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РЕЗЮМЕ

СРАВНИТЕЛЬНЫЕ ИССЛЕДОВАНИЯ НАД D-ГЛИЦЕРАЛЬДЕГИД-3-ФОСФАТ ДЕГИДРАЗАМИ. VIII

Исследование эссенциальных ионов цинка энзимов

Т. КЕЛЕТИ и М. ТЕЛЕГДИ

Авторы исследовали возможность 1,10 фенантролином тормозить окисление D-глицеральдегид-3-фосфата (PGA) и D-глицеральдегида (GA), D-глицеральдегид-3-фосфат дегидразой (PGAD), кристаллически изолированной из мышц кроликов, рогатого скота и свиньи, из пивных и спиртовых дрожжей, как и из мышцы рака. Они установили, что эти энзимы — независимо от применяемого субстрата — можно тормозить приблизительно в одинаковой мере. Если в реакционной смеси вместо арсената применять фосфат, то торможение происходит медленнее, а если вместо восстановленного дифосфопиридино нуклеотида (DPN) применять аллоксан, то D-глицеральдегид-3-фосфат дегидраза не способна окислять D-глицеральдегид-3-фосфат; при таких условиях окисление D-глицеральдегида также кажется сомнительным.

ЭЛЕКТРОФИЗИОЛОГИЧЕСКИЙ АНАЛИЗ ПОВТОРНЫХ ОТВЕТОВ НА СКРЫТОМ НЕРВЕ (n. saphenus) КРЫС

Я. ПОРСАС

Применение вератрина на поврежденной поверхности кожи облегчает действие происходящих под областью повреждения соприкосновений. Наступлению вызванных вератрином облегчений можно препятствовать динитрофенолом (1 : 1000).

Вызванный применением на поврежденной поверхности кожи ионов калия, натрия и лития повторный ответ состоится вследствие непосредственного действия не на рецепторы, а на аксоны.

Этилендиаминтетраацетат, связывающий кальций в комплексной форме, — в противоположность цитрату натрия — не вызывает повторных разрядов и, следовательно, вызванная цитратом натрия авторитмическая деятельность, по всей вероятности, является непосредственным действием аниона цитрата.

Вызываемым ионами повторным ответам можно воспрепятствовать динитрофенолом.

Автор обсуждает данные результаты с точки зрения новых литературных данных о процессах нервного возбуждения.

ДЕЙСТВИЕ ПОВРЕЖДЕНИЯ ГИПОТАЛАМУСА НА ФОСФАТИДНЫЙ ОБМЕН ВЕЩЕСТВ ПЕЧЕНИ

Ш. КИШ, А. Г. Б. КОВАЧ, М. ИРАНЬИ, Я. АНТАЛ, М. ДОДА и Э. МОНОШ

Количество встроенного в фосфатидах арсенохолина на действие голодания в течение 24 или 48 часов повышается ($P = 0{,}01$).

У крыс с удаленными надпочечниками на действие голодания не состоится сильного повышения фосфатидного обмена веществ.

Три недели после электролитического разрушения передне-медиальных областей гипоталамуса на действие голодания не состоится повышенного встраивания арсенохолина, наблюдаемого при нормальных условиях.

Авторы не наблюдали подобного действия в случае повреждения других областей гипоталамуса или при внегипоталамических повреждениях.

ИССЛЕДОВАНИЯ В ЦЕЛЯХ ДИФФЕРЕНЦИРОВКИ ГОЛОДАНИЙ ОТ НЕДОСТАТКА БЕЛКОВ

Й. ШОШ, А. ДЁКЛЕН и Т. КЕМЕНЬ

Качество белков, поедаемых белыми крысами во время изокалорических голоданий, имеет для них большое значение.

Потребление клейковины пшеницы (недостаток лизина) меньше всего усиливает последствия голодания. Кормление клеем (недостаток этионина), дрожжевыми белками (недостаток S — аминокислоты) и желатин + казеин-гидролизатом (недостаток триптофана) в большой мере ухудшает состояние животных.

В случае повышения количества клейковины, вредное действие кормления последней, при одинаковых условиях уменьшается, в то время как при повышении количества остальных трех недостаточных для питания белков процесс ухудшается.

При возмещении недостатков четырех недостаточных белков только вредного действия дрожжевых белков не удалось прекратить.

ГУМОРАЛЬНАЯ ПЕРЕДАЧА РЕНАЛЬНОЙ ГИПЕРТОНИИ

Т. ТОТ

На одной из соединенных в парабиозе крыс, автор вызвал почечную гипертонию по методу *Лёринц—Горац*, а на другой он проводил одностороннее удаление почки. Кровяное давление животных повышалось сопряженно. На крысах с ишемизированной почкой удалось вызвать повышение кровяного давления в среднем на 75 Hgmm, а у парабиотической пары на 62 Hgmm. Это доказывает, что почечные гипертонии можно передать на животные с нормальным кровяным давлением гуморальным путем. Согласно литературным данным, а также и по результатам собственных опытов автора, на парабиотические крысы нельзя передать гипертоний при удалении почек, и они развиваются гораздо медленнее. Из этого обстоятельства автор заключает о наличии различных механизмов двух видов гипертоний.

ИССЛЕДОВАНИЯ ПРОМЕТАЗИНОМ. II.

Антигистаминовое действие оптических видоизменений фенергана

Я. БОРШИ, Г. ЛАЗАРНЕ, Ж. ЧИЗМАДИЯ и Л. ТОЛЬДИ

Авторы исследовали токсичность, антигистаминовую активность и наркозоповышающее действие оптических изомеров фенергана.

Они установили, что :

между токсичностью, антигистаминовой активностью и наркозоповышающем действии рацематов, (+) и (—) фенерганов на подвздошной кишке морских свинок, кровяном давлении кошек и астме морских свинок не наблюдается разницы.

Следовательно, между антигистаминовой активностью исследованных другими авторами структурных изомеров (изо-фенерган) и той исследованных авторами оптических видоизменений существует разница.

ФАРМАКОЛОГИЧЕСКИЕ ДЕЙСТВИЯ ЭЛАСТАЗЫ ПОДЖЕЛУДОЧНОЙ ЖЕЛЕЗЫ

Й. БОРШИ, Ж. ЧАК, Г. ЛАЗАР и Д. БАГДИ

Авторы исследовали фармакологические действия эластазы поджелудочной железы и установили :

Токсичность энзимовых препаратов различной активности при внутривенном введении крысам и мышам является функцией их эластолитической эффективности — чем больше активность препарата, тем и больше его токсичность.

Характерный для энзима симптом острой токсичности — возникновение легочного кровотечения и отека.

Следовательно, при внутривенном введении энзима, токсические и субтоксические действия проявляются, главным образом, на богатых эластическими волокнами легких и селезенке. Энзим предположительно играет роль при содержании коллагенного состояния и повышает проницаемость капилляров.

При хлоралоз-уретановом наркозе дозы в 120—480 Е/кг вызывают у кошек понижение кровяного давления. Степень и длительность понижения кровяного давления зависят от величины дозы.

Эти исследования указывают на то, что понижающее кровяное давление, действие эластазы является, главным образом, следствием расширения периферических сосудов и капилляров. Однако, нельзя исключить также наличие центрального действия, оказанного на вазомоторный центр.

