity was about *40-times* and non-pregnant one 10-times higher than that of newborn plasma. Perinatal samples bound 3 times more testosterone than oestradiol. There was no detectable sex difference in the binding capacity.

The ^{3}H-testosterone uptake of mature newborn plasma was significantly reduced by non-labbelled testosterone and oestradiol. Progesterone, corticosterone and hydroxycorticosterone suppressed the ^{3}H-testosterone uptake in high concentrations only.

Radioimmune assay of ACTH in rat plasma

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Anti-serum to ACTH was produced in white New-Zealand rabbits. The animals were injected at multiple intradermal sites with bovine gamma globulin conjugates of synthetic α_{p}^{1-28} ACTH (Richter Budapest; 1 mg ACTH/mg BGG/rabbit), emulsified in complete Freund's adjuvant. Primary immunization was followed by two "reminder" injections at two-month intervals, and the animals were bled 4 weeks after the last injection. α_{h}^{1-39} ACTH was labelled with ^{125}I according to the chloramine-T method of *Hunter* and *Greenwood* (1962) as modified by *Berson* and *Yalow* (1968). The average yield of iodination was 66% on 7 consecutive occasions with a calculated specific activity of 360—850 μCi/μg ACTH. Antisera were selected on the basis of dilution and standard curves plotted with a constant amount of labelled corticotrophin (10 pg). Native porcine ACTH (3rd International Working Standard) served as standard. Incubation procedure and separation of free and bound antigens were made according to *Rees* et al (1971). The limit of sensitivity was about 15 to 30 pg (75—150 pg ACTH/ml buffer). Methods for extraction of ACTH from rat plasma were studied.

Tissue specificity of endogenous mitosis-regulator substances of hepatic origin

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Six polypeptide (pp) fractions of a molecular weigth between 2000 and 3000 were isolated from intact (I) and regenerating (R) rat livers by combination of Dowex chromatography and Sephadex G-25 gel filtration. The effect on DNA synthesis of these pps was studied in a concentration of 6 μg/ml in *vitro* test systems prepared from R livers and kidneys. Pps stimulating DNA synthesis in a tissue-specific manner (tsp) and those inhibiting DNA synthesis in a tissue-aspecific (tasp) manner were detected in about equimolar amount in I livers. The ability of pps isolated from R livers to stimulate tsp DNA synthesis was considerably increased,

an event accompanied by a practically total loss of tasp DNA synthesis inhibitor properties of the corresponding pp fraction.

The results indicate that pps of this type are participating in the regulation of the rate of DNA synthesis in the liver. In the mitotically resting state, the effects of tsp DNA stimulating pps are counterbalanced by those of the tasp DNA synthesis inhibitors. The mitotically active state is characterized by changes in the composition of the pp fractions which lead to a substantial increase in tsp DNA synthesis stimulation accompanied by a virtually complete loss of tasp DNA synthesis inhibition. The pps described in the present paper are assumed to be mediators of the mitotic regulatory system responsible for the maintenance of mitotic homeostasis in the liver.

Effect of motor response on conditioned evoked potential in humans

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It was shown previously that conditioned evoked potentials can be established in humans by pairing sound and delayed light stimulus. The response appeared not only in the specific but also in the associative cortical areas. In the present study it was examined how motor activity associated with learning would influence the development of conditioned evoked potential. By using tasks of different degrees of difficulty, a more generalized appearance of conditioned evoked potential was demonstrated in the course of acquisition of different tasks. In the case of motor activity, the shape of sound-evoked potentials recorded monopolarly by the C_z and F_z electrodes became reversibly similar in the course of learning.

The data prove that, as a function of the task, the evoked response of the associative cortical areas can be regarded as a sensitive indicator of the process of learning.

Excitatory activity of some adrenergic agonists on guinea pig terminal ileum

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Adrenaline, noradrenaline and phenylephrine are known to evoke contraction on the terminal part of the isolated guinea pig ileum. The question has been studied whether other adrenergic agonists would exert such an effect. The reactivity of the adrenergic receptors to the drugs studied was also examined. Beside the above-

mentioned drugs also phenylephrine, tyramine, amphetamine and dopamine caused contraction of the terminal ileum. The excitatory effect of adrenaline, noradrenaline and phenylephrine was only due to activation of alpha receptors. Pholedrine, sympethamine, ephedrine and methoxamine did not evoke contractions.

Effect of picrotoxin and strychnine on blood pressure reflexes elicited by intense stimulation of cutaneous afferents in anaesthetized cats

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Alterations due to anaesthesia, picrotoxin and strychnine of vasomotor reflexes evoked by prolonged (4 min) intense (8—16 V, 1—16 imp/sec, 0.5 msec) electrical stimulation of cutaneous afferents were investigated in cats immobilized by gallamine under artificial respiration. Reflex sensitivity to cutaneous input, being fairly high in waking animals, almost completely disappeared under anaesthesia. Partial restoration of the reflexes, with inverse dependence on the frequency of stimulation, was achieved by picrotoxin. Under the combined effect of picrotoxin and strychnine, however, stimulations elicited blood pressure responses of high amplitude, usually displaying slow wave oscillations. The results indicate that the spinal input of cutaneous afferents is controlled by pre- and postsynaptic inhibition.

Pharmacological analysis of the effects evoking eyelid reaction in the rat

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Muscular elements of the rat's eyelid possess both somatic (n. facialis) and autonomic (sympathetic) innervation. After elimination and/or depression of the somatic functions (section of the facial nerve, administration of neuromuscular blocking agents, anaesthesia) the eyelid can be used in nearly similar manner and for the same purpose as the nictitating membrane of the cat.

The eyelid reaction evoked by sympathetic stimulation is blocked by intravenous TEA, hexamethonium and obidoxim. The ED_{50} values (dose-ranges) for the ganglion blocking activity are: TEA = 8—12 mg/kg; hexamethonium = 1—2 mg/kg; obidoxim = 20—30 mg/kg. The doses refer to the corresponding salts.

Acetylcholine, adrenaline and noradrenaline, given intravenously, exert the reaction by a direct action on the eyelid. The doses applied were, acetylcholine = 3.75—120 μg/kg, adrenaline and noradrenaline = 0.5—64 μg/kg. Adrenaline was twenty, and noradrenaline five times as potent as acetylcholine. Anticholinester-

ases and cocaine potentiate the effect, while alpha-blocking or para-sympatholytic drugs prevent it. Obidoxim, up to a dose of 25 mg/kg, potentiated the effect of acetylcholine.

Intravenous administration of DMPP and TMA stimulated both the superior cervical and the adrenal medulla (catecholamine mobilization). These two actions can be separated by using the eyelid as the target organ.

Effect of vasopressin on dynamic biomechanic properties of the arterial wall

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The dynamic elastic modulus in arterial wall (common carotid and femoral arteries of the dog) has been studied after topical and systemic arginine-vasopressin administration (30—150 μU/ml blood) by harmonic analysis in pulsative frequency and amplitude range before and after hypophysectomy.

For the first five harmonics, a decrease was found in carotid and femoral elastic moduli (25—35 and 17—24% respectively), and in local blood flow impedance (10—17 and 10—18%), 1—2 hours following hypophysectomy. Topically applied vasopressin at stop-flow did not significantly influence the carotid elastic modulus, but changed the femoral modulus by 22—26% before, and by 21—25% after, hypophysectomy. Vasopressin administered intravenously increased elastic moduli and local impedances in both arteries; the effects were enhanced after hypophysectomy.

It is concluded that vasopressin, in concentrations within endogenous limits, significantly influences the elasticity of arteries.

Rachitogenic effect of some anticonvulsants in rats kept on a high cadmium or high strontium diet

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Vitamin D deficiency is a recently discovered side effect of anticonvulsant drugs. The mechanism of the rachitogenic effect is not clear.

Diphenylhydantoin (0.8 g/kg) and phenobarbital (0.5 g/kg) mixed into a diet of low calcium and vitamin D content failed to produce rickets in weaning rats during a 5-week period (0.8 g/kg). Cadmium (0.2 g/kg) or strontium (14 g/kg) in the diet

resulted in rachitic symptoms. The anticonvulsants added to the high-cadmium or high-strontium diets significantly increased the rachitogenic effect of these diets.

Thus, an experimental model has been found for studying the rachitogenic effect of certain anticonvulsants. The significance of the simultaneous effect of some environmental contaminants and anticonvulsants is emphasized.

Reflex changes in blood pressure and renal sympathetic activity evoked by sustained stimulation of cutaneous afferents

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A+C fibres of the medial cutaneous nerve of the forelimb and the saphenus nerve were activated by long train (1—2 min) electrical stimuli in gallamine immobilized cats under artificial respiration. Renal sympathetic activity and systemic blood pressure were recorded simultaneously.

1000—2000 ms long burst of high amplitude and frequency, which were followed by a sudden rise in blood pressure, proved to be a characteristic component of the sympathetic reaction. Alterations in this constituent of the response were studied after spinal cord transsection and/or administration of drugs (picrotoxin, strychnine, anaesthetics) as well as following deafferentation of the baroreceptors.

Recent investigations have shown that the patterns of somato-vegetative reactions are determined by the actual balance of the integrative excitatory and inhibitory processes. An equilibrium of these processes, characteristic of conscious animals, is required for the appearance in the sympathetic reaction of the constituent investigated. The balance is affected by interventions such as spinal cord transsection, anaesthesia, etc, manifesting themselves with an altered reaction pattern.

Causal correlation between gastric H^+ secretion and the ATP-ADP system of gastric corpus mucosa in humans

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Na^+—K^+-dependent ATP-ase activity and ATP and ADP content of gastric corpus mucosa have been studied in patients with different basal acid output (BAO) and maximum acid output (MAO). The enzyme activity was expressed in nmoles of inorganic phosphate/mg membrane protein/hour, and the ATP and ADP contents in terms of nanomoles/mg DNA.

In patients with different BAO values the following positive and mathematically significant correlations were found. (1) Between BAO value and Na^+—K^+-dependent ATPase activity ($r=0.88$, $p<0.001$, $n=45$); (2) Between BAO value and tissue ATP content ($r=0.53$, $0.01>p>0.001$, $n=28$); (3) Between BAO value and tissue ADP content ($r=0.59$, $p<0.001$, $n=28$); (4) Between Na^+—K^+-dependent ATPase activity and tissue ATP content ($r=0.41$, $0.05>p>0.01$, $n=19$); (5) Between tissular ATP and ADP contents ($r=0.99$, $p<0.001$, $n=31$).

In patients with different MAO values the following positive and mathematically significant correlations were found. (1) Between MAO value and Na^+—K^+-dependent ATPase activity ($r=0.77$, $p<0.001$, $n=32$); (2) Between MAO value and tissue ATP content ($r=0.60$, $p<0.001$, $n=35$); (3) Between MAO value and tissue ADP content ($r=0.51$, $0.01>p>0.001$, $n=35$); (4) Between Na^+—K^+-dependent ATPase activity and tissue ATP content ($r=0.77$, $p<0.001$, $n=11$); (5) Between tissue ATP and ADP contents ($r=0.72$, $p<0.001$, $n=35$).

The results prove a causal correlation to exist between gastric H^+ secretion and gastric mucosal ATP—ADP system in humans.

Cleavage of platelet action by thrombin

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Thrombin-induced platelet aggregation is probably mediated through the proteolytic effect of thrombin on platelet membrane. As contractile mechanism play an important role in the initial events of platelet aggregation, the effect of thrombin on platelet actin was investigated. Two types of actin differing in their polymerization properties can be prepared from bovine platelets. In the presence of CaATP neither type was split by thrombin, while EDTA pretreatment made them susceptible to limited thrombic hydrolysis. In the course of fragmentation, first two N-terminal actinopeptides were split off by thrombin, then the larger fragment was further cleaved into parts of 26,500 and 11,000 daltons respectively. It is suggested that the thrombin-platelet actin interaction may have an importance in thrombin-induced platelet aggregation.

ADH-reserve capacity after deafferentation of the supraoptic nucleus in the rat

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The supraoptic nucleus is known to play an important part in the central regulation of water metabolism. Since the consequences of deafferentation of the nucleus have not been fully elucidated, diuresis and ADH-reserve capacity were studied in rats after supraoptic deafferentation.

Deafferentation was performed with Halász's knife. The daily water intake of operated animals was about 60% higher than that of the non-operated controls. Diuresis did not significantly change in deafferentated animals after water loading but following administration of physiological saline the decrease of diuresis was less marked. ADH excretion with urine and hypothalamic ADH content were lower in the deafferentated group. Osmotic stimulus induced by administration of hypertonic salt solution increased ADH secretion to a lesser extent in the operated than in the control rats, and ADH depletion of the hypothalamus was slighter.

The results indicate that the ADH-reserve capacity of the rat decrease after deafferentation of the supraoptic nucleus.