На сердце лягушек 24—48 Е вызывают повышение амплитуды, большие дозы имеют следствием остановку сердца в состоянии диастолы.

Проведенные на изолированных органах исследования указывают на то, что поведение эластазы во многих отношениях подобно действию трипсина.

СВЯЗЬ МЕЖДУ СТРУКТУРОЙ И ДЕЙСТВИЕМ МОРФИНА И ЕГО ПРОИЗВОДНЫХ

Я. РАУШ, Й. СЕГИ, И. СЛАМКА, Ю. НАДЬ

Авторами была исследована связь между структурой и действием следующих изготовленных в дебреценском Институте органической химии производных морфина: морфин, дихидроморфин, 6-ацетилморфин-метобромин, норморфин, кодеин, дигидрокодеин, 6-ацетилкодеин, 6-ацетилкодеин-метобромид, норкодеин, героин, И-аллин, норморфин и диацетин-И-аллин-норморфин. Авторы исследовали болеутоляющее действие, эффект на дыхательный объем, привыкание, антагонизируемость болеутоляющего действия И-аллил-норморфином и диацетил-И-аллил-норформином, также как и токсичность вышеназванных средств. Они установили, что

1. кватернеризация третичного И повышает болеутоляющее действие, хотя согласно Эдди необходимым элементом болеутоляющего действия является присутствие рядом с четвертичным атомом С, также и третичного атома И, связанных между собой цепью $CH_2—CH_2$.

2. Степень болеутоляющего действия не меняется параллельно с токсичностью, с действием на уменьшение дыхательного объема и привыканием.

3. Исследованные действия нор-соединений весьма слабые.

4. И-аллил-норморфин антагонизирует в большей мере чем диацетин-И-аллил-норморфин болеутоляющее действие производных морфина со свободной группой ОН, чем производных, у которых это место было замещено.

5. И-аллил-норморфин повышает дыхание, в то время как диацетил-И-аллил-норморфин его снижает.

6. Болеутоляющее действие последнего средства на 1,63 раза сильнее действия И-аллил-норморфина.

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ON THE FUNCTION OF THE DENERVATED KIDNEY

By

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1. The sodium and water output by the kidney denervated by splanchnicotomy is higher in the anaesthesized animal than that by the innervated, contralateral kidney.

2. In the majority of cases hypersaluria and polyuria are associated with higher inulin and creatinine clearances, respectively.

3. There is no correlation between the differences in natruresis and inulin clearance. In some periods the higher sodium output is accompanied by lower inulin clearance. The higher diuresis of the denervated kidney predisposes technically for higher clearances. On this basis a reduced tubular sodium reabsorption is believed to be one of the factors involved in denervation hypersaluria and polyuria.

4. It is characteristic for the denervated kidney that a greater percentage of the total osmols is sodium than in the case of the innervated organ.

5. In the unanaesthesized animal we found no appreciable difference in function between the denervated kidney and the innervated one.

Since CLAUDE BERNARD it has been confirmed by many authors that in the anaesthesized animal the diuresis of the denervated kidney exceeds that of the contralateral, innervated kidney. (For the literature the reader should consult the monographs by SMITH [1,2]). On the other hand, some authors (RHOADS, VAN SLYKE, HILLER and ALVING [3], HIATT [4], BERNE [5], SURTSHIN, MUELLER and WHITE [6], SURTSHIN and HOELTZENBEIN [7], VASILIJEVA [8]) have found no difference in function between the innervated kidney and the denervated one in the unanaesthesized animal. BIKOV and ALEXEJEV-BERKMANN [9], as well as KLISIECKI, PICKFORD, ROTSCHILD and VERNEY [10] have stated that in the unanaesthesized animal the innervated and the denervated kidneys responded in the same way to water loading. In contrast with this, other authors (HARA [11], MEILMAN and WINER [12], SARTORIUS and BURLINGTON [13], BLAKE [14], KAPLAN, WEST and FOMON [15], FISCHER, SZÉCSÉNY and VIRÁNYI [16]) found that the denervated kidney excreted an increased amount of water and sodium even in the unanaesthesized animal. In a few, meticulously conducted experiments on unanaesthesized dogs SELLWOOD and VERNEY [17] showed that in response to peroral loading with water and physiologic saline the blood flow increased more markedly in the denervated kidney than in the innervated one. There was no difference in the increase of filtration following water loading, but after salt loading the filtration of the denervated kidney increased more markedly.

There is no agreement of opinion as regards the mechanism of the so-called denervation hypersaluria and polyuria. According to one team of workers, general anaesthesia and the other conditions associated with the experiment would cause a sympathetic excitation, which leads to vasoconstriction in the kidney; as a result of this, renal blood flow and glomerular filtration rate decrease, leading secondarily to a reduction in the excretion of salt and water. Denervation of the kidney abolishes this increased constrictor tone, the blood flow and filtration increase in the denervated organ and, secondarily to that, salt and water diuresis increases. As according to the now widely accepted view (SMITH [1, 2], MUELLER, SURTSHIN, CARLIN and WHITE [18], SELKURT [19]), a slight, hardly measurable change in filtration would give rise to a major change in salt and water diuresis in the same direction. The above group of authors attributed no significance to the tubules in the development of the denervation effect and explained it with an increased filtration.

BERNE [5], SURTSHIN, MUELLER and WHITE [6], PAGE, BAXTER, REEM, SCOTT-BAKER and SMITH [20] have found denervation hypersaluria and polyuria, respectively, in association with increased filtration. After loading with hypertonic mannitol solution, KAPLAN, FOMON and RAPOPORT [21] showed for hydropenic dogs that on the denervated side a major percentage of the osmols fell to sodium than on the innervated side, *i. e.* the sodium concentration of the urine produced by the denervated kidney was higher. As at the same time also filtration was higher in the denervated side, these authors were unable to conclude that the phenomenon would involve a direct tubular nervous effect. KAPLAN, WEST and FOMON [15] found that the hypersaluria of the denervated side was associated with increased filtration also in the unanaesthesized dog. They concluded from the results that following unilateral denervation the increased tone of the intact side, too, may play a role in the difference of the responses.

MARSHALL and KOLLS [22, 23] found an increased output of salt and water on the denervated side. As clamping of the renal artery diminishes salt and water diuresis, it is suggested that the denervation effect is connected with an increase in renal blood flow. There was, however, no appreciable difference in creatinine excretion between the two sides, suggesting that the denervation hypersaluria and polyuria would develop in the presence of an unchanged filtration. KRISS, FUTCHER and GOLDMAN [24] think that the slight increase in filtration is no satisfactory explanation for the hypersaluria of the denervated side and suggest that also a decrease in tubular reabsorption may be involved.

KAPLAN and RAPOPORT [25] stated for hydropenic dogs loaded with hypertonic NaCl that the difference in saluresis between the denervated and innervated sides exceeds in measure that expectable on grounds of the difference in filtrated quantities. From this they draw the conclusion that one of

the causes of the increased salt output by the denervated kidney is the decreased Na-reabsorbing power of the denervated proximal tubule. Likewise, in their perfusion experiments SARTORIUS and BURLINGTON [13] find denervation hypersaluria in the presence of unchanged filtration. FISCHER, SZÉCSÉNY and VIRÁNYI [16] showed in 152 clearance periods that in the anaesthesized dog the denervated side excreted more salt and water, and in 69 periods the filtration of the denervated kidney was smaller than that of the innervated organ. FISCHER, TAKÁCS and VARGA [26] clamped the aorta between the two renal arteries, reducing thereby the blood flow and filtration of the denervated kidney. In many periods the sodium excretion of the denervated kidney was greater, in spite of the reduction in filtration. From these they conclude that denervation has a direct nervous influence on the tubular sodium reabsorption.

In the light of these contradictions in published evidence it was deemed necessary to re-investigate the correlation between the filtration and salt—water excretion of the denervated kidney in larger material. In addition, we wished to elucidate eventual differences between the innervated and denervated kidneys in their responses to various effects (water deprivation, drawing of minor volumes of blood, various infusions).

Methods

Experiments on anaesthesized dogs. Mongrels of both sexes were used. 2 or 3 weeks before experiment the left splanchnic nerve was transected under morphine-ether anaesthesia, from an abdominal incision. The last food intake took place about 20 hours before experiment and in the majority of the experiments the animals were withheld water for 14 to 16 hours (exceptionally for 48 hours). Anaesthesia was induced by the intravenous infusion of 0.1 g/kg of a 1 per cent solution of chloralose. The appropriate arteries and veins were cannulated for measuring blood pressure, for taking blood and for the administration of infusions, respectively. The urines from the two kidneys were collected separately, by means of supravesically introduced catheters.

The clearance substances were administered in the form of a priming dose (0.15 g/kg of inulin or 0.05 g/kg of creatinine and 0.015 g/kg of PAH, dissolved in 50 ml 0.9 per cent NaCl solution) and in the form of a maintenance dose (1.5 per cent inulin or 0.5 per cent creatinine and 0.15 per cent PAH solution in 0.9 per cent saline, infused at a rate of approximately 1 ml/min throughout the experiment). Inulin was determined according to HARRISON [27], creatinine according to POPPER, MANDEL and MEYER [28], PAH according to SMITH, FINKELSTEIN, ALIMINOSA, CRAWFORD and GRABER [29]. The concentration of sodium in plasma and urine was determined by flame photometry *(Zeiss)*, osmolar concentration in the urine by measuring the reduction in the freezing point and in the plasma by WESSON's formula [30].

The experiment was begun about 30 minutes after the completion of the preparatory work. The urine was collected in 15-minute periods, taking arterial blood samples for chemical analysis at about the middle of each period.

In some experiments the animals, which had been thirsted 14 to 16 hours, were given 0.85 per cent NaCl solution equivalent to about 3 per cent of the total body weight in the form of intravenous infusion about 50 ml/min in rate, after 2 control periods, 15 minutes in duration each. The experiment was then continued for 60 to 90 minutes. In an other series of experiments the control periods were followed by the intravenous infusion of an isoosmotic and isooncotic solution of dextran, equivalent to 1.5 per cent of the total body weight, then the experiment was continued for further four periods, 15 minutes in duration each.

In the third series both food and water had been withheld for 48 hours. Then 4 ml/kg of a 20 per cent mannit solution was infused and the plasma mannitol concentration was maintained by infusing 0.04 ml/kg of a 20 per cent mannitol solution every minute. At this dose the mannitol concentration of the extracellular space is about 20 mmol/l.

In the fourth series acute hypovolemia was induced in dogs by taking samples of blood equivalent to 0.5 per cent of the total body weight at the end of the 15-minute experimental periods. This procedure was continued until the arterial blood pressure was reduced to about 80 mm Hg. (In this type of experiment the blood withdrawn was not replaced, whereas in the other experiments the blood taken for chemical analysis was replaced by fresh dog blood by infusion.)

The success of splanchnicotomy was controlled by autopsy, after the experiment. At the end of the experiment polyuria was induced by the rapid administration of a hypertonic anelectrolyte solution, then the tracheal cannula was clamped. Denervation was considered successful only when a significant difference occurred in the number of urine drops coming from the right (innervated) and the left (denervated) kidneys during asphyxia ; the innervated kidney stopped producing urine within 2 minutes.

Experiments on unanaesthesized dogs. The right and left ureters of a bigger female dog were transplanted one by one to the abdominal wall. The bladder was extirpated. After complete wound healing the functions of the two kidneys were compared, then under morphine-ether anaesthesia the left kidney was denervated by splanchnicotomy. The main experiment was begun 2 weeks after this operation. The successful denervation of the left kidney was determined by the following method. At intervals (about 10 days) the diuresis of the two kidneys was determined, a slight ether anaesthesia was applied, in response to which the diuresis of the innervated kidney diminished to a minimum, whereas that of the denervated one did not change.

Conscious animals were experimented on while standing on the Pavlov stand, slightly banded. Urine was collected in test tubes bound under the abdomen. Experiments were conducted after ad libitum water intake and after 24 to 30 hours of thirsting. In some experiments the infusions of physiologic saline and of mannitol were made into a limb vein, as described for the anaesthesized dogs. (No clearance studies were made in the unanaesthesized animals.)

Results

The results are presented in Tables I to VI and Figures 1 to 4. The tables contain the means for the innervated and denervated periods of the corresponding series. To save space, no detailed data are presented. In the tables we can find the so-called D/I quotient, which is the mean of the quotients computed from the numerical results (denervated and innervated) of the same period. The significance analysis concerns the difference between the innervated and denervated results, on the one hand, and the deviation of the D/I quotient from 1, on the other.

In all the tables and figures the data are computed for 1 square metre of body surface area and 1 kidney. The value of V is given in ml/min, of $U_{Na}V$ in μEq/min, of $U_{osm}V$ in μosm/min, of C_{in} and C_{PAH} in ml/min. The four vertical columns of the tables show the sodium excretion in percentage of the total osmol output. Statistical analysis was by FISHER's t-test, taking conventionally a $P < 0.05$ as a criterion of significance.

In Figs. 1 to 3 are illustrated the means of the changes resulting from loading with mannitol, physiologic saline and dextran, respectively. In Fig. 4 is presented a type-experiment, with single periods lasting 15 minutes, each ending with the withdrawal of blood equivalent to 0.5 per cent of the total body weight. In the upper part can be seen the arterial blood pressure, which

is still as high as 135 mm Hg after the fifth blood taking, *i. e.* after the loss of blood equivalent to 2.5 per cent of the total body weight.

In the experiments on anaesthesized animals we determined the inulin clearance, except for the dextran series in which the exogenous creatinine clearance technique was employed. (Dextran interferes namely with the chemical determination of inulin). In the conscious series no clearance tests were made and GFR was assessed on the basis of the excretion of endogenous creatinine.

Discussion

In the various types of experiments on anaesthesized dogs we have compared the function of the innervated kidney with that of the denervated one in a total of 143 periods. In 17 cases the sodium output by the denervated kidney was slightly lower than that by the innervated one, whereas in 126 instances it was higher. Thus, in agreement with the data in the literature, we too, demonstrated the existence of a denervation hypernatruria. In the control (water-deprivation), physiologic saline and dextran loading series the correlation between natruresis and water diuresis is a linear one, *i. e.* the increased water output may be considered to be a secondary sequel to hypernatruresis. After loading with mannitol the size of the diuresis is determined by the mannitol loading employed.