Correlation between quantitative changes of the rat anterior pituitary lactic and pyruvic acid content and lactate dehydrogenase (L-lactate -(NAD)-oxydoreductase E.E.1.1.1.2.7.) activity

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Lactic acid content of the anterior pituitary in female rats exceeded that of male animals by about 60%. Pyruvic acid content was higher in the males. The quotient of pituitary lactic: pyruvic acid was twice as high in females than in males. After ovarectomy the same values were found for both sexes. Pituitary lactic and pyruvic acid content exhibited a moderate decrease in orchidectomized rats. The quantity of the mentioned substrate or metabolite, respectively, agreed with pituitary LDH activity and alterations in enzyme subunits. The results corroborate the view that peripheral hormone actions determine decisively the characteristics of pituitary metabolism.

Site of the anticonvulsant action of diazepam

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Using the intracerebral injection technique, the site of the anticonvulsant action of diazepam was studied in rats and rabbits against chemically and electrically induced convulsions. In the rat the convulsant effect of intraperitoneal pentetrazol was inhibited in a dose-related manner by diazepam injected into the anterior amygdaloid nucleus while phenobarbital was ineffective.

Spike-discharges were evoked in waking rabbits by slow intravenous infusion of pentetrazol; the threshold was measured in cortical and subcortical EEG leads. The convulsant threshold of pentetrazol was markedly elevated after injection of Diazepam in the anterior amygdaloid nucleus while application of the drug into the basal amygdaloid nucleus or the dorsal hippocampus was ineffective. Phenobarbital proved ineffective in all the investigated regions.

In the rat, a protective effect against electroshock-seizures was seen only in the case of injecting diazepam into the anterior amygdaloid nucleus, while intrahippocampal application had no effect. Phenobarbital was also ineffective in both regions.

In waking rabbits after-discharges were evoked by subcortical electrical stimulation and the threshold of this action was studied after intracerebral application of diazepam. The hippocampo-amygdaloid after-discharges were left unaltered by diazepam, while the threshold of amygdalo-hippocampal after-discharges was markedly elevated provided the drug had been injected into the anterior amygdaloid nucleus; intrahippocampal application failed to influence after-discharges.

It is concluded that the amygdaloid complex, in particular the anterior amygdaloid nucleus, plays a fundamental role not only in the tranquillizing action but also in the anticonvulsant effect of diazepam.

Alterations in gastric secretion and serum gastrin level in pylorus-ligated and antrectomized rats after prostaglandin E_2 treatment

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The effect of 15, 30 and 60 μg of prostaglandin E_2 (PGE_2) has been studied on the gastric secretory response, HCl secretion and serum gastrin level in pylorus-ligated and antrectomized rats.

(1) PGE_2 inhibits gastric secretory response and HCl secretion in pylorus-ligated rats in a dose-related manner; (2) PGE_2 inhibits the gastric secretory response and HCl secretion in antrectomized rats in a dose-related manner; (3) there occurs

no characteristic change in the serum gastrin level in pylorus-ligated and antrectomized rats; (4) the serum gastrin level changes insignificantly after pyloric ligature and antrectomy; (5) PGE_2 does not produce any characteristic alteration in the serum gastrin level of pylorus-ligated and antrectomized rats.

It is to concluded that the gastrin system has no significant role in eliciting gastric hypersecretion in pylorus-ligated rats. The serum gastrin level does not change significantly after surgical antrectomy. PGE_2 does not induce any characteristic change in either pylorus-ligated or antrectomized rats. The gastrin system has no significant role in the inhibition of the gastric secretory response and HCl secretion of rats treated with PGE_2.

Quantitative evaluation of coronary reactive hyperaemic responses

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A method has been developed for the quantitative evaluation of reactive hyperaemic flow curves. As a first step conductance is calculated from flow, and pressure signals are recorded on magnetic tape; then "normalized" flow values are obtained which are independent of the effects of blood pressure changes. By transforming the zero axis to the normalized flow curve and integrating appropriate parts of the curve, a so-called reactive hyperaemic index (I_h) was obtained; it is a quantitative measure of the repayment of flow deficit arising during occlusion. Application of the method is demonstrated on coronary reactive hyperaemia. It was shown that I_h significantly decreased after coronary occlusion lasting 90 and 180 minutes. Elevation of blood pO_2 to several times the normal value did not significantly influence I_h. The method seems suitable for the study of the reserve capacity of coronary circulation and offers information on the viability of transiently ischaemic areas of the myocardium.

Effect of anoxia on mechanical activity, metabolic processes and ultrastructure of the perfused rat heart

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The effect of different types of anoxia (N_2; CO) was investigated on the perfused isolated Langerdorff rat heart. Maximum anaerobic performance was determined by measuring the isovolumic work. Functional and structural changes of mitochondria were analysed by electron microscopy and by measuring pyridine nucleotide fluorescence.

Isovolumic performance decreased by 80% in the first 15 minutes of anoxic perfusion, and stabilized at that level by the first hour. Isovolumic performance was doubled on increasing heart rate, perfusion pressure, or the calcium concentration in the perfusion fluid. The calculated ATP consumption was near the theoretical maximum of anaerobic glycolytic ATP production of the rat heart (40—42 μM/g dry weight/min). According to the fluorometric and electron microscopic findings, mitochondrial destruction started in the third hour and became widespread and severe by the end of the fifth hour.

Denervation natriuresis in dogs after indomethacin treatment

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The decrease of tubular reabsorption of sodium and water after renal sympathectomy leads to diuresis and natriuresis. Prostaglandins exert a similar effect while indomethacin, by inhibiting the endogenous synthesis of prostaglandins, produces antidiuresis and antinatriuresis.

In the present experiments the effect of indomethacin pretreatment has been examined in pentobarbital-anaesthetized, unilaterally splanchnicotomized dogs, and in control animals.

In control dogs urine flow (V), sodium excretion ($U_{Na}V$) and the fraction of filtered sodium (E_{Na}) were 3.1 ± 0.4 ml/min, 345 ± 56 μEq/min and $3.42 \pm 0.47\%$, respectively, on the innervated side; and 6.5 ± 0.8 ml/min, 742 ± 92 μEq/min and 7.2%, respectively, on the denervated side. The differences were highly significant.

In the group with indomethacin pretreatment V, $U_{Na}V$ and E_{Na} were 1.8 ± 0.3 ml/min, 239 ± 43 μEq/ml and $2.49 \pm 0.38\%$, respectively, in the innervated kidney; and 3.71 ± 0.4 ml/min, 556 ± 72 μEq/min and $5.45 \pm 0.56\%$, respectively, in the denervated kidney. Thus, indomethacin pretreatment had no substantial effect on the denervation phenomenon since marked denervation diuresis and natriuresis occurred also in this group.

Thus, endogenous prostaglandins do not seem to play any major role in the denervation phenomenon.

Phospholipid content of human fetal brain and fatty acid composition of its individual phospholipid components during development

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Phospholipids have an important role in the development of the functions of individual cells during intrauterine development. Cerebral tissue from 6—24-week fetuses was studied. Up to the 24th week of pregnancy there was no essential change in the phospholipid content. The fatty acid composition of the individual phospholipid components underwent a significant change with the advance of pregnancy. The proportion of polyunsaturated fatty acids containing 18—22 carbon atoms increased with increasing gestation time in the phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol fractions. The changes are assumed to be due to the action of specific acyltransferases.

The formation of early human fetal brain phospholipids was also studied by estimating the incorporation of labelled ethanolamine, choline, serine and methionine. The data obtained prove that phospholipid synthesis already takes place in 6—8-week old fetal cerebral tissue. It is concluded that an interconversion is possible between the phospholipids.

Effect of corticosterone given to new-born rats on adult behaviour and some biochemical correlates in the brain

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It has been shown that administration of moderate doses of corticosterone to new-born rats results in a permanent change in passive avoidance learning behaviour. Recently, an increased exploratory activity (rearing) could be observed following neonatal corticosteroid treatment in male and, especially, in female rats. The increased exploratory activity coincided with an elevation of the noradrenaline and serotonin level in the mesencephalic-posterior hypothalamic region.

In further experiments the ^{3}H-corticosterone uptake by hippocampus homogenate was measured in vitro; a diminished uptake was observed in the nuclear fraction.

A strong correlation was found between rearing activity and the noradrenaline content of the rostral brain stem on the one hand and the ^{3}H-corticosterone uptake in the nuclear fraction of the hippocampal homogenate on the other.

Effect on cerebral blood volume of changes in arterial pCO_2 and blood pressure

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A method has been developed for determining cerebral blood volume (rCBV) localized throughout the brain in three dimensions, using transverse section scanning of radionuclides given intravenously. The method requires an intravenous injection of approximately 15 mCi of ^{99m}Tc labelled red cells. Subsequently, multiple scans can be made over a 4—5-hour period without further injection of radionuclide. During this time the physiological state of the subject may be altered and the subsequent effect on rCBV can be documented by repeated transverse section scans. Each scan requires 2—20 min for completion, depending on the precision of data required. A blood sample is obtained from the subject at the time of the scan and assayed to determine μCi/ml of red cells. Later, a scan is made on a phantom containing a known uniform distribution of the same radionuclide. The section scans are processed in a digital computer, corrected with the scan data of the uniform distribution, and then directly converted to millilitres of red cells per 100 g of tissue using the known concentration of radionuclide in the blood. The result is a two-dimensional map of rCBV which represents a cross-section at a certain level of the brain. We have now tested method in 5 anaesthetized baboons under controlled conditions of $paCO_2$ and arterial blood pressure. Analysis of the results obtained led to the regression equation, CBV/ml/100 g = 3.14+0.049 ($paCO_2$, mm Hg)—0.013 (MABP, mm Hg).

The baboon rCBV data in this study showed a close correspondance to the absolute value and response to $paCO_2$ and blood pressure reported in the literature. It was therefore concluded that the section method is capable of yielding accurate maps of rCBV which respond appropriately to physiological stimuli.

Synchronizing effect of basal forebrain stimulation in unanesthetized cats

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Distribution and functional properties of the basal forebrain synchronizing areas were studied in unanesthetized, immobilized cats stimulated at one-millimetre steps. The effects were evaluated on the basis of changes in amplitude of the EEG as well as the latency and phase relationships of the evoked potentials.

Stimulation with 10 cps of the region revealed several points which produced synchronization. The majority of synchronizations was frequency-follower, and of increment or sustained in character. Certain responses were followed by synchronizing, while others by desynchronizing rebound. The projections to neocortical and limbic cortical areas were not diffuse, and their topographical distribution could be determined. Areas synchronizing the anterior and posterior parts of the cortex could be distinguished. It is assumed that, since the functional characteristics of the frequency-follower synchronizations evoked from the basal forebrain are varying, they cannot be due to a uniform mechanism.

Insulin transport in lymph

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The concentration of immunoreactive insulin (IRI) was measured by radioimmune assay in the blood plasma and the thoracic duct lymph in the following groups of rats, all starved for 18 hours: intact rats, rats subjected to bilateral adrenalectomy and treated with physiological saline solution, and rats rendered hypocalcaemic by isolated bilateral parathyroidectomy.

In the intact rats, IRI concentration was the same in plasma and lymph.

In the adrenalectomized rats IRI concentration was significantly lower in both plasma and lymph than in intact animals. IRI concentration was the same in the plasma and lymph.

In the parathyreoidectomized rats, IRI concentration in plasma and lymph was significantly lower than in the intact animals. However, IRI concentration in the thoracic duct lymph was significantly lower than in the plasma.

It is suggested that calcium deficiency due to parathyroidectomy affects not only the release of insulin but also the direction of its flow, so that less insulin is passing into the lymphatics.

Effect of diethyl-imino-malonate on the slow anion permeability of human red blood cells

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The possibilities offered by diimino-esters for studies of membrane transport are largely unutilized. This group of compounds, for instance diethyl-imino-malonate (DEM), has the important advantage over other bifunctional protein amino reagents that it introduces imino groups of the same number at the site of action, and balancing thus the total number of positive charges. The effect of DEM was examined

on the transport of sulphate, phosphate and chromate. Phosphate and sulphate were measured as unidirectional flux at Donnan-equilibrium, while for chromate the net uptake was estimated. In a 25% suspension of washed red cells in 125 mM phosphate buffer pH 8.0 at 0 °C, 20 mM DEM caused the total hypotonic and detergent-induced haemolysis to cease and inhibited the transport of phosphate. Under these conditions the velocity of chromate uptake was inhibited by 40%, and no further change was observed after prolonged exposure to DEM. In contrast, the transport of 10 mM sulphate in chloride medium pH 8.0 was not influenced in cells treated with DEM for two days. Supplementary experiments showed that the acetylcholinesterase located on the external surface of the membrane also remained practically unaltered.

The findings indicate that the anions examined pass across the erythrocyte membrane by different molecular mechanisms and that sites other than the amino groups near the external surface are also involved into the effect of DEM.

Effect of aloxan diabetes on carraghenin-induced inflammation in rats

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Anaphylactoid reaction, anaphylactic shock and adjuvant arthritis are known to be prevented by alloxan diabetes in the rat. The present experiments showed that the inflammatory response to carraghenin was enhanced by alloxan diabetes. Insulin that exerts an anti-inflammatory activity on carraghenin paw oedema in normal rats, failed to prevent the inflammatory response in diabetic animals.

The results indicate that insulin and diabetes have an opposite effect on acute, non-immune inflammation as compared to allergic reactions.

Magnesium level of erythrocytes and plasma in liver diseases

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The blood magnesium level was studied in various liver diseases and its diagnostic value was analysed. The plasma magnesium level was increased in obstructive diseases of the liver. The elevated plasma magnesium content is of diagnostic value in various forms of cholestasis when factors such as drugs etc. can be excluded. The magnesium level is erythrocytes showed no significant alteration.

Animal experiments revealed that the increase in plasma magnesium level was not due to the high plasma bile acid or the elevated plasma corticosteroid content.

Effect of cervical lymph blockade on the rats food uptake

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Food intake and body weight were studied for 14—20 days prior to, and for 15 days after, ligature of cervical lymph nodes. The animals were fed for 2 hours daily; this feeding period was followed by 22-hour fasting. Food intake decreased after operation. The decrease had two distinct phases: after an initial fall on the 1st and 2nd days it reached the control level. Later, by the 6th—9th day a new decrease was observed.

The first phase was present both in sham-operated and control animals treated with a standard dose of pentobarbital. The first phase is a non-specific event, while the second one a specific consequence of the ligature. The same phases were found in the increase of duration of slow-wave sleep in rabbits on the same postoperative days. These effects as well as the autonomic and behavioural changes observed earlier point to a hypothalamic dysfunction due to the cervical lymph blockade.

Effect of vincanol (RGH 4406) on the central nervous system

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Vincanol, a synthetic vincamine derivative, exerts a vasodilator action on cerebral vessels and also other effects on the CNS.

The drug increased the spontaneous motility of mice; large doses induced transitory ataxia. Beside its antimetrazol effect the drug has a strong anti-aggressive action as judged by the paw-shock test. Its analgesic effect could be demonstrated by the writhing test and Haffner's method, but not by D'Amour-Smidt test. The analgesic effect of morphine was potentiated. Vincanol exerted no anticholinergic effect; in fact, it increased the toxicity of physostigmine. Hexobarbital sleeping-time was reduced, amphetamine toxicity and stereotype potentiated, reserpine hypothermia reduced or reversed by Vincanol.

Vincanol exhibited anticataleptic effect in reserpine, haloperidol or tetrabenazine-induced catalepsy. MAO activity was not inhibited.

Catecholamine sensitivity of swimming rats

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It was shown previously that in the catecholamine resistance due to regular physical training the response to alpha-adrenergic stimulation decreased while the response to beta-adrenergic stimulation remained unaltered.

The problem was studied in trained and control albino rats; training consisted of swimming daily for 8 weeks. Blood pressure and heart rate responses elicited by various doses of noradrenaline, adrenaline and isoprenaline as well as the effect of various antagonists were investigated under urethan anesthesia.

The main differences between the two groups were as follow: (1) the lower resting heart rate of the trained rats was not reduced by beta-blocking agents; (2) the pressor response elicited by noradrenaline and adrenaline was smaller in the trained rats; (3) the tachycardia elicited by adrenaline and isoprenaline was higher in the trained than in the control group.

The results support the hypothesis that the sensitivity of the two types of adrenergic receptor changes in a dissimilar manner after training. As regards response amplitudes, an altered equilibrium of the autonomic nervous system in the exercise-adapted organism must also be taken into account.

Effect of anesthetics on regional blood flow and metabolism of the brain

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The effect of two different anaesthetics, alpha-D/+/-gluco-chloralose and phencyclidine was investigated on cerebral blood flow, CO_2 responsiveness, cerebral autoregulation and metabolism.

The experiments were performed on immobilized baboons of both sexes under artificial respiration. Anaesthesia was induced with 100 mg/kg chloralose (Merck) or with 1 mg/kg phencyclidine (Bio-Ceutic Laboratories). Arterial and cerebral venous pressures as well as end tidal CO_2 concentration of the expired air were monitored throughout the experiment. Cerebral blood flow in 8 different brain regions was measured by the intra-arterial ^{133}Xe-clearance technique, using on-line computer system for data analysis. Arterial and sagittal sinus blood samples were taken at predetermined intervals for blood gas, glucose, lactate and pyruvate estimation. CO_2 responsiveness of the cerebral vascular bed was tested by giving a gas

mixture containing 5% CO_2. To investigate autoregulatory responses, the arterial blood pressure was reduced by withdrawing blood.

Chloralose depressed both total and regional cerebral blood flow as compared to phencyclidine and caused a shift toward lower flow values in both CO_2 responsiveness and the pressure-flow relationship of the cerebral vascular bed. In deep hypotension (below 60 mm Hg) there was no difference in cerebral blood flow between the two groups as.

Effect of disruption of T-tubules on K-exchange in frog skeletal muscle

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An attempt was made to differentiate between the exchange of potassium ions across the muscle surface membrane and through the tubular system. Frog sartorius muscles were kept in Ringer solution made hypertonic by the addition of 400 mM glycerol for one hour. After substituting the bath for normal Ringer containing 5 mM $CaCl_2$ and 5 mM $MgCl_2$, a disruption and disconnection of the transverse tubular system from the surface membrane occurred.

^{42}K uptake, K-exchange, water and ion content of muscles were measured 0.5, 1, 1.5, 2, 4, 5 hours later. Water and Ca content of treated and untreated muscles did not differ significantly, but a significant Na gain and K loss as well as a decrease of the Mg level were found at 1 to 5 hours. The initial potassium influx rate as well as the amount of K in muscle cells exchanged for ^{42}K during 60 min were reduced by 30% after glycerol treatment. The ouabain-sensitive fractions of the total K influx in treated muscle were increased and they decreased as compared to untreated muscle. Thus the detubulated muscle had lost a substantial part of its capacity to exchange K through an ouabain-insensitive K : K exchange transport mechanism.

Effect of some muscle relaxants in alloxan diabetic and cortisone-treated rats

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The ED_{50} values of five peripheral muscle relaxants (d-tubocurarine, pancuronium, gallamine, decamethonium and succinylcholine) were compared on the anterior tibial muscle of in normal, alloxan diabetic and cortisone-treated rats. Except succinylcholine, a single injection of the ED_{50} of all the other drugs studied exerted

a less pronounced inhibition of synaptic transmission in diabetic and cortisone-treated than in untreated normal animals. Diabetes and cortisone treatment increased the effectiveness of succinylcholine. The phenomenon can partly be explained by an altered elimination of the drugs and partly by an increased excitability of the motor end-plate caused by diabetes or cortisone.

Sensitivity to biogenic amines of isolated organs of swimming-trained and control rats

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As shown previously, both regularly trained animals and athletes under intense training exhibited a reduced sensitivity to adrenaline, noradrenaline and histamine. In order to clarify the underlying mechanism, the responses were studied in isolated organs. The inhibitory effect of catecholamines on acetylcholine contraction of the uterus was studied in trained and untrained Wistar rats. In order to investigate the dose-effect relationships the concentrations ranged from 3×10^{-10} to 1×10^{-6} g/ml for adrenaline, from 3×10^{-8} to 3×10^{-6} g/ml for noradrenaline and from 1×10^{-10} to 3×10^{-7} g/ml for isoprenaline. Pindolol caused a dose-related inhibition of the responses.

The greatest difference between the trained and control groups was found with isoprenaline, where a dose ratio of about 1 :10 was observed between the respective curves. This indicated a rise in beta-receptor sensitivity in the swimming animals. The smallest deviation from the control was seen in the noradrenaline response while adrenaline exerted an effect opposite to that of isoprenaline.

Effect on myocardial contractility of carbutamide, gliclazide and glibenclamide

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The original sulphonylurea compounds used in the treatment of diabetes elevate arterial blood pressure, increase myocardial contractility and enhance the cardiovascular mortality of diabetic patients. Carbutamide gliclazide and glibenclamide have not been tested in this respect.

In dogs anaesthetized with chloralose carbutamide and gliclazide increased arterial blood pressure, myocardial contractility, the acceleration of contractility, cardiac output and the speed of aortic flow. The effect was a direct cardiac action

since it was dose-related and could be elicited after pancreatectomy and adrenalectomy. On the other hand, glibenclamide had no such effect. Thus, a significant difference was demonstrated between the cardiovascular effects of glibenclamide and the other sulphonylureas tested.

Modifying effect of clonidine and levodopa on sympathetic reflex activity

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Several authors ascribed the antihypertensive effect of clonidine to a central alpha-mimetic action.

An untoward side-effect of levodopa is its hypotensive action of central origin, just as its inhibitory action on sympathetic efferent activity.

The efferent sympathetic activity and its reflex responses mirror the functional state of the medullary vasomotor centre.

In cats anaesthetized with chloralose-urethan both clonidine and levodopa were found to increase somato-excitatory reflexes and inhibit somato- and viscero-inhibitory reflexes. When administered after levodopa the effect of clonidine was much more marked than when given by itself.

It is concluded that the two substances also inhibit the interneurones assumed to be located between inhibitory and excitatory neurone-networks.

Peripheral action of clonidine

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Intravenous injection of clonidine causes an initial rise in blood pressure followed by lasting vasodepression. At the same time the drug permanently contracts the nictitating membrane. The author succeded in producing tachyphylaxis to the hypotensive action of clonidine, just like to the blood pressure effect of indirect sympathomimetic amines. The hypotensive action of clonidine is central in origin. The contractions of the nictitating membrane persist also in animals which had developed tachyphylaxis.

The present investigations were aimed at deciding whether the clonidine-evoked contraction of the nictitating membrane was brought about by a direct or an indirect effect. The nictitating membrane of the cats was denervated on the one side 7—11 days before the experiment. Then the effect of clonidine was investigated under chloralose-urethane anaesthesia. Clonidine was found to behave in an amphetamine-like manner.

Fatty acid esters in the serum of hyperlipaemic patients

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In subjects with primary hyperlipaemia of WHO types II/A and V, about 5—6% of the total serum lipids belonged to the fatty acid ester fraction as estimated by thin-layer chromatography. The R_f value for the fraction was between the triglyceride and cholesterol-esters.

The R_f for glycerol-ether-lipids were similar to those of fatty acid esters, but it was possible to separate them in the usual class-separation system.

The fatty acid composition of these fractions was investigated by GLC, and a difference in composition was found between the two groups.

Redox state of different tissues and the effect of acetylcholine

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By simultaneous recording of myograms, cardiomyograms and tissue redox state potentials (RSP) it was established that in tissues with high RSP level (+170 mV or higher: rectus muscle, gastric smooth muscle) acetylcholine (ACh) exerted a depolarizing type of action, while in the frog heart ventricle (with RSP values lower than +170 mV) the mediator evoked a hyperpolarizing action.

Since after appropriate adjustment of RSP values by exogenous redox agents both the depolarizing and the hyperpolarizing ACh effects can be modelled in any organ, independently of the original type of ACh effect, it is assumed that the actual redox state plays an important role in the determination of depolarizing and/or hyperpolarizing type of the ACh effect.

Gradual development of blood pressure responses after hatching in chicks

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It was shown previously that blood pressure of the chick embryos responded to drugs in a highly selective manner during the second half on the incubation period. Drugs evoking a fall in blood pressure generally caused irreversible and fatal effects, while adrenaline, noradrenaline produced real pressor responses. On

repeated administration, however, these pressor effects decreased and were finally reversed into depressor ones. Embryo with growth of the tolerance of the circulatory system improved, but during the first period of post-hatching life the pattern of adrenaline effects reminded of the embryonal responses.

In the present experiments, the adrenergic and cholinergic responses of young chicks was followed daily during the first week, then weekly up to the end of the third month of life. Many significant quantitative alterations could be observed during this period. For instance, the blood pressure responses to anticholinesterase agents gradually underwent a total inversion during ageing.

Effect of chlorpromazine on tyrosine hydroxylase in rat brain and beef adrenal medulla

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The effect of chlorpromazine (CPZ) and chlorpromazine free radicals (CPZ^+) was studied in vitro on the tyrosine hydroxylase activity of bovine adrenal medulla and the rat striatum. CPZ^+ caused a stronger inhibition than CPZ in both tissues. With bovine adrenal medulla tyrosine hydroxylase the inhibitory constants (K_i) were 23×10^{-4} M for CPZ and 6.0×10^{-4} M for CPZ^+, and with the rat striatal tyrosine hydroxylase 9.0×10^{-4} M and 5.0×10^{-4} M, respectively.

Chronic CPZ treatment (20 mg/kg for 12 days) increased by 20% the rat's striatal tyrosine hydroxylase activity.

ATPase activity in dissociated nerve cell cultures as a function of cell maturation

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Dissociated nerve cells prepared by the method of *Booher* and *Sensenbrenner* from the brain of 6—19 day old chick embryos were cultured for 7—28 days. The ratio of glial to neuronal cells as well as cell maturity in the different cultures varied in dependence on cultivation time and on the age of chick embryos whose brains had been dissociated.

The total, the Mg^{2+} and the Na^+—K^+—ATPase activities in the cells were estimated according to the slightly modified method of *Kimelberg*. Before homogenization the "ecto-ATPase" activity of the cells too was assayed in most experiments. Enzyme activities in the cultures were compared with those in the brain of the embryos.