The question arises: is the increased sodium excretion merely an obligatory sequel to the increased filtration or may our data be interpreted as indicating that also a reduced tubular sodium reabsorption is involved in the increased sodium output by the denervated kidney?

Thus, in the first approach we should analyse whether we have any periods in which the increased natruresis of the denervated kidney is associated with reduced or identical filtrations, *i. e.* the difference in filtration between the two kidneys does not explain the difference in natruresis.

Table I

14—16 hours water deprivation

	V	$U_{Na}V$	$U_{osm}V$	$(U_{Na} : U_{osm})100$	C_{in}	C_{PAH}
I	0.25 ± 0.14	29 ± 26	137 ± 89	17.7 ± 9.7	27 ± 12	105 ± 42
D	0.68 ± 0.36	104 ± 68	414 ± 164	29.0 ± 7.7	36 ± 13	122 ± 41
P	0.001	0.001	0.001	0.01	0.05	0.05
D/I	3.26 ± 2.08	8.71 ± 12.1	4.18 ± 2.73	—	1.43 ± 0.41	1.30 ± 0.37
P	0.001	0.01	0.01	—	0,001	0.01
n	22	22	10	10	22	21

Table II

48-hours water deprivation

	V	$U_{Na}V$	$U_{osm}V$	$(U_{Na} : U_{osm})100$	C_{in}	C_{PAH}
I	0.18 ± 0.09	34 ± 32	191 ± 151	13.6 ± 7.7	24 ± 13	111 ± 59
D	0.51 ± 0.24	136 ± 92	380 ± 204	29.9 ± 12.0	34 ± 7	137 ± 58
P	0.02	0.05	0.05	0.02	0.05	0.05
D/I	2.96 ± 0.74	4.53 ± 2.99	2.90 ± 1.54	—	1.89 ± 1.43	1.49 ± 0.76
P	0.01	0.05	0.05	—	0.05	0.05
n	6	6	6	6	6	6

In the water deprivation (control) periods (total number: 39) the sodium output by the denervated kidney exceeds that of the innervated one in 35 cases. The inulin clearance is higher in 28 cases in the denervated kidney, it shows no difference in 3 cases and is smaller in 4 cases. The means in Table I and Table II show that the D/I quotient of the inulin clearance is higher than 1, indicating that (with due regard to the methodological possibilities of the inulin clearance test) the increased Na output by the denervated kidney is usually associated with a higher inulin clearance.

The increased sodium excretion by the denervated kidney is associated with a higher diuresis. On the basis of extensive experiments BÁLINT, FEKETE and HAJDU [32], as well as BÁLINT, KISS and SZALAY [33] have emphasized the already known fact that in oliguric periods the clearances cannot be relied upon. Thus, at low diuresis the lower inulin clearance is a technical consequence of the low diuresis. In our opinion this statement reduces much from the evaluability of the D/I quotient of the inulin clearance in this series. Thus, for technical reasons the denervated kidney shows a higher inulin clearance as a result of polyuria.

This objection cannot be raised in the series in which the loading with physiologic saline and mannitol, respectively, results in a higher diuresis in both kidneys than the limit below which the clearances cannot be evaluated reliably. After loading with mannitol the sodium output by the denervated kidney is higher in all the 37 periods (Table IV) and at the same time the inulin clearance is higher in 23 periods and lower in 7 periods in the denervated kidney and there is no difference in 7 periods. At any rate, this indicates that with sufficient diuresis the D/I quotient of the inulin clearance is smaller than 1 more often than in the cases in which the diuresis of the innervated kidney is low. After loading with physiologic saline the denervated kidney excreted more sodium with higher inulin clearance in 22 cases, whereas in 4 cases the higher Na output was associated with a lower inulin clearance. After loading with dextran increased sodium output by the denervated kidney was asso-

ciated with higher inulin clearance in 9 cases and with lower clearance in 4 cases.

In every series of experiments attempts have been made to find a correlation between the inulin clearance and natruresis in both the denervated and innervated kidney. No correlation of any kind was demonstrable. In general, the sodium output by the denervated kidney was higher and this was associated with slightly higher inulin clearances, as it can be seen from the means and D/I quotients presented in the tables as well.

Furthermore, attempts have been made to find a correlation between the differences of the inulin clearances and those of the sodium outputs. No correlation whatsoever was demonstrable. If we accept the view that denervation causes an increase in the sodium output exclusively by the fact that the haemodynamics and in connection with it the filtration of the kidney increase in the presence of an unchanged tubular function, we may expect some kind of a quantitative correlation to exist between filtration and the difference in sodium output.

On the basis of what has been elaborated above we do not think it to be justified simply to ascribe to the difference in filtration the increased sodium output by the denervated kidney, though in the majority of the cases the inulin clearance is higher in the denervated kidney. Experience has shown us that particularly at lower diuresis levels the difference in diuresis has a more profound influence on the clearance values than it is generally believed and thus the denervation polyuria predisposes technically for higher clearances in every series. This statement applies in particular to those periods in which the low diuresis of the innervated kidney made its clearance absolutely unreliable. Attention should be called to the fact that at a normal plasma level a filtration difference of 1 ml means an about 150 μEq difference in filtrated Na and the means in the tables make it clear that the difference in sodium output between the innervated and denervated kidneys did not exceed this value in the order of magnitude.

According to Tables I, II and IV, the most characteristic for the denervated kidney is the fact first emphasized by KAPLAN, FOMON and RAPOPORT [21] that on the denervated side a significantly higher percentage of the osmol output falls to sodium than on the innervated side. This we believe to be a sign of a reduced sodium reabsorption by the denervated tubules. It is most remarkable that this difference between the two kidneys disappears after loading with physiologic saline.

Figs. 1 to 3 show the responses of the innervated and denervated kidneys to different infusions. The means for the control periods after loading with mannitol (dogs deprived from water for 48 hours) are presented in Table II. After the administration of mannitol the curves run essentially parallel courses, thus it may be stated that there is virtually no difference between the

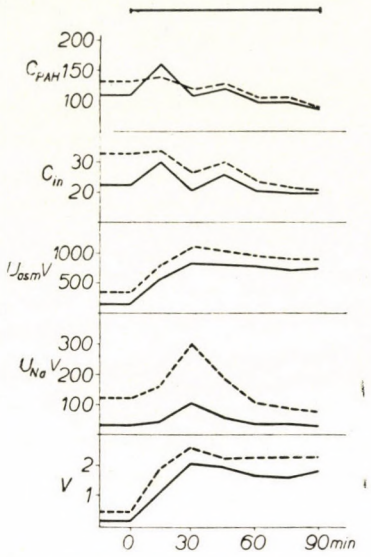

Fig. 1. Renal response to mannitol loading. Solid line: innervated kidney. Broken line: denervated kidney

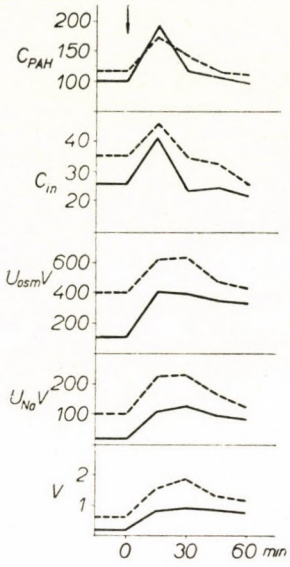

Fig. 2. Renal response to the infusion of physiologic saline equivalent in quantity to 3 per cent of the body weight. The time of infusion is marked by an arrow. Otherwise the signs are as in *Fig. 1*

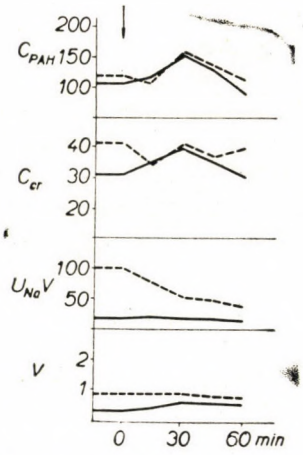

Fig. 3. Renal response to the infusion of dextran equivalent in quantity to 1.5 per cent of the body weight. The time of infusion is marked by an arrow. Otherwise, the signs are as in *Fig. 1*

innervated and denervated kidneys in their response to loading with mannitol. In this experimental setup the hypervolemia of the extracellular space is associated with intracellular hypovolemia.

Likewise, the curves run parallel after loading with physiologic saline (Fig. 2, 14 to 16 hours water deprivation). Thus, the denervated kidney re-

Table III

Intravenous physiological saline

	V	$U_{Na}V$	$U_{osm}V$	$(U_{Na} : U_{osm})100$	C_{in}	C_{PAH}
I	0.85 ± 0.58	114 ± 89	387 ± 154	36.0 ± 8.3	29 ± 14	132 ± 62
D	1.50 ± 0.91	192 ± 132	562 ± 271	35.8 ± 7.5	35 ± 13	143 ± 52
P	0.01	0.01	0.01	—	0.05	0.05
D/I	2.43 ± 1.94	3.71 ± 2.58	1.68 ± 0.81	—	1.38 ± 0.70	1.18 ± 0.4
P	0.001	0.001	0.001	—	0.01	0.05
n	35	35	23	23	35	35

Table IV

Mannitol loading after 48-hours water deprivation

	V	$U_{Na}V$	$U_{osm}V$	$(U_{Na} : U_{osm})100$	C_{in}	C_{PAH}
I	1.76 ± 0.60	60 ± 65	762 ± 225	7.0 ± 5.4	23 ± 8	114 ± 54
D	2.41 ± 0.68	168 ± 164	961 ± 299	16.3 ± 12.3	27 ± 9	117 ± 49
P	0,001	0.001	0.01	0.001	0.05	0.05
D/I	1.39 ± 0.22	3.03 ± 1.32	1.27 ± 0.22	—	1.20 ± 0.36	1.08 ± 0.30
P	0.001	0.001	0.001	—	0.01	0.05
n	36	36	36	36	36	36

sponds to the hypervolemia of the extracellular space in the same way as the innervated one. The means for the control and the post-saline periods are to be found in Table I and III, respectively. In response to loading, the water, sodium and total osmotic diuresis increase significantly in the innervated and denervated kidneys alike, but there is no significant change in the inulin and PAH clearances. The sharp rise in clearances observable immediately following salt infusion is, in our opinion, a direct technical consequence of the sudden increase in diuresis.

Table V

Intravenous dextran

	V	$U_{Na}V$	C_{creat}	C_{PAH}
I	0.55 ± 0.26	21 ± 21	36 ± 17	124 ± 60
D	0.85 ± 0.40	56 ± 51	38 ± 13	130 ± 58
P	0,01	0.01	0.05	0.05
D/I	1.94 ± 1.57	6.53 ± 10.50	1.15 ± 0.30	1.11 ± 0.30
P	0.02	0.05	0.05	0.05
n	20	20	16	20

In response to loading with dextran (Fig. 3, water deprivation for 14 to 16 hours) the increased sodium output by the denervated kidney slightly decreases, approaching that of the innervated kidney. There is practically no change in diuresis and in the sodium output of the innervated kidney. The difference in creatinine clearance observable during the control period disappears practically after loading with dextran.

Fig. 4 illustrates the typical response to repeated blood taking. In the first period the water and sodium output increases in the denervated kidney and

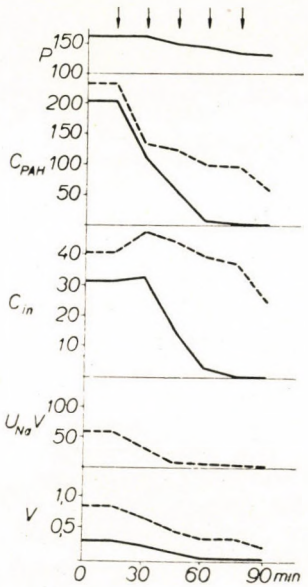

Fig. 4. Renal response to the loss of blood equivalent to 0.5 per cent of the body weight. The times of blood taking are marked by arrows. Otherwise the signs are as in *Fig. 1*

in conjunction with it the C_{in} and C_{PAH} also increase. After blood taking the diuresis is significantly reduced in both kidneys. After the second blood taking the innervated kidney is practically anuric, thus its clearances can be ignored. BÁLINT and STURCZ [34] have shown that in such an experimental setup diuresis and in connection with it the clearances strongly decrease, when the direct measurement of the renal blood flow still indicates only slight changes or no change at all in the haemodynamical relations. Thus, oliguria or anuria is a direct sequel to oligemia and the low clearance value is a technical consequence of the former. The diuresis of the denervated kidney is reduced at a much slower rate and as a result its clearances, too, are reduced in a significantly smaller measure. Thus, in this type of experiment it is not a change in the haemodynamics that is the primary factor and the sequence of events is not that the innervated kidney becomes ischaemic, thus oliguric,

but reversed: the diuresis of the innervated kidney is diminished faster than that of the denervated one and it is the technical consequence of this that the clearances behave as outlined above.

Table VI

Experiments on conscious dogs

	V	$U_{Na}V$	$U_{osm}V$	$(U_{Na} : U_{osm})100$	$U_{creat}V.100$
			6a. Water ad libitum		
I	0.45	21	201	11.4	34
D	0.53	26	209	11.9	33
D/I	1.20 ± 0.38	1.18 ± 0.36	1.05 ± 0.25	—	0.95 ± 0.14
P	0.05	0.05	0.05	—	0.05
n	16	16	16	16	16
			6b. 48-hours water deprivation		
I	0.09	4.4	125	3.4	28
D	0.10	7.1	124	5.6	26
D/I	1.10 ± 0.14	1.82 ± 0.48	0.99 ± 0.12	—	0.91 ± 0.16
P	0.05	0.001	0.05	—	0.05
n	11	11	11	11	11
			6c. Saline infusion		
I	0.68	96	471	20.3	35
D	0.86	121	519	23.1	39
D/I	1.26 ± 0.17	1.28 ± 0.16	1.14 ± 0.27	—	1.12 ± 0.09
P	0.01	0.01	0.05	—	0.01
n	8	8	8	8	8
			6d. Mannitol infusion		
I	1.84	109	1080	9.5	32
D	1.70	107	977	10.8	29
D/I	0.94 ± 0.11	1.14 ± 0.04	0.92 ± 0.10	—	0.94 ± 0.15
P	0.05	0.05	0.02	—	0.05
n	12	12	12	12	12

Relatively few experiments have been made on unanaesthesized dogs (2 animals). With unrestricted water intake (Table VIa) the various excretions from the innervated and denervated kidney are practically identical. Neither does the D/I quotient deviate significantly from 1. After 30 hours of water deprivation (Table VIb) the difference of water and Na output is significant mathematically, but, owing to the very low diuresis, we do not

believe it to be significant biologically. After the infusion of physiologic saline (Table VIc) the output by the denervated kidney is somewhat higher, but the order of magnitude of the difference is substantially smaller than it is in similar experiments on anaesthesized animals. After mannitol infusion (Table VId) there is no appreciable difference between the innervated kidney and the denervated one. There was no difference in the percentage ratio of sodium and total osmolar output between the two kidneys in either type of the experiments, unlike it happened in the ones on anaesthesized dogs.