The results showed a clear-cut parallelism between the time-course of enzyme activities in cultures and the evolution of ATPases in the developing brain. The total ATPase activity was low and no trace of oubain-sensitive ATPase was detected either in very immature cells in cultures or the brain of young embryos. After an identical period of maturation cultured cells and embryonic brain both developed a significant level of oubain-sensitive ATPase activity. This period coincides with the hatching time of the embryos and means a corresponding cell cultivation period of 19—23 days.

Autoradiographic study of ^{14}C-amino acid incorporation into the cerebral cortex during physiological stimulation

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Thin paper strips soaked in ^{14}C-amino acid solution were applied to the auditory cortex of anaesthetized cats bilaterally and kept there for 40 min. Thereafter, the underlying cortical tissue on the right side was excised and the left auditory cortex stimulated by clicks of 2/sec frequency for 40 min and similarly prepared for autoradiography.

1. Leucine, glutamic acid and aspartic acid were distributed rather homogeneously, while GABA and glycine showed a marked preference for nerve cells. 2. Stimulation enhanced the incorporation of leucine and aspartic acid into the superficial cortical layers. 3. GABA was incorporated intensely into the II and III layers; the labelling diminished gradually toward the deeper regions. Superimposed on the diffuse labelling many patches were seen indicating a cellular localization of GABA. The latter was depressed by stimulation. 4. The concentration of glycine in nerve cells somewhat diminished troughout the stimulated cortices.

Alteration of vascular resistance in isolated kidney

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Previous experiments suggested the occurrence of an intrarenal redistribution of blood flow in the isolated kidney. In the present work the mechanism of that redistribution has been investigated. Total renal blood flow (TRBF) was measured by means of electromagnetic flowmeter, venous outflow, as well as by ^{42}K and ^{86}Rb isotope fractionation methods (Rb—RBF). After the control periods noradrenaline was infused into the renal artery at a rate of 0.5—1.0 μg/min/kidney for 20 min. The ratio of Rb—RBF:TRBF increased from 0.75 ± 0.03 found in the control

to 0.95 ± 0.03, while TRBF decreased moderately, and Rb–RBF cortical and medullary blood flows remained unaltered. The Rb–RBF/TRBF ratio of 0.95 corresponds to the value obtained in the innervated kidney in situ. The higher reactivity of the renal medullary vessels to sympathetic stimuli and sympathetic mediators suggests that the increase of the Rb—RBF:TRBF ratio may be the result of a decreased medullary blood flow and of the improvement of Rb extraction in the medulla. The results are in agreement with the assumption that the differences between Rb—RBF and TRBF may be attributed to the enhanced medullary flow.

Effect of $NiCl_2$ on the isolated heart

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The effect of $NiCl_2$ at various concentrations (0.001–1 mM) in the perfusion fluid was investigated on mechanical performance, coronary resistance and O_2 consumption of the perfused rat heart. After administering 1 mM $NiCl_2$ the hearts immediately stopped beating and coronary resistance increased by 50%, though with a certain delay in time (10 min). Decreasing $NiCl_2$ concentration to 1 μM, performance became gradually less damaged but coronary resistance increased to the same extent or even more. Increasing the calcium concentration of the perfusion fluid fully antagonized the myocardial effect of $NiCl_2$, but coronary resistance continued to increase by 50%. It is assumed that the electromechanical coupling was different in the two types of muscle. Simultaneous infusion of 2 mM $NaNO_2$ did not change the resistance-increasing effect of $NiCl_2$. O_2 consumption was decreased by 1 mM $NiCl_2$ but increased by 1 μM $NiCl_2$.

Effect of endogenous pyrogen on granulocyte count

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Endogenous pyrogen was prepared from the rabbit's peritoneal granulocytes and its effect was examined on the granulocyte count and fever response in rabbits. The endogenous pyrogen increased the granulocyte count; the peak was reached by the second hour. After heat treatment, the pyrogen failed to alter the granulocyte count and to cause fever. In endotoxin-tolerant animals, however, the pyrogen elicited both granulocytosis and fever. It is assumed that the endogenous pyrogen and the granulocyte-mobilizing factor are identical.

Effect of Panax ginseng on neuromotor functions in humans

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The effects of alcoholic extracts of Panax ginseng and placebo were compared in randomized, self-control, double-blind acute experiments. The parameters were simple visual reaction time and two types of combined attention-reaction tests: stop of a running light-spot according to a given program, and observing the turn-off of the direction of a running light-spot. The Sákovits–Alexits electronic apparatus was used. The test lasted 25—30 min and 560 data were registered. Eight healthy subjects were examined first at rest and than during 50 Watt load 10 min after administration of the substances.

Ginseng abolished the expected changes during physical load: neither the reaction time nor the vigility of attention were decreased, in contrast to the placebo-treated subjects.

Single neuronal responses to chemical stimulation of lip receptors in the snail Helix pomatia L.

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The effect of afferent impulses evoked by chemical stimulation of the lip receptors was investigated in CNS neurones of the snail, using an isolated lip-brain preparation. Electrical activity was recorded extracellularly from the lip nerve and intracellularly from one or two unidentified neurone(s) of the suboesophageal ring.

Activity of a number of neurones was affected by distilled water, NaCl, KCl, glucose, sucrose and several plant protecting chemicals. These changes followed the increase in the activity of the lip nerve. Synaptic potentials, increase or decrease of activity, usually occurred during application of the drug, but after-effects were also observed. The same neurone could give different responses to different test solutions, and different neurones could respond to the same substance in a different manner. This is the physiological basis of the central chemo-discriminative process determining the behavioural reactions evoked by food and other chemicals.

The neurones in the cerebral and suboesophageal ganglia responding to chemical stimulation of the lip are either secondary sensory neurones or interneurones participating in integrative processes; but some of them could be motoneurone regulating motor functions connected to feeding, escape, secretion, or other functions.

Body-plethysmographic measurements in coal miners exposed to prolonged dust inhalation

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Airway resistance, viscous work and intrathoracic gas volume were measured by body-plethysmography in about 900 smoking and non-smoking coal miners.

Intrathoracic gas volume was higher in smoking than in non-smoking coal miners, in agreement with previous results.

A pathological increase in airway resistance could be demonstrated in 28% of non-silicotic coal miners exposed to dust. This proportion was somewhat lower in coal miners with silicosis. No difference was found between smoking and non-smoking coal miners in the degree of increase of the airway resistance.

Similarly, no difference could be demonstrated in the increase of viscous work between smoking and non-smoking coal miners.

Propinylamine-type monoamine oxidase (MAO) inhibitors

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The propinylamine derivatives of aliphatic, phenylethylamine and isoquinoline-type compounds were examined as to their MAO-inhibitory effect on rat brain and liver preparations both in vivo and in vitro. ^{14}C-tyramine, ^{14}C-benzylamine and ^{3}H-serotonin served as substrate.

Among the phenylethylamine structures, N-propinyl-N-methyl-2-phenylethylamine (TZ 650 a_1) and N-propinyl-2-phenylethylamine (TZ 652 f_2) proved to be the most potent MAO inhibitors.

TZ 652 f_2 has the same substrate specificity as deprenyl both in vivo and in vitro. It potently inhibits the oxidative deamination of benzylamine and less effectively that of serotonin. The substrate specificity of TZ 650 a_1 differs from that of deprenyl. It is less potent in inhibiting the metabolism of tyramine.

The experiments revealed that the propinyl structure is important for the inhibition of MAO but the inhibitory potency and substrate specificity are determined by the complex structure of the molecule.

Ascorbic acid-induced lipid peroxidation in subcellular fractions of the rat brain

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Lipid peroxidation can be induced by ascorbic acid in microsomes and nerve-ending membranes isolated from the rat brain. The effect shows an optimum curve dependence on the concentration of ascorbic acid. Lipid peroxide formation is fully antagonized by 2×10^{-3} M ascorbic acid. High concentrations of dehydroascorbic acid act in a similar manner, indicating that not the antioxidant but the structural properties of ascorbic acid are responsible for the inhibitory effect. Since the concentration of ascorbic acid in the rat brain is 2×10^{-3} M, it can be assumed that ascorbic acid has a role in the regulation of processes involving free radical intermediates.

Characterization of mucosal pseudocholinesterase

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The pseudocholinesterase (pChE) activity of the intestinal mucosa was described by Donhoffer. The physiological function of the enzyme is unknown; it is supposed to exert some effect on intestinal absorption. It was demonstrated previously that inhibition of pChE did not alter the absorption of Ca^{++}, methionine or glucose, and influenced Na^{+} absorption in an uncertain way.

The aim of the present experiments was 1) to characterize the mucosal enzyme; 2) to compare it with the bile ChE enzyme; 3) to examine the effect of a direct, irreversible ChE inhibitor (DDVP) on sodium absorption.

1) The rat's mucosal pChE proved to split BSCh faster than ASCh, the optimum substrate concentration being around 10^{-2} M. No substrate inhibition was found. The enzyme was inhibited by 10^{-5} M IOMPA. It is concluded that a pChE enzyme was responsible for more than 90% of the ACh-splitting activity of the mucosa.

2) The bile cholinesterase was characterized as a mixture of pChE and AChE with high AChE content.

3) Male albino rats of 120 g body weight were treated with 20 mg/kg and 5 mg/kg of DDVP administered by the oral route. Two hours later, pChE activity of the jejunal and ileal mucosa was inhibited by about 75%, and after 20 hours by 25—40%. Sodium absorption did not change in either the jejunum or the ileum.

Mechanism of the spasmogenic action of pentetrazole and some pyridine-tetrahydroisoquinolines

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The physicochemical properties of pentetrazole and of some pyridine-tetrahydroisoquinolines, their biological activity on isolated frog skin and fundus strips of the rat stomach were studied, and model experiments were carried out on ether-0.1 N NaCl "liquid chain".

A correlation was found between the degree of intermolecular compensation and orientation of the compounds at the interphase and their membrane activity. Exposure of the solution of the substances to changing magnetic and static electric fields and ultrasonic treatment strongly influenced their activity. A monolayer was built up by the spasmogenic molecules at the interphase and, after saturation, they formed floating lens and crystals whose anisotropy influenced the pharmacological activity and depended on the concentration.

Influence of the sympathetic and parasympathetic system on excitability of the heart

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The effect of stellate ganglion, carotid sinus nerve and vagal nerve stimulation was studied on the electrical systole (Q-T) and effective refractory period of the dog heart. Stimulation was performed by the radio-frequency principle using a Medtronic Baristat electrical barostimulator. The effective refractory period of the heart was measured by delayed coupled stimulation of the right ventricle. The Q-T (electrical systole) duration was determined by the aid of ECG tracings using I—III bipolar limb leads, the local Q-T duration from epicardial unipolar ECG tracings. The investigations were repeated during electrical pacing of the right ventricle and in local myocardial ischaemia induced by ligation of the descending branch of the left coronary artery.

Sympathetic stimulation shortened, parasympathetic stimulation lengthened Q-T time, due to changes in frequency. At constant heart rate (electrical stimulation of the right ventricle), electrical stimulation of the stellate ganglion or of vagal fibres failed to affect the duration of Q-T. Stimulation of the stellate ganglion decreased, while that of the vagal fibres or the carotid sinus nerve increased the duration of the refractory period. In local myocardial ischaemia a significant difference was observed in Q-T time of the ischaemic and intact zone (inhomogeneous repolarization); the difference increased during sympathetic stimulation and decreased during carotid sinus nerve stimulation.

Some characteristics of the acetylcholine release from the rat vas deferens

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The vas deferens of the rat contains high amount of ACh (10.9 ± 1.3 nmol·g^{-1}) that is released on electrical stimulation. In the present experiments the organ bath was perfused with eserinized Krebs solution at a constant rate, and 5 and 10 min effluents were collected. The released ACh was bioassayed on guinea pig ileum. When electrical stimulation of 10 Hz and supramaximal voltage was applied the released ACh increased from 31 pmol·g^{-1}·min^{-1} (resting value) to 225 ± 25

225 ± 25 pmol·g^{-1}·min^{-1} in 15 min and was still

294 ± 31 pmol·g^{-1}·min^{-1} 45 min later. The ACh content of the stimulated organ (11.8 ± 1.4 nmol·g^{-1} did not significantly differ from the control. However, when choline uptake was inhibited by 10 μM of hemicholinium (HC-3) the synthesis of ACh ceased and the effect of stimulation decreased exponentially to the resting level (41 ± 7 pmol·g^{-1}·min^{-1}). The ACh content of the tissue also decreased by 56% (4.8 ± 0.7 nmol·g^{-1}). The ACh content of the vas deferens was not reduced to the same extent as the ACh release. When a minimum amount of ACh is released from the organ, much ACh is still pooled in the vas, showing that a great amount of the stored ACh is not available for release by electrical stimulation.

Increased K^+ concentration (47.3 mM) caused a long-lasting and steady increase in ACh release (110 ± 9.0 pmol·g^{-1}·min^{-1}). If Na^+-free isotonic solution was perfused, the release of ACh was doubled, while 20 mM of oubain was ineffective. Thus, inhibition of the membrane ATPase, unlike in the intestine (Auerbach's plexus) and cortex, did not increase the release of ACh from the rat's vas deferens.

Prostaglandin synthesis

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Prostaglandins were isolated from various tissues. According to previous investigations the prostaglandins seem to play an important role in various physiological and pathophysiological events. The questions studied were as follows.

i) The amount of prostaglandin synthetase in various tissues;

ii) do the prostaglandin synthetases isolated from different tissues need the same bivalent metal ion for activation;

iii) the subcellular distribution of prostaglandin synthetase.

The stomach, small intestine, kidney and lungs contain the enzyme in large amount. The highest specific activity of prostaglandin synthetase was found in the microsomal fraction. The enzyme was easy to solubilize.

The active enzyme was contained in the 100,000 *g* tissue supernatant fraction.

Organization of the neuronal network implicated in the regulation of heart beats in Helix pomatia L.

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The central neurones implicated in the regulation of heart function can be interpreted as a system interconnected by their regulatory function. In the central nervous system of *Helix pomatia*, 21 cells building up this neuronal network were identified according to morphological and physiological properties. In the present study the connections of central elements and the changes in their interactions were analysed under the influence of sensory inputs from the heart.

In *Helix pomatia*, the neuronal network implicated in the regulation of heart beats is constituted by pacemaker and non-pacemaker cell. This network proved to be over-protected, of the convergent type where the inputs predominated and the outputs formed the independent parallel pathways (by motoneurones V12, V13, etc.) being coordinated by dual-action interneurones (V20, V21). The neuronal network can be divided into information collector and coordination levels, depending on the role played in conducting or analysing the impulses.

Strophanthin-sensitivity of K-influx in skeletal muscle of the frog Rana esculanta

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The steady level of K in skeletal muscle is maintained by a balance of the passive permeability of the cell membrane and the active transport mechanism. The latter can be blocked by strophanthin (Str), a potent inhibitor of Na-K-ATPase. There are, however, considerable discrepancies in the results concerning the Str-sensitivity of K-influx in skeletal muscle. In the present study, Str (5×10^{-5} M) exerted a negligible effect on ^{42}K-influx in freshly dissected sartorius muscles at $[K]_0 = 2{\cdot}5$ mM, while physostigmine (10^{-3} M) inhibited it by 60%. When the external K concentration was reduced to 0.15 mM in Na-free choline Ringer, the K-influx was 0.28 $\mu M/g^{-1}/hr^{-1}$; 80% of this was abolished by Str and 40—45% by physostigmine. The inhibitory effect of Str and physostigmine could equally be observed in muscles with both low and high $[Na]_i$. In Na-free choline Ringer containing 10 mM Li and 0.15 mM K, the influx was reduced to 0.16 $\mu M/^{-1}/hr^{-1}$. In muscles pretreated with Str, lithium ions had no effect, while they caused an additional inhibition of K-influx in physostigmine pretreated muscles. The data indicate that

a large Str-sensitive fraction of K-influx becomes unmasked at low (0.5 mM) $[K]_o$ in muscles with both low and high $[Na]_i$. It is concluced that Str affects the energy supply to the uphill K movement and physostigmine influences the K-complex formation of carriers, regardless of the energy requirement of ion translocation.

Castration-induced changes in œstradiol-binding capacity of the rat uterus

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Data in the literature are contradictory as regards the oestradiol-binding capacity of the uterus after castration. In the present investigations the influence of ovariectomy was studied on oestradiol uptake by uterine slices of intact, ovarectomized and oestradiol-treated rats. The dissociation constant of the receptor-oestradiol complex was the same in both intact and castrated animals (2×10^{-9} M/litre at 0 °C). The binding capacity of the cytosol fraction was higher in ovariectomized than in intact uterus. In the latter group however, the endogenous oestradiol supply must also be taken into consideration. "Pulse-injection" of oestradiol resulted in a significant increase in the binding capacity of the uterus in castrated rats.

Effect of catecholamine on protein synthesis in the rat heart

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The effect of in vivo and in vitro catecholamine treatment on protein synthesis of rat heart tissue slices was studied. The rate of protein synthesis was measured by the incorporation into proteins of ^{3}H-leucine the incorporation rate was proportional to the quantity of ^{3}H-leucine and to the time of incubation (up to 4 hours).

Intraperitoneal administration of isoprenaline, adrenaline and noradrenaline increased the labelling of myocardial proteins. The effect was most intensive with isoprenaline, while that of adrenaline and noradrenaline was practically the same. The magnitude of increase was a function of the dose and the number of injections.

Since addition of the drugs studied to heart tissue slices from untreated animals did not affect the rate of protein synthesis, an indirect action may be supposed on cardiac proteins. After in vivo isoprenaline treatment an increased synthesis of myocardial ribonucleic acids (*Wood* et al., J. Molec. cell. Cardiol. *3*, 127, 1970) and an enhancement of myocardial protein synthesis (*Gordon* et al., J. Molec. cell. Cardiol. *4*, 543, 1972) were demonstrated.

Effect of renal denervation on tubular PAH and glucose transport in the dog

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Renal denervation is followed by a decreased reabsorption of sodium and water. Tm phosphate is also depressed.

In the present study the effects of PAH and glucose loading were examined in pentobarbital-anaesthetized dogs previously subjected to unilateral splanchnicotomy.

Denervation diuresis and natriuresis were consistently observed. PAH secretion did not differ in the intact and the denervated kidney below a plasma PAH level of 20 mg per dl, whereas Tm of PAH was 13.5 ± 0.5 mg/min on the innervated (I) and 11.4 ± 0.7 mg/min on the denervated (D) side ($P < 0.001$).

Glucose loading resulted in a significantly decreased tubular reabsorption at every plasma concentration (I: 263 ± 12; 325 ± 11; 334 ± 21 349 ± 31; 366 ± 25 mg/min. D: 229 ± 12; 283 ± 13; 298 ± 19; 326 ± 27; 319 ± 23 mg/min (100 ml of GF; $P < 0.001$).

It is concluded that renal sympathectomy not only inhibits proximal tubular reabsorption of sodium but also alters other active transport mechanisms.

Metabolism of rat liver cells in malnutrition

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Blood sugar level, serum total protein level as well as pyruvate content and pyrophosphatase activity in liver homogenates of rats kept for two weeks on daily 8 g and then for three further weeks on daily 4 g of a semisynthetic diet. The results were compared with those measured in rats allowed the same diet *ad libitum*. Oxygen consumption of liver mitochondria in the presence of 3-hydroxybutyrate or succinate was also measured.

Most of the animals were hypoglycaemic. Oxygen consumption of liver mitochondria and the pyruvate content of liver homogenates decreased, while the pyrophosphatase activity of liver mitochondria increased. The role of these changes in the development of hypoglycaemia is discussed.

A new drug for enzyme induction: m-trifluoromethyl-α-ethylbenzhydrol (RGH 3332)

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RGH 3332 is a new compound that causes induction of drug-metabolizing liver microsomal enzymes. The biological activity and/or metabolism of the following drugs was studied in rats and mice pretreated once or 4-times with 40 mg/kg of RGH 3332 by the oral route: hexobarbital, chlorzoxazone, methaqualone, meprobamate, carisoprodol, mephenesine, propanidide, hydroxydione, nikethamide, strychnine, picrotoxin, hydroquinone, acenocumarol, ethylmorphine, aminopyrine, diphenylhydantoin, pancuronium bromide, oestradiol, testoterone, spironolactone, metyrapone, triamcinolone, bromsulphalein and bilirubin.

The action on drug metabolism was biphasic and dose-related.

RGH 3332 increased the cytochrome P_{450} content of liver microsomes and the urinary excretion of ascorbic acid.

The effect of RGH 3332 was inhibited by d,l-ethionine.

RGH 3332 in an oral dose of 900 mg/kg, did not exert toxic and central nervous effects.

Effect of physical exercise on cytochrome-P_{450} content of the rat liver

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CHEMICAL WORKS OF GEDEON RICHTER LTD., AND HUNGARIAN SCHOOL OF PHYSICAL EDUCATION, BUDAPEST

In previous experiments an accelerated elimination of hexobarbital and canrenone was observed in swimming-trained rats. In the present study the cytochrome-P_{450} content of the liver was determined after one single swimming and during regular swimming exercise.

Cytochrome-P_{450} content was not affected by a single swimming exercise. There was a slight increase after 2 to 4 weeks physical training, and a marked one after the 5th week.

The results are in favour of the suggested relationship between the previously observed changes and the higher activity level of microsomal enzyme systems of the liver.

Effect of food deprivation on the compensatory hypertrophy of endocrine organs and kidneys

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Food deprivation for 94 and 144 hours after unilateral ovarian, testicular and renal extirpation in CFY rats reduced the compensatory hypertrophy of the residual organs, Compensatory hypertrophy was also decreased in fasting female animals. In contrast, an increase of compensatory adrenal hypertrophy was observed in male rats. In starved animals the weight loss was more conspicuous than the disturbance of compensatory hypertrophy inasmuch as the relative weight of the organs was identical to or even exceeded that of the control organs. Following food deprivation, growth hormone concentration of the anterior pituitary was increased in males and unaltered in female animals. Thus, caloric supply decisively modifies, while growth hormone fails to affect, compensatory hypertrophy after unilateral extirpation of paired organs.

Beta-blocking and antiarrhythmic activity of a new alcanolamine derivative (Chinoin-103)

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A series of alcanolamine derivatives with beta-blocking and antiarrhytmic properties was synthetized by Harsányi and Korbonits. One of these compounds, Chinoin-103, was found to possess favourable properties. A study was made to check the beta-blocking and antiarrhytmic activities of the drug.

Chinoin-103 was three times as potent as practolol in inhibiting the inotropic effect of isoprenaline on electrically driven guinea-pig left atrial preparations. On isolated tracheal ring preparation of the guinea pig, the $beta_2$-inhibitory effect of the drug was weaker than that of practolol.

Chinoin-103 was more potent in inhibiting the appearance of arrhythmia induced by aconitine in the rat than were the widely used beta antagonists propranolol, oxprenolol, and practolol. The effectiveness of the drug in the prevention of arrhythmia induced by digoxin, and in the aboliton of arrhythmia induced by strophanthin in cats was nearly the same as that of practolol. In view of its favourable cardioselective properties Chinoin-103 is considered a valuable drug in the treatment of cardiac arrhythmias.

Pathomechanism of pyrogen-induced fever in the new-born rabbit

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In a few hours old rabbits, fever develops in response to the bacterial pyrogen Coli 0 111 1838 S_4 at thermoneutral environmental temperature. An increase in heat production accounts for the increase in body temperature. Brown fat thermogenesis is enhanced as judged from the temperature difference between interscapular brown fat and colon. Changes in heat loss by vasoconstriction or depression of respiratory rate do not contribute to the rise in body temperature until the age of about 7 to 10 days. The course of fever does not show qualitative differences from that in the adult rabbit.

The adrenergic receptors of the myocardium and the coronaries

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CHINOIN PHARMACEUTICAL WORKS BUDAPEST, AND CASSELLA FARBWERKE, MEDIZINISCHE FORSCHUNG, FRANKFURT/MAIN

As shown previously in the dog's hind limb circulation carbocromene (CA) transforms alpha receptors into beta ones, presumably by releasing a modulator substance.

On the dog heart in situ CA (0.1—1.0 mg/kg I.V.) increased the effect of isoprenaline (ISO) and noradrenaline (NA) on dp/dt max, heart rate and CBF. This latter was reflected in the end-diastolic resistance (CEDR). Thus the same increase was found in beta potency as previously in hind limb circulation.

Seventeen dogs were pretreated with 5—10 mg/kg CA for 5—14 days. This treatment had previously been shown to cause complete depletion of the modulator substance. After this treatment NA and phenylephrine evoked a considerable increase in CEDR that could be blocked by alpha antagonists. At the same time neither the myocardial effect nor the coronary dilation caused by ISO underwent any change. Thus the beta modulator substance is only "stored" in the coronaries and can be depleted by CA pretreatment. The myocardium may be the site of a continuous synthesis of the modulator substance. Hence, CA pretreatment does not influence either the cardiac effects of ISO or the secondary metabolic dilation caused by ISO.

Relation between hypoxia and the myocardial effects of adenosine

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Adenosine plays a key role in the regulation of coronary blood flow. The substance is continuously released from the normal heart; under conditions of myocardial hypoxia the release is enhanced. In the present experiments a paradoxical effect of adenosine was observed after intravenous injection in anaesthetized cats; the substance evoked ischaemic electrocardiographic signs. On electrically-driven guinea-pig left atrial preparations incubated in the presence of oxygen, 0.1 mM adenosine caused a loss of myocardial K^+ and Mg^{++}. Similar results were obtained on atrial preparations incubated anaerobically without added adenosine. Glycolytic activity of the atrial muscle was studied by measuring lactate production. A distinct increase of lactate production was found in atrial muscle incubated anaerobically, and also under aerobic conditions in the presence of 0.1 mM adenosine.

The possible role of adenosine in the pathogenesis of ischaemic heart disease is discussed.