Summing up the results of our experiments, it may be stated that in the anaesthesized animal the denervated kidney excretes more sodium and water than the contralateral, innervated organ. This increased output is associated with a higher inulin clearance in the majority of the cases. Nevertheless, we do not think that the increased sodium output by the denervated kidney would be due exclusively to an increased filtration, because 1. there undoubtedly are periods in which Na output was higher, though filtration was lower, 2. no correlation whatsoever is demonstrable between the differences in filtration and natruria, 3. the lower diuresis of the innervated kidney predisposes technically for lower clearances, which are not reliable at all under a certain level of diuresis, thus, it is clear that the clearance of the denervated kidney (the diuresis of which is higher) is higher also for this technical reason. We think that the denervation hypernatruria is connected with a reduced tubular sodium reabsorption, which is characterized by the fact that a significantly higher percentage of the total osmolar output by the denervated kidney is in sodium than in the urine produced by the innervated organ. We do not wish to doubt that, in general, the haemodynamics and filtration of the denervated kidney are greater, yet we do not think it justified to claim schematically that this would be the sole cause of hypernatruria.

The innervated and denervated kidneys respond in practically the same way to loading with mannitol, physiologic saline and dextran, respectively. After the gradual withdrawal of small volumes of blood the diuresis of the innervated kidney diminishes more strongly and faster, the technical consequence of which is a decrease in clearances.

We have made relatively few experiments on unanaesthesized dogs. The differences found are not significant mathematically, thus we have no right to claim that in the animal allowed water ad libitum or in the one on water deprivation the sodium and water output by the denervated kidney exceeds significantly that by the contralateral, innervated organ. Nevertheless, a tendency to increased output is evident in the denervated kidney. This is even more marked after salt infusion and disappears altogether after mannitol infusion. Insofar as we are justified to assess differences in filtration on the basis of the creatinine output (BÁLINT, KISS and SZALAY [35]), it appears that these small differences in output result at the same levels of filtration.

And if there does exist a difference in salt and water diuresis between the denervated and the innervated kidneys in the unanaesthesized animal, the order of magnitude of this difference is substantially smaller than in the anaesthesized ones.

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ON THE STORAGE OF INULIN AND PAH IN RENAL TISSUE

By

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With constant infusion of inulin and PAH and at a constant inulin and PAH concentration in plasma the quantities excreted per minute in the urine are lower than the doses infused. Thus, some part of the infused inulin and PAH is deposited in the organism, or is utilized in some other way. A smaller part of the calculated quantity was found to be stored in the kidney.

Anuria was induced by bleeding in dogs, to which subsequently inulin and PAH were administered by intravenous infusion. After two hours of anuria the left kidney was transplanted into the neck of a recipient dog and the amounts of inulin and PAH were determined in the urine of the transplanted kidney, in the urine of the recipient dog and in the tissues of the transplanted kidney. The inulin and PAH content of the contralateral, non-transplanted and "not washed" kidney was also determined. It has been found that the "washed" left kidney also contained inulin and PAH.

The amount of deposited inulin and PAH does not explain the difference between the clearences determined by the usual urinary and other techniques. The results cannot be interpreted as proving the existence of inulin secretion.

It is well-known that renal blood (plasma) flow can be determined by dividing the minute output of some substance in the urine ($U \times V$) by the difference in the concentration of that substance in arterial and renal venous blood (plasma) ($P_a - P_v$). This is the application of the Fick principle, well-known in the determination of cardiac output, to the kidney. If this formula is converted according to the terminology of renal physiology so that both the output per minute and the arterio-venous concentration difference are divided by the arterial concentration (P_a), the numerator means the clearance of the substance in question and the denominator the intrarenal extraction of that substance. In other words renal plasma flow is obtained if the clearance of some substance is divided by the extraction of that substance ($RPF = C/E$). It follows from this formula that if by some method we determine directly the renal plasma flow and the extraction, the clearance of the substance in question can be computed ($C = RPF \times E$). According to PHILLIPS, DOLE, HAMILTON, EMERSON, ARCHIBALD and VAN SLYKE [1], the clearance calculated by the classical method will agree with that computed from the above formula if (i) diuresis is sufficiently copious to make the dead space negligible; (ii) the substance reaches the kidney exclusively in the arterial blood and leaves it exclusively with urine and venous blood; (iii) the substance is not produced, metabolized or stored by the kidney; (iv) the distribution

of the substance between erythrocytes and plasma does not change in the blood flowing through the kidney.

If by constant infusion we administer a substance, which is neither metabolized nor stored in the organism, sooner or later an equilibrium will be reached, *i. e.* the quantity infused per minute will be the same as that excreted in the urine in one minute. In the numerator of the classical clearance formula $U \times V$ will be replaced by $I \times V$, where I is the quantity of the substance in 1 ml of the infused solution and V is the volume infused in 1 minute. On this principle is based the so-called constant infusion clearance test first recommended by EARLE and BERLINER [2], that has been shown by BERGER, FARBER, EARLE and JACKENTHAL [3], as well as by SCHWARTZ, BREED and MAXWELL [4], to yield both in dog and man the same inulin and PAH clearance values as the classical urine clearance technique does. Should the clearance substance be excreted also extrarenally and/or be metabolized in the body and/or be deposited in the organism, the value yielded by the infusion test will naturally surpass that of the classical urinary clearance.

BÁLINT, FEKETE and HAJDU [5], as well as BÁLINT, KISS and SZALAY [6], have shown that in posthaemorrhagic hypotension, as well as in water deprivation oliguria the direct ($RPF \times E$) and the infusion ($I \times V/P$) clearance tests yielded markedly higher values than that obtained by the classical urine clearance ($U \times V/P$). On the other hand, with sufficient diuresis (above 1 ml per minute in the dog) the results of the different techniques agree satisfactorily. In contrast with this, in oliguric states the difference exceeds the one expectable on grounds of the so-called dead-space effect.

One of the possible explanations of the latter phenomenon is that the clearance substances (inulin and PAH) would be stored in the kidney. The fact that inulin is metabolized or stored extrarenally has been proved in nephrectomized dogs (BÁLINT and FORGÁCS, [7]). In the present report we wish to deal with the intrarenal storage of inulin and PAH.