Oedema induced by triethylstannous sulphate

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According to data in the literature, selective cerebral oedema is induced by triethylstannous sulphate (TSS). The conditon was investigated by several authors, but no data were found as to the mechanism of action. In the present experiments the effect of TSS on metabolism was examined. Oxygen consumption as well as colon temperature were decreased. Electrical activity of the brain in the acute phase was slowed down significantly, a phenomenon seemingly temporal connection with the change in blood pressure as well as with the time curve of the hyperglycaemic reaction. On the basis of the results, TSS does not induce selective cerebral oedema.

Effect on carbon tetrachloride toxicity of substances influencing reticuloendothelial activity

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Di Luzio in 1962 reported that reticuloendothelial stimulation induced by zymosan decreased the toxicity of carbon tetrachloride. Several authors have then shown that the toxicity of certain hepatotoxic agents can be influenced by substances

acting on the reticuloendothelial system. On this basis the effect of different reticuloendothelial stimulants and depressants has been studied on the toxicity of carbon tetrachloride.

It was shown that in the rat the reticuloendothelial stimulant, zymosan, decreased, while another reticuloendothelial stimulant, triolein, did not influence the intensity of carbon tetrachloride liver injury. Both of the reticuloendothelial depressants investigated, methylpalmitate and gadolinium chloride, decreased the toxic effect of carbon tetrachloride.

Correlation of vital capacity with body dimensions in 6 to 16-year-old children applying for admission to a school of sports

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Maximum inspiratory, expiratory, and end-tidal chest girths, vital capacity, body weight and height were measured, and decimal age and maximum chest excursion were calculated in 800 children. Relationships were studied by total and partial correlation and by multiple regression. Though total correlations between vital capacity, body dimensions and age were high ($r = 0.77$—0.92), even the derived multiple regression equations failed to yield estimates of adequate accuracy for vital capacity; the standard errors of individual estimates were too large: ± 270—350 ml. The virtually close total correlations were brought about by general growth. Individual differences in developmental rate, however, may give rise to considerable (100—600 ml) differences between actual and predicted values. Accordingly, in children and adolescents estimates relying on body dimensions and age cannot substitute for a direct measurement of vital capacity.

Fatty acid composition during the growth of Ehrlich ascites tumour in mice

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Fatty acid composition was determined in different periods of hyperlipaemia accompanying the development of ascites tumour. The lipids isolated from ascites tumour cells, ascites fluid, omental adipose tissue, liver and serum were separated by thin-layer chromatography to polar (phospholipid) and neutral (triglyceride) groups. The fatty acid composition of each fraction was estimated by means of gas chromatography.

The changes in lipid flux between host tissues and cancer cells in the different periods of tumour growth are discussed.

Effect of hypokinesis on the properties of contractile muscle proteins

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The effect of hypokinesis on the properties of functionally different striated muscles was studied in white New-Zealand rabbits weighing 2.5—3.0 kg. The right hind legs were immobilized by plastering. The experiments were carried out on the 14th, 28th and 42nd days on 16, 16 and 12 animals, respectively. Samples were taken from the immobilized and contralateral gastrocnemius (G) and soleus (S) muscles under pentobarbital anaesthesia. G and S muscles of intact animals served as controls. The parameters studied were, water content, wet weigh, myofibrillar-sarcoplasmic proteins, nucleic acid content and superprecipitation of actomyosin.

A progressive decrease was found in the native weight of G and S, which was more marked in S than in G muscles (36.2 and 19.6%, respectively). The alteration of DNA content followed the decrease of muscle weight. The change in RNA content differed in the two types of muscle: a 10% decrease was found in the G and a 66% decrease in the S muscle.

Metabolism-dependence of membrane potential oscillations elicited by veratrine on skeletal muscle, I.

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The time-scale of oscillation processes connected with metabolic activity changes within wide ranges. The phenomenon described previously, the membrane potential oscillation elicited by veratrine, belongs to the glycolytic oscillation rhythm-domain in order of magnitude. In the present experiments it was shown that the phenomenon can only be observed on muscles under anaerobic conditions, i.e. on glycolyzing ones, as non-oxygenated muscles kept in Ringer solution for 40 hours responded to veratrine treatment with membrane oscillation just as did fresh muscles. The significance of glycolytic metabolism in the process of oscillation is further proven by the fact that phloridzin, but also sodium fluoride and monoiodoacetic acid, inhibit the membrane potential oscillation elicited by veratrine. Cyanide and azide, which influence oxidation, do not inhibit the phenomenon under similar experimental conditions, an event proving that the muscle is indeed under anaerobic conditions and calling attention to the significance of glycolytic metabolism in the mechanism of oscillation.

Aromatic acid excretion in rats treated with phenylpyruvic acid (PPA)

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It has been shown that in phenylketonuria (PKU) PPA was mainly responsible for the damage to the central nervous system. It was supposed that a condition similar to PKU could be induced by means of PPA treatment. In four groups of six Wistar rats each of the animals were treated with phenylalanine (PAL), p-chlorophenylalanine (CPAL) and PPA, respectively, the controls were given physiological saline. Urinary aromatic acid excretion was estimated by gas-liquid chromatography. The results obtained were, in terms of aromatic acid mg/creatinine g,

	Physiol. saline	PAL	PPA	CPAL
mandelic acid	—	—	20	20
2-OH-phenylacetic acid	—	20	—	15
phenylacetic acid	—	70	1560	500
4-OH-phenylacetic acid	100	80	100	50
PPA	—	180	1650	1540

After PPA treatment the urinary aromatic acid excretion was similar in the CPAL-treated animals.

Retransfusion acidosis after haemorrhagic hypotension of different duration in dogs

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The sudden fall of pH in arterial blood after retransfusion is well known in both experimental and clinical shock. As it is attributed to lactic acid outflow from the hypoxic tissues, it is called "washout-acidosis".

In chloralose-anaesthetized dogs haemorrhagic hypotension of different duration was induced. i) Blood pressure was reduced to 40 mm Hg and stabilized at this level allowing 30% of the shed blood to flow back spontaneously. ii) Bleeding was performed at a rate of 50 ml/min to a total of 25 ml/kg. The interval between bleeding and retransfusion was about 3 min. In both types of haemorrhage, the hypotension was followed by quick arterial retransfusion of an adequate volume of blood, extran or Locke solution.

(1) After retransfusion in both types of hypotension arterial pH decreased (-0.08, -0.07) and pCO_2 increased (13—14 mm Hg) in two min. Lactate level or pO_2 tension did not change significantly. Similar changes were found in the dextran and Locke-groups. (2) A close correlation was detected between the changes

in pH and pCO_2. (3) The phenomenon could be demonstrated after bilateral transection of the carotid sinus nerves.

Retransfusion acidosis after haemorrhagic hypotension of different duration seems to be correlated with the rise of CO_2 in arterial blood and occurs without changes in the lactate level; so it cannot be attributed to a washout of lactate from the hypoxic tissues.

Effect of hypokinesis on the submolecular properties of muscle proteins

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The cellular and protein fractions of control and immobilized muscles were analysed as follows. The myofibrillar and mitochondrial fractions were solubilized in a medium containing 1% SDS + 6 M urea + 1% mercaptoethanol. The lyophilized sarcoplasmic preparation was dissolved in the same solution at a final concentration of 5 mg/ml.

Samples were run in monotone and gradient polyacrylamide gels according to the method of Weber and Osborn. The electrophoretic mobility of the individual components was compared with that of the standards. Relative quantity of the identified components was analysed by densitometric scanning.

Immobilization was found to influence the submolecular composition of muscle proteins as early as 14 days after plastering. A significant change was found in the relative proportions of myosin and tropolon subunits. The alteration was more expressed in the soleus muscle.

Effect of picrotoxin on blood pressure responses to intense stimulation of cutaneous afferents with different segmental inputs in conscious cats

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The qualitative and quantitative changes and differences in the reflex blood pressure reactions elicited by stimulation of somatic afferents identical in function but different in spinal segmental input have been reported previously.

In the present experiments cutaneous afferents of cervical and lumbosacral segmental inputs were stimulated in conscious, gallamine-immobilized cats under artificial respiration. Intense stimulation (16 V, 4—8 imp/s, 0.5 ms) acitivating both

A and C fibres was applied and repeated until the responsiveness of the animal had decreased considerably.

Subconvulsant doses of picrotoxin administered at this stage led to a marked enhancement of the pressor responses evoked by sustained stimulation of fibres with cervical input. At the same time, reflexes due to nerves of lumbosacral origin were weakened. This difference could not be observed following spinal cord transection.

The findings point to differences in irradiation of activity in the CNS; they depend on the level of activation and the animal's condition.

Brain stem neuronal responsiveness during early postnatal life in the rat

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Multiple unit activity (MUA) of the mesencephalic reticular formation was recorded in unanaesthetized, curarized rats and in freely moving rats during the first 15 postnatal days. Neuronal pools in new-borns responded to acoustic, visual and somatosensory stimulation by a significant increase in firing rate. Somatosensory responsiveness showed a high rate in new-born rats, to changed slightly in the course of maturation. Depending on the age of the animals and on the type of sensory stimulation, MUA responses exhibited different functional properties. Opening of the external auditory meatus and eyes resulted in a sharp elevation of the MUA basal level and a marked alteration of responsiveness. It is suggested that neonatal CNS is capable of information processing. The stable high level of somatosensory responsiveness is of great importance in the survival of new-born animals, since this is the neuronal mechanism possibly underlying elementary learning processes and memory formation.

Intestinal phase of human gastric secretion

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In previous studies, oral application of caffeine and bicarbonate produced significant changes in the serum gastrin level. These results were explained by a negative feed-back mechanism between gastric HCl and the gastrin system. The role of the intestinal phase in the regulation of gastric HCl secretion was emphasized.

The question arose whether the gastrin system had any role in the regulation

of gastric HCl secretion produced in the intestinal phase. The following solutions were applied intraduodenally to elicit alterations in gastric HCl secretion: hypertonic glucose, distilled water, isotonic saline solution, 0.01 N hydrochloric acid and sodium bicarbonate. The gastric secretory responses were measured, and the serum gastrin level was determined by radioimmune assay.

No characteristic correlation was found between gastric HCl secretion and serum gastrin level under the experimental conditions employed.

Effect of drugs on the adrenal catecholamine content

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It has been shown that intestinal paralysis was a consequence of sympathetic hyperactivity and thus an increased catecholamine production. Intestinal motility could be restored by sympatholytic drugs; patients with intestinal paralysis were treated effectively with the alphalytic drug trifluperidol and the betalytic practolol. The mechanism of action of these drugs is not fully understood. In the present experiments the effect of sympatholytic agents on the catecholamine content of the adrenals was studied in cats under normal conditions and in stress elicited by manipulation of the intestinal tract.

Trifluperidol was found to decrease the adrenaline and noradrenaline contents of the adrenals. Stress decreased the adrenaline content but did not influence the noradrenaline level. Stress applied after trifluperidol pretreatment did not further decrease the adrenaline level while it caused an increase in the noradrenaline level. Practolol only decreased the noradrenaline content. The results suggest that sympatholytic agents may influence the intestinal paralysis ileus not only by their receptor blocking action but also through effects on catecholamine production or release.

Renal function in hypotension

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Renal function was studied in anaesthetized mongrel dogs after exposing the left renal hilum through a left lumbar incision, and anastomosing the renal vein to the left external jugular vein via a siliconized rubber tubing.

Values for 18 dogs obtained in 3 clearance periods served as control. Arterial blood pressure was 124 ± 14 mm Hg, renal blood flow (RBF_{dir}) 436 ± 98ml/min/100 g, and vascular resistance ($R_{kidney/kg}$) 1.78 ± 0.43. The extractions were; E_{PAH} 0.78 ± 0.09,

E_{creat} 0.30 ± 0.09 and E_{inulin} 0.33 ± 0.09; the direct clearances ($E \times RPF$): C_{PAH} 187 ± 40 ml/min, C_{creat} 75 ± 24 ml/min and C_{inulin} 81 ± 25 ml/min. The difference between C_{inulin} and C_{creat} was not significant ($p > 0.10$). Urinary output was 0.76 ± 0.35 ml/min.

In 8 dogs the same surgical manipulation on the hilum evoked an arterial hypotension with a mean blood pressure of 74 ± 7 mm Hg. The renal parameters were: RBF_{dir} 432 ± 142 ml/min, R/kidney 1.16 ± 0.40, E_{PAH} 0.69 ± 0.08 and E_{creat} 0.16 ± 0.05. The clearances were, C_{PAH} 170 ± 50 ml/min, C_{inulin} 54 ± 28 ml/min, C_{creat} 39 ± 18 ml/min.

The difference between C_{inulin} and C_{creat} was highly significant ($p < 0.01$), urinary output was 0.22 ± 0.26 ml/min.

The cause of the observed hypotension is not clear; the results point to a considerable rediffusion of creatinine into the nephron during the hypotensive period.