It has been generally accepted that inulin is excreted exclusively by glomerular filtration, and is neither resorbed nor secreted by the tubules. In contrast with this, PAH is excreted by filtration and tubular secretion. (For the pertaining literature, the monograph by SMITH [8] should be consulted). Inulin clearance can be accepted as the measure of GFR only when inulin is excreted by the above mechanism. According to FREY [9], part of the inulin administered is secreted; its clearance cannot therefore be accepted as a measure of GFR. Among the indirect proofs against inulin secretion there is the evidence published in the early literature that inulin is not stored in the tubular cells, whereas PAH, which is excreted by secretion, is demonstrable also in those cells. RICHARDS, WESTFALL and BOTT [10] perfused the dog kidney with blood containing inulin and phenol red, at a pressure of 25 to 30 mm Hg. At such a low pressure no urine is formed. Comparing the blood entering and

leaving the kidney it was revealed that 20 per cent of the inulin and as much as 50 per cent of the phenol red infused were retained. Then the same kidney was perfused at high pressure with blood containing neither inulin nor phenol red. The urine subsequently formed contained ample amounts of phenol red, but no inulin. From this it was concluded that at pressures too low to make filtration possible, inulin does not reach the tubular cells and leaves the kidney with the venous blood. In contrast with this, the secreted phenol red is taken up by the tubular cells which secrete it when urine is not formed. Thus, according to these data the tubular cells do not store inulin.

There is, however, some more recent evidence of inulin being stored in the renal parenchyma. GAYER [11] administered inulin and PAH to rabbits by a single intravenous injection. Twenty minutes later the left kidney was removed and perfused with Tyrode's solution through the renal artery. The fluid leaving through the renal vein and the ureter was collected until its inulin and PAH concentration had became negligible. The right kidney was analysed without perfusion, the left kidney after perfusion. The mean inulin content of the left, "washed", kidney was 0.73 mg/g, 54 per cent of the inulin content of the right kidney. The left kidney contained 0.34 mg/g of PAH, 17 per cent of the amount contained by the right kidney. From this GAYER drew the conclusion that the tubular cells store not only PAH, but also considerable amounts of inulin. Similar results have been obtained by FREY [12] who following administration of inulin found 3 to 12 times more inulin in the rabbit's renal tissue than what would have been expected on the basis of the inulin concentration of the blood and urine in the kidney.

In his monograph, JANCSÓ [13] wrote that in certain oliguric states some of the macromolecular substances are precipitated in the tubular urine, then are deposited in the preformed intracellular canals of the tubular cells. Substances not resorbed by the tubules are demonstrable in the tubular cells after ligation of the ureter or in shock. Under such conditions, inulin stored in the tubular cells can be revealed by histochemical techniques, under the polarization microscope. This means that storage is in a causal relationship with the process of tubular concentration: the excessive reabsorption of the solvent predisposes to precipitation and storage of the solute (*e. g.* inulin).

Experimental

The storage of inulin and PAH by renal tissue has been investigated in two types of experiment.

(i) *So-called infusion experiments.* Dogs of various breeds and both sexes were allowed to drink water according to the desired level of diuresis or were thirsted for 24 to 48 hours. The experiments were made under chloralose anaesthesia. Depending in quantity on the body weight of the animal and on

the plasma level to be attained, inulin and PAH were administered in a preliminary dose and then by constant infusion (see DEANE, [14]). Infusion was made at an even rate, by means of the usual apparatus. Urine was collected by means of ureter catheters introduced supravesically through a suprapubic incision, taking great care to ensure reliable estimation of clearance by the classic technique. About 1 hour after starting the constant infusion plasma concentration became constant, as determined by analysing 3 blood samples taken at about 20-minute intervals.

Next, the left kidney was removed from median laparatomy and the diuresis of the right kidney was enhanced by infusing about 200 ml of 20 per cent mannite. After further two clearance periods the right kidney was also removed. The data yielded by the chemical analysis of the kidney, plasma and urine will be discussed later.

(ii) *So-called transplantation experiments.* The experiment began as described above. Except for experiments Nos. 34, 44 and 43, posthaemorrhagic hypotension of 2 hours duration was induced by lowering blood pressure to about 50 mm Hg by bleeding. At this blood pressure there was practically no diuresis. The infusion of inulin and PAH was continued at a reduced rate. At the end of the 2-hour hypotensive period (in the above-mentioned 3 experiments after the usual 2-hour clearance procedure) the left kidney was removed through a median laparatomy incision and was transplanted into the neck of a previously anaesthesized recipient dog. The urine from the transplanted kidney and from the recipient dog was collected. The recipient dog was given neither inulin nor PAH. Prior to transplantation, urine was collected from the recipient dog to determine the blank inulin and PAH values. After transplantation osmotic diuresis was induced by the infusion of 20 per cent mannite, in response to which urine output increased in both the transplanted kidney and the normal kidneys of the recipient dog. Urine collection lasted about 1 hour; 2 to 3 portions were collected, pooled and then analysed.

Immediately after transplanting the left kidney, the right kidney of the animal was removed for inulin and PAH analysis. At the end of the experiment the transplanted left kidney was also subjected to the same analysis.

Determination of inulin and PAH. Inulin in plasma and urine was determined according to LITTLE [15], and PAH according to SMITH, FINKELSTEIN, ALIMINOSA, CRAWFORD and GRABER [16]. The LITTLE test determines only the so-called alkali-resistant inulin.

Inulin and PAH in renal tissue were determined by the technique of ROSS and MOKOTOFF [17]. The capsule of the kidney was pulled off, the hilar structures and fatty tissue were removed by scissors. A longitudinal cut was made and the renal surface was thoroughly rinsed with running tap water. After drying with filter paper the kidney was weighed and the weight of an about 1 g specimen of cortex and medulla was measured with 10 mg accuracy.

The specimen was placed in a glass-stoppered flask and was digested with 6 ml of 0.85 *N* NaOH for 20 minutes, in a boiling water bath. After cooling, 1 ml of 5 *N* HCl was added, then followed neutralization with 0.5 *N* HCl against lithmus. Deproteinization was made with 10 ml 10 per cent zinc sulphate and 10 ml 0.5 *N* NaOH. After making up to 50 ml, the mixture was thoroughly shaken and filtered. Adequate volumes (for inulin, 2 ml; for PAH, 5 ml) of the filtrate were measured and subjected to the inulin test of LITTLE and the PAH test of SMITH.

The inulin preparation used was about 90 per cent alkali-resistant.

Results

The results of the infusion experiments can be found in Table I, in which column 4 shows the difference between the quantities of inulin and PAH infused per minute and the quantities excreted with the urine at equilibrium, *i. e.* after the plasma level became constant. A loss of the clearance substance occurred even with satisfactory diuresis. In general, the difference between