Effect of para-substituted amphetamines on the synaptosomal uptake of ^{3}H-dopamine

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P-bromomethamphetamine (V-111) is a selective and long-acting inhibitor of the neuronal reuptake of ^{3}H-serotonin (^{3}H-t-HT) in rat brain synaptosomal preparations (*Knoll* and *Magyar*, 3rd I.S.N. Meeting, Budapest 1971, Abstracts p. 271; *Knoll* et al. in: Symposium on Pharmacological Agents and Biogenic Amines in the C.N.S. Ed.: *Knoll, J.* and *Magyar, K.*, Budapest 1973. pp. 13).

In the present study the effect of V-111 and other substituted amphetamine derivatives (methylamphetamine, p-bromoamphetamine, o-bromomethyl-amphetamine) was investigated on the synaptosomal reuptake of ^{3}H-dopamine (^{3}H-DA). V-111 strongly inhibited the uptake process; in this respect, the (+)-isomer was more effective. Among the compounds investigated, amphetamine and p-bromo-amphetamine proved to be the most potent inhibitors of ^{3}H-DA uptake.

According to the results, N-methyl substitution of amphetamine is not favourable as regards dopamine uptake.

Similarly to the uptake of ^{3}H-5-HT, ^{3}H-DA uptake too was inhibited by V-111 for a protected period recovery took three weeks. In contrast, amphetamine, the most potent inhibitor of dopamine uptake, became ineffective in 12 hours.

The prolonged effect of V-111 on ^{3}H-DA uptake should be considered an important factor in the complex pharmacological action of the drug.

Absorption of C-terminal pentapeptide of gastrin from the jejunal and rectal mucosa

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Seventy per cent of the intravenously injected C-terminal pentapeptide (1-^{14}C-pentapeptide amide) is excreted by the liver of the rat. The pentapeptide amide and metabolites excreted in the bile were collected and injected into the jejunum and rectum of recipient rats. Radioactivity of blood samples from the aorta and portal vein was measured and the values were plotted against time.

In other experiments pentapeptide was directly administered into the jejunum and rectum, and serum radioactivity was determined in the aorta and the portal vein.

The results were compared with the biological effectiveness of pentapeptide administered in the two different parts of the digestive tract.

Effect of substantia nigra lesion on the rat's escape avoidance behaviour

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Much evidence has recently been accumulated suggesting a major role of dopamine in the performance of conditional avoidance response (CAR). Animals treated with 6-hydroxydopamine failed to display acquisition of a shuttle-box or a one-way avoidance response (*Cooper, B. R.;* J. Pharmacol. *185:* 358, 1973).

It is assumed that the catecholaminergic and serotoninergic systems antagonistically modulate the learning and retention performance of rats. Newly-synthetized amphetamines with proper substitution at para-position improve learning and retention, either by increasing the catecholaminergic or by decreasing the serotoninergic tone of the brain (*Knoll, J.:* Proc. 5th Int. Cong. Pharmacology, Vol. 4, pp. 55—68, Karger, Basel, 1973).

Two simple one-way avoidance techniques have been developed for studying the effect of different amphetamine derivatives on learning and retention in the rat (*Knoll, B.:* Symposium on the Pharmacology of Learning and Retention, Akadémiai Kiadó, Budapest 1974).

Experiments were carried ont by using the authors' one-way technique and a two-way avoidance apparatus (shuttle-box) in order to investigate the effect of the substantia nigra (SN), lesion. Three weeks after unilateral SN lesion the learning ability of the rats was poorer than that of the sham-operated animals in both forms of acquisition.

Biochemical determination of the striatal dopamine level and histological verification were made parallel with the behavioural testings.

Mechanism of frequency generators in cases of intact and impaired electrogenesis

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Cerebral electrical activity was studied by correlation analysis and power density spectrum in control cases and in cases of petit mal and temporal lobe epilepsy in different vigilance states.

The basic spectrum of the EEG was complex even in wakefulness. A certain energy content of the delta generator was present over all areas. In SWS-2, apart from the well-pronounced energy of delta activity, a band of 15—18 cps with high energy content appeared over all brain regions. Subcortical (thalamic) structures of wide-spread cortical representation were assumed to be responsible for the activity of both generators. The energy content of the whole spectrum was highest in REM sleep, having a wide frequency range and equal distribution, with a slight maximum in the delta band.

In cases of petit mal epilepsy, a well-pronounced difference was found between the power density spectra of the non-epileptogenic "Stable" REM-phase and the epileptogenic "unstable" REM-phase. The power density spectrum of the latter was similar as that of SWS-2: high energy content in the delta band and almost the same energy content in the 15—18 cps band. In temporal lobe epilepsy, a difference was found in SWS-2 around the spike focus, first of all in the values of the 15—18 cps band, while the energy content in REM differed from that of the intact areas.

Effect of incompatible blood transfusion on regional cerebral blood flow

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The effect of incompatible blood was studied on regional cerebral blood flow and blood coagulation in anaesthetized dogs.

The haemolytic reaction was produced by intravenous infusion of human blood. Regional cerebral blood flow was measured in deep nuclei of the brain (ventral posterolateral nucleus of the thalamus, ventromedial nucleus of the hypothalamus) and in the white matter, by the H_2-gas clearance technique. Eight different parameters of the blood coagulation system were determined and blood gas analysis was made in arterial blood samples.

Cerebral blood flow was significantly reduced following transfusion in each area studied. Local blood flow decreased more in the grey than in the white matter. The diminished blood flow values did not return to the control level in two hours following transfusion, in spite of the normalization of MABP. The activity of the coagulation factors and the fibrinogen content of the blood decreased while fibrinolysis markedly increased after transfusion.

Renal function after increasing the functioning renal mass

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An isolated kidney was transplanted into the neck of anaesthetized dogs whose left kidney was acutely denervated, and the effect of the increase in functioning renal mass was studied on the function of the in situ kidneys in two series of experiments. In the first group the animals were kept on a sodium-rich diet (0,5 g/kg day NaCl); those in the other group were fed normal food. Data for animals with a low sodium excretion during the control period were only evaluated.

In animals kept on sodium-rich diet, the increase of the functioning renal mass was not followed by any significant increase in glomerular filtration rate, while urine and sodium excretion decreased slightly. In the other group, transplantation of the isolated kidney was followed by a marked decrease of GFR, sodium and urine excretion, while mean arterial blood pressure remained unchanged.

The results suggest that the isolated kidney releases some humoral material responsible for the decreased function of the in situ kidney. Production of the substance is assumed to depend on the sodium balance of the animal.

HbA- and HbS-subunit valine-codon multiplicity in the vicinal double valine of tryptic oligopeptides

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Among the tryptic oligopeptides of HbA and HbS, only T4 and T14, contain double valine in vicinal position. In the other tryptic oligopeptides the valine residues are in other arrangements.

It was investigated whether the vicinal valines were incorporated by the same, or by a different, way into the neighbouring codons of the mRNA.

In both amino acid loci of the T4 oligopeptide, valine was incorporated by baker's yeast (BaY2) and E. coli MRE 600-t-RNA-Val(^{14}C), i.e. the direction of

valines in these positions was due to GUA codons (Amino acid loci Nos 33 and 34).

In positions 133 and 134 of the T14 oligopeptide BaYl and brewer's yeast (BrY1/-t-RNA-Val/^{14}C) directed valines. BaY1-t-RNA-Val(^{14}C) incorporated Val(^{14}C) in 1.3-times higher amounts than did BrY1-t-RNA-Val(^{14}C). Both of these t-RNAs incorporated Val(^{14}C) in the presence of codons GUU and GUC; therefore, the question cannot be answered whether one of the codons acted specifically as a difference in affinity to the reticulocyte membrane-bound enzymes may have also been responsible for the phenomenon.

It is concluded that valine codon structures in HbA- and HbS-mRNAs are as follows: in loci Nos 33 and 34=GUA, while in loci No. 133 and 134=GUU or GUC. A. simultaneous occurrence of the two codons in both possible sequences (-GUUGUC- or -GUCGUU-) cannot be excluded either.

In vitro sulpho-conjugation of 4-^{14}C-dehydroepiandrosterone and 4-^{14}C-5-androstene-3β, 17β-diol in healthy and pathological human skin slices

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It was shown that human skin exhibits Δ^5-3β-hydroxysteroid sulphokinase (Δ^5 = 3β-HSS) activity, an enzyme catalyzing the sulpho-conjugation of 4-^{14}C-dehydroandrosterone and 4-^{14}C-5-androstene-3β, 17β-diol in vitro. In the present work the quantitative relations of the sulpho-conjugation were studied.

Abdominal skin slices of healthy males and females synthetized dehydroepiandrosterone sulphate (DHA-S) from 4-^{14}C-dehydroepiandrosterone. Abdominal skin slices of two patients with idiopathic hirsutism and one patient with hirsutism of ovarian origin displayed normal enzyme activities. Addition of ACTH to the incubates enhanced the formation of DHA-S in abdominal skin slices from healthy females and also in those from a female with hirsutism.

Healthy male and female abdominal skin slices transformed 4-^{14}C-5-androstene-3β, 17β-diol to 5-androstene-3β, 17β-diol-3-sulphate. Abdominal skin slices from a complete testicular feminization patient synthetized DHA-S and 5-androstene-3β, 17-β-diol-3-sulphate in normal amounts. For some unknown reason formation of these sulphate ester steroids was decreased in the incubate of abdominal skin slices from an agonadal patient.

Effect of external sodium ions on the K efflux from striated muscle cells

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K efflux was reported to be halved in Na-free, $MgCl_2$-substituted Ringer solution. It could not, however, be established whether this effect was due to the absence of sodium, to the presence of magnesium, or to both factors.

In the present study, K efflux from the frog sartorius muscle was measured at different external Na concentrations, replacing external NaCl by choline chloride. The rats constant for potassium loss and K efflux in K-free Ringer were directly proportional to the external Na concentration. This effect on K efflux indicates a stimulatory action of external Na ions. At an external K concentration of 0.25 mM, K efflux was inversely proportional to the external Na concentration. If the Ringer solution contained 0.75 mM potassium, the K efflux was independent of the external sodium ions.

The results suggest that a large part of K efflux from muscle cells is mediated by a K : K exchange mechanism. In the absence of external K ions sodium ions promote the leakage of K ions out of muscle cells, by occupying the carrier sites for K : K exchange and induce a Na : K exchange. At a low (0.25 mM) K concentration, high concentrations of Na ions will inhibit the K exhange by competition for the carrier sites. The competitive effect of external Na ions is lacking in Ringer solution with $[K]_o$ of 0.75 mM or more, because of their low affinity to the carrier sites of the K : K exchange process.

Investigation on the law of initial value at neuronal level

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In his law of initial value, Wilder formulated a biological rule of universal validity, according to which living systems react to the same effect in a different way if they have been affected previously. The role has been investigated in relation of dose-effect in pharmacology and in that of stimulus sensation in psychophysics.

The question arose of whether the law was valid at the level of an organism only, or also in unit activity. The present experiments evaluated in the case of neurones how the law of initial value would prove to be valid. The measurements were carried out on the so-called silent cells of the central nervous system of the garden snail Helix pomatia L. In case of hypo- or hyperpolarization of different degree the neurones were stimulated intracellularly be means of microelectrodes.

The neurones were supposed to form a regulatory system. The responses were elicited by the usual jump and speed functions. Evaluation of the obtained series of action potentials revealed that the neurones did not function as a linear system. The non-linearity derived from the regulation of the inner state of the system was, first of all, a function of the output frequency.

Electrophysiological study on sensory inputs activated by chemical stimulation of the oral receptor areas in Helix pomatia L.

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Differences in chemical sensitivity to various substances of the oral receptor area were observed during recording the electrical activity of the internal n. labiales in the isolated mouth and a preparation comprising mouth and brain. During application of KCl that causes generalized depolarization, the amplitude of the nerve activity was variable but the selectivity of the receptors could be demonstrated by using glucose or sucrose. Physiological solution and distilled water excited the receptors in a different manner. Significant changes were also caused by NaCl, glucose and sucrose, as well as Desomet, metifonate and malathion, substances used in plant protection.

In the completely isolated mouth, basic activity of the internal n. labiales was higher than that of the preparation comprising mouth and brain. This finding emphasizes the importance of the central nervous system in the regulation of sensory cell activity. Presence of neurones in nerves dissected from the ganglion could be demonstrated electrophysiologically; these neurones were active for a certain period of time also after isolation of the nerve.

Leao's spreading depression in the rat's cerebral cortex

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The initiation and propagation of cortical spreading depression (SD) is generally explained by a supposed liberation of potassium ions and glutamic acid from cortical cells. As tetrodotoxin fails to block SD the depolarization is ascribed to the decrease of the resting potassium potential. In the present experiments it was examined whether potassium ions and glutamic acid elicited SD by an identical mechanism.

Quabain, in a concentration of 10^{-3} g/ml, prevented KCl and glutamic acid in evoking SD while hydroxylamine, that interferes with the decarboxylation of glutamic acid, proved to be ineffective in this respect. It is concluded that SD is brought about in basically the same way in the case of both KCl and glutamic acid.

Possibility of restrictive blood flow in the hepatic arterial system: acute experiments

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Cats of both sexes weighing 3—5 kg were anaesthetized intraperitoneally with 40 mg/kg pentobarbital sodium. After median laparotomy splenectomy and porto-jugular by-pass were performed. The left coeliac ganglion was prepared for electrical stimulation. Blood flow in the hilar and peripheral areas of the left liver lobe was simultaneously and continuously measured by flexible thermocouples according to *Hensel* et al. (1954) and recorded by means of a two-channel Fluvograph. A Hellige multiscriptor with Statham transducer was used for recording pulse rate and pressure in the femoral artery. A cannula inserted into the left femoral vein was used for intravenous injections. Histamine (Peramin^{R}, Richter) was given in doses of 2, 4 and 8 μg/kg. Electrical stimulation of the left coeliac ganglion was performed for two min using a Multistim (DISA) apparatus, at 20 cps, 1 msec impulse duration and 2.5, 5 and 10 V.

Stimulation of the left coeliac ganglion and intravenous injection of histamine resulted in different flow changes in the central and peripheral regions. Local blood flow either increased, decreased or unaltered in the central region, and significantly diminished in the peripheral region. In cats with porta-jugular by-pass the phenomenon was similar as the restrictive flow redistribution found in the liver of dual blood supply.

Effect of PGE_2 and $PGF_{2\alpha}$ on local blood flow and progesterone ovarian secretion

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The experiments were performed on dogs anaesthetized with chloralose-urethane.

1. In anoestrous dogs treated with HCG for 10 days the ovaries were isolated, perfused in situ with blood from the femoral artery at a constant rate and controlled pressure. Progesterone was determined by the competitive protein binding assay in blood samples taken from the ovarian vein of HCG-treated animals.

2. In anoestrous dogs, without any injury of the ovarian veins and arteries, thermocouples were introduced into the stroma of both ovaries in order to measure the changes in blood flow. Prostaglandins were administered through a cannula built into the ovarian bursa.

In the in situ isolated ovary, intraarterial PGE_2 decreased, and $PGF_{2\alpha}$ did not influence the perfusion pressure. When infused into the bursa, both PGE_2 and $PGF_{2\alpha}$ increased blood flow in the stroma of the ovary. $PGF_{2\alpha}$ seems to be capable of changing the distribution of flow in the ovary without altering the mean vascular resistance of the ovarian artery.

Intraarterial infusion of PGE_2 or $PGF_{2\alpha}$ did not significantly affect the progesterone level in the venous blood of the ovaries.

Significance of the extracellular environment in the changes elicited by veratrine in the skeletal muscle's membrane potential

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The characteristic parameters of the oscillation and depolarization of the membrane potential elicited by veratrine were investigated on the frog sartorius muscle as a function of external sodium concentration. Latency time increased gradually at decreased Na-concentration while the depolarizing effect of veratrine and the frequency of oscillation decreased logarithmically. Oscillation could even be observed at 10 mM $[Na]_o$, but its mean frequency decreased to 1/5 when compared with that of the control. Finally, the amplitude of the oscillation showed an inverse ratio to, but a logarithmic correlation with, the $[Na]_o$ concentration: at a Na-concentration of 10 mM, it was more than twice as high than in the control.

The depolarizing effect of veratrine was invariably observable on isolated fibres or small bundles, but no oscillation of the membrane potential could be demon-

strated. This observation agrees with the finding that, if the experiment is carried out on whole muscle, a fibre displaying oscillation is rarely found in the uppermost layer of the sartorius while in the second layer every fibre shows marked oscillation. This observation calls attention to the significance of the interfibrillar space in the mechanism of oscillation.

Effect of furosemide on renal blood flow in the conscious dog

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The ability of furosomide to increase renal blood flow in both anaesthetized and conscious dogs seems to be well-established. In the present experiments the effect of furosemide was investigated in conscious dogs with a previously implanted electromagnetic flow-meter head.

Furosemide, in a dose of 0.1 mg/kg given intravenously, increased renal blood flow after an initial fall. Renal blood flow reached the maximum value, 125% of the control, 4—5 min after the injection and declined below the control level by the 20—25th min. After indomethacin pretreatment furosemide failed to increase renal blood flow but its diuretic and saluretic effects remained unchanged. Thus, the effect of furosemide on renal blood flow is partly prostaglandin-dependent.

Effect of immunization on RNA polymerase activity of macrophages

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Nuclei were isolated from peritoneal macrophages obtained from guinea pigs immunized with sheep red blood cells. DNA-dependent RNA polymerase activity was tested in the presence or absence of α-amanitine measuring polymerase A and B activity. Immunization increased the activity of both RNA polymerases above the control level. To study the nature of the newly synthetized RNA experiments with dual labelling were carried out. They revealed that more DNA than RNA was synthetized after antigen stimulation. The data are consistent with the assumption that specific antigen stimulation leads to increased RNA synthesis in macrophages.

Identification of the component of calcium transport in the rat duodenum in vitro

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Time-course and the role of passive and active components of intestinal calcium transport were investigated in the everted rat duodenum. During the initial first 5—10 min of incubation a considerable calcium exchange took place between the medium and the tissues, together with a calcium release from the intestinal wall. All these processes appeared to be insensitive to metabolic inhibitors, and fairly independent of the vitamin D state of supply. Later the rate of calcium transport became more pronounced in systems rich in vitamin D; in addition, it became dependent on tissue metabolism. Both findings indicate that, by this time, active processes are necessary for the vitamin D-sensitive component of the calcium transport system.

The results presented suggest that greater caution is needed whenever the mechanism of initial uptake or transfer of radioactive calcium is studied by this technique, as passive components may be superimposed upon active transport processes.

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РЕЗЮМЕ

ГОМОСИНАПТИЧЕСКИЕ И ГЕТЕРОСИНАПТИЧЕСКИЕ ВЗАИМОДЕЙСТВИЯ В ЯДРЕ ОТДЕЛЬНОГО ТРАКТА

ДЬ. ЗУХ и Э. ТЕЖЛА

В опытах на кошках под смешанным хлоралозно-нембуталовым наркозом изучалось взаимодействие потенциалов в ядре отдельного тракта на уровне обекса при раздражении низкопороговых афферентных волокон блуждающегося нерва. Латентное время пика отрицательной волны при ипсилатеральном раздражении равнялось $6{,}38 \pm 0{,}56$ мсек, при контралатеральном – $6{,}92 \pm 0{,}49$ мсек. Вызванный потенциал гомолатеральной стороны примерно в отношении 4:3 больше, чем контралатеральной. Вызванный потенциал чувствителен к асфиксии, его амплитуда зависит от частоты и уменьшается на половину первоначальной величины при 30 имп/сек. Вызванный потенциал не показывает посттетанического потенцирования. Гомосинаптическая серия раздражений сопровождается послевозбудительной депрессией, что проявляется не только в уменьшении амплитуды, но и в удлинении латентного времени.

Реакции, полученные в ответ на билатеральное раздражение ($B_{\mu V}$), были больше, чем в ответ на отдельные раздражения, но отставали от суммы последних ($S_{\mu V}$). Имеется отрицательно направленная корреляция между значениями $S_{\mu V}$ и $\Delta S_{\mu V}$($\Delta S_{\mu V} = B_{\mu V} - S_{\mu V}$). Зависимость можно выразить уравнением регрессии. $\Delta S_{\mu V} = -0{,}369 \cdot S_{\mu V} + 8{,}635$. Большая часть взаимодействий окклюзионного происхождения, аддиция наблюдалась лишь в нескольких случаях, фацилитацию вызвать не удалось.

Суммация без уменьшения амплитуды осуществляется лишь в интервале ± 1 мсек, исчезает при $\pm 2{,}5$ мсек. Дальнейшее увеличение интервалов между раздражениями вызывает торможение, которое достигает максимума при 5 мсек. Первоначальная амплитуда тестируемого ответа возвращается примерно через 30 мсек. Время возвращения определяется величиной реакции, вызванной кондиционированным раздражителем. Инстрасолитарное торможение авторы связывают с удлиненной позитивностью, сопровождающей отрицательный компонент вызванного потенциала.

ИЗМЕНЕНИЯ БИЛИАРНОЙ СЕКРЕЦИИ БЕНГАЛЬСКОЙ КРАСНОЙ У КРЫС В ОТВЕТ НА ВВЕДЕНИЕ ФЕНОБАРБИТАЛА

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Определялась плазменная концентрация внутривенно введенной краски (бенгальская красная), прием ее печенью и выделение с желчью после введения фенобарбитала (ежедневно 50 мг/кг внутрибрюшинно, в течение 4-х дней). Быстрое начальное падение (примерно 0–16 мин.) концентрации краски в плазме после однократного ее введения внутривенно соответствует приему краски печенью. У контрольных и получавших фенобарбитал крыс кривые концентрации бенгальской красной в плазме не отличаются друг от друга ни в случае введения малых (5 мг/кг), ни в случае введения больших доз (50 мг/кг). Концентрация краски в печени у животных, получивших фенобарбитал, была достоверно ниже, чем у контрольных животных. Общее количество принятой печенью краски, однако, было одинаковым в обеих группах, поскольку печень животных, получавших фенобарбитал, была больше, чем у контрольных. Экскреция с желчью бенгальской краски при введении малых

доз (5 мг/кг) была статистически достоверно больше у крыс, получивших фенобарбитал, чем у контрольных. Однако при введении 50 мг/кг и 100 мг/кг этой разницы уже не отмечалось. Это можно объяснить тем, что большие дозы краски тормозили эффект фенобарбитала, выражающийся в увеличении желчеотделения.

ИЗУЧЕНИЕ ПРОСТРАНСТВЕННОГО ВЗАИМОДЕЙСТВИЯ РЕФЛЕКСОВ КРОВЯНОГО ДАВЛЕНИЯ

ДЬ. ЗУХ, Ж. ХИДВЕГИ и К. БОДА

Мы изучали пространственное взаимодействие депрессорных и прессорных рефлексов, вызванных односторонним и двусторонним раздражением разных висцеральных и соматических афферентных нервов у кошек, находящихся под хлоралозным и нембуталовым наркозом. Как показывают результаты опытов, динамику пространственного взаимодействия можно изобразить уравнениями регрессии в зависимости от величины реакций, вызванных унилатеральным раздражением. На угол наклона прямых, полученных в различных состояниях при постоянных условиях, а также на его положение в системе координат оказывают влияние тип рефлекса, форма наркоза и функциональное состояние животного. При сагиттальном разделении по средней линии продолговатого мозга и моста характер взаимодействий существенно не меняется. На форму взаимодействия предыдущие раздражения не влияют.

ХИМИЧЕСКАЯ ПРИРОДА ФУНКЦИОНАЛЬНЫХ ХОЛИНОВЫХ РЕЦЕПТОРОВ НЕРВНЫХ КЛЕТОК LYMNEA STAGNALIS

В. Я. ИЛЬИН и П. Д. БРЕГЕСТОВСКИЙ

1. С помощью техники *voltage clampe* с микроэлектродами авторы изучали влияние pH и некоторых алкализующих агентов на свойства холинорецепторных мембран нервных клеток *Limnea stagnalis*.

2. Уменьшение значения pH инкубирующей среды ниже 7,5 снижало ответную реакцию нервной клетки на ацетилхолин. При значениях pH $= 6{,}7 \pm 0{,}1$ проводимость холинорецепторной мембраны уменьшалась наполовину. Увеличение pH до 10,6 не изменяло величину реакции на ацетилхолин.

3. Влияние pH осуществляется не путем действия на ионные каналы, а, повидимому, является результатом протонации групп, функционирующих на активных холинорецепторах.

4. Наличие реактивных групп SH у холинорецепторов доказать не удалось, но были обнаружены важные с функциональной точки зрения карбоксильные группы.

5. Влияние pH, повидимому, связано с протанацией имидазольных групп и групп $-COO^-$ при значениях pK_a около 6,7.

АНТИАРИТМИЧЕСКИЙ ЭФФЕКТ ДИЗОПИРАМИДА ПРИ ВВЕДЕНИИ В АРТЕРИЮ СИНУСНОГО УЗЛА

Д. ГАРСИА-БАРРЕТО и ПЕРЕ МЕДИНА

В опытах на наркотизированных собаках авторы изучали эффект антиаритмического препарата дизопрамида, вводимого в дозе 10—200 мкг/мл в артерию, снабжающую синусный узел. Низкая концентрация этого препарата вызывала атропиноподобный эффект, увеличивая, но не достоверно, синусную частоту сердцебиений, а при введении в артерию синусного узла в дозе 10 мкг/мл дизопирамид не был в состоянии купировать фибрилляцию предсердий, вызванную ацетилхолином. Лишь дозы выше 100 мкг/мл вызывали брадикардию и предохраняли от аритмии. Авторы приходят к выводу, что антиаритмическое действие дизопирамида при синусовой аритмии является следствием его прямого хронотропного эффекта, противоположного его хорошо известному атропиноподобному действию.

ЭФФЕКТ PGE_1 И $PGE_{2\alpha}$ НА НЕРВНО-МЫШЕЧНУЮ ПРОВОДИМОСТЬ В ПОРТНЯЖНОЙ МЫШЦЕ ЛЯГУШКИ

П. ИЛЛЕШ, Л. Г. МАГАЗАНИК и Й. КНОЛЛ

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