Table I

Storage of inulin and PAH in infusion experiments

No.	V/min	Plasma level mg per 100 ml	Difference, infused/excreted mg/min	Quantity found in the kidney			
				left kidney		right kidney	
				mg/g	total, mg	mg/g	total, mg
			Inulin				
73	1.52	80	12.0	1.53	54	1.17	47
85	1.94	96	19.9	2.55	96	1.83	71
54	0.31	97	19.3	2.76	96	1.44	50
59	0.33	75	5.2	2.92	110	1.48	65
69	0.22	47	11.7	2.64	127	2.49	112
65	0	293	46.0	6.98	195	4.03	122
68	0.08	178	49.6	6.80	340	2.84	160
60	0	350	34.4	10.80	332	—	—
			PAH				
73	1.52	3.2	1.1	0.19	6.8	0.14	5.8
85	1.94	1.1	0.7	0.06	2.3	0.04	1.8
54	0.31	3.2	3.4	0.39	13.4	0.18	6.1
59	0.33	1.9	1.3	0.22	8.3	0.10	4.3
69	0.22	0.95	0.7	0.14	6.6	0.11	4.9
65	0	8.3	1.9	0.59	16.7	0.45	13.5
68	0.08	3.6	2.6	0.71	36.0	0.21	11.8
60	0	6.9	5.3	1.57	48.5	—	—

the infused and excreted amounts was the greater the lower was the diuresis, but the correlation was not linear. A constant plasma level may naturally develop even in total anuria, when all the infused quantity remains in the body. The difference between the infused and excreted amounts was not proportionate to the amounts of inulin and PAH detected in the kidney by analysis. Neither was there a demonstrable correlation between the plasma level and the stored amount of clearance substances. The inulin and PAH content of the right kidney was somewhat less than that of the left kidney. As it has been pointed out above, this was due to the fact that in the mannitol diuresis most of the material were removed from the urinary tract. It is therefore conceivable that that amount of inulin and PAH, which is present also in the right kidney, is located in the renal parenchyma and the interstitium. We must call attention to the fact that, as related to the plasma levels and to the difference between the infused and excreted quantities, the amounts of PAH deposited in the left and right kidney, respectively, were greater than the amounts of inulin.

In Table II are presented the results of the transplantation experiments. In these the amounts of inulin and PAH contained in the whole kidney were determined by analysing the right kidney. From the blood vessels and the interstitium of the left kidney the clearance substance being removed by the urine, the amounts of substance found in that organ must have been bound to the tubular cells. The columns of Table II show these three kinds of inulin and PAH values. Their sum, provided storage in the two kidneys is identical, must equal the amount found in the right kidney. It is seen that, as calculated from the sum of the three columns, the left kidney contained on the average 22 per cent more inulin and 5 per cent more PAH than the right kidney. The agreement is, however, satisfactory, if we consider the great sources of error involved in the test, and also the circumstance that the right kidney can be rinsed before the vascular bed and urinary tracts are "washed", whereas the left kidney can be rinsed only after most of the inulin and PAH had been removed *per vias naturales*.

Several blank inulin and PAH determinations were made in the kidneys. The mean blank values were 0.05 mg of inulin and 0 to 0.02 mg inulin and PAH, per g kidney. When calculating the amounts of clearance substance contained in renal tissue, the blank values were ignored. The urine from the transplanted kidney cannot be subjected to blank tests for technical reasons. The blank value was taken into account when computing the inulin and PAH content of the recipient's urine.

In Fig. 1 the amount of inulin and PAH in the right kidney was taken to be 100 per cent and to this amount have been related the amounts stored in the left kidney, excreted with the urine of the transplanted kidney and with that of the acceptor animal.

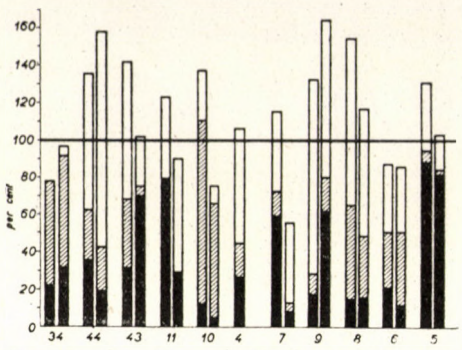

Fig. 1. Percentage distribution of inulin and PAH storage in renal tissue. Of the pairs of columns the left one shows the values for inulin, the right one those for PAH. The inulin and PAH content of the right kidney is taken to be 100 per cent. The solid parts of the columns show the amount of substance which had remained in the left kidney, the shaded parts show the quantity found in the urine of the transplanted kidney and the empty parts show the amount in the urine of the recipient dog

Table II

Storage of inulin and PAH in transplantation experiment

No.	In urine of transplanted left kidney mg	In urine of recipient dog mg	In transplanted kidney mg	Sum of columns 2—3—4 mg	Right kidney mg	Storage mg/g	
						Left	Right
			Inulin				
34	20	0	8	28	36	0.22	1.14
44	11	31	15	57	42	0.38	1.21
43	20	40	17	77	54	0.38	1.03
11	0	25	45	70	57	1.84	2.30
10	64	10	7	81	59	0.30	2.00
4	11	38	16	65	61	0.40	2.00
7	10	37	49	96	83	0.77	1.20
9	11	102	17	130	98	0.82	4.46
8	58	102	17	177	115	0.30	2.05
6	57	69	39	165	188	0.83	4.80
5	13	81	194	288	220	5.10	5.75
			PAH				
34	4.9	0.4	2.5	7.8	8.0	0.08	0.26
44	4.8	25.0	4.4	34.2	21.5	0.11	0.62
43	1.6	8.5	22.4	32.5	32.2	0.46	0.65
11	0	15.3	7.4	22.7	25.2	0.30	1.03
10	44.7	6.8	2.9	54.4	72.0	0.12	2.45
7	1.3	13.2	2.5	17.0	30.0	0.04	0.43
9	1.9	9.1	6.7	17.7	10.8	0.31	0.50
8	11.4	24.4	5.6	41.4	35.2	0.10	0.62
6	17.8	16.3	5.6	39.7	46.0	0.12	1.18
5	1.5	14.3	61.0	76.8	74.0	1.60	2.00

Discussion

The results obtained in both types of experiment unequivocally indicate that renal tissue does in fact store inulin and PAH. A comparison of the mg/g values for the left kidney in Table I with those for the right kidney in Table II shows the total amounts stored to be comparable in the order of magnitude. These total amounts correspond to the quantities present in the urinary ducts, vascular bed, interstitium and cells. According to Table I, the right kidney contained 64 per cent of the amount of inulin detectable in the left kidney and 59 per cent of the amount of PAH. This means that with ample diuresis 36 and 41 per cent, respectively, of the corresponding totals were removed, *i. e.* those portions of inulin and PAH which had remained in the tubules and urinary tracts. The data in Table II and Fig. 1 show that from the transplanted left kidney 32 per cent of the total amount of inulin and 25 per cent of the total amount of PAH were removed by the urine. The urine of the recipient's kidney contained (Table II and Fig. 1) 53 per cent of the total amount of inulin and 47 per cent of the total quantity of PAH. These quantities had originated from the blood and the interstitium of the transplanted kidney. This excreted quantity was, however, greater than what may be present in dissolved form in the extracellular space of the kidney. Assuming that the plasma concentration of inulin 100 mg per 100 ml and that of PAH 3 mg per 100 ml, and, further, that in the kidney 20 per cent of the inulin and 30 per cent of the PAH fall to the extracellular space, in a kidney weighing 40 g the extracellular space may contain 8 mg of inulin and 0.36 mg of PAH. Column 3 in Table II shows that amounts far greater than these had entered the recipient's urine (although the mean plasma inulin concentration was much lower than 100 mg per 100 ml). This means that the quantity present in the recipient's urine had originated not only from the extracellular space of the transplanted kidney but also from its parenchymal cells. Columns 4 and 7 in Table II show that after two "washings" there still remained some inulin and PAH in the transplanted kidney and that can be nowhere else but in the cells of the renal parenchyma (tubules).

To some extent the first three experiments in Table II (Nos. 34, 44 and 43) can be looked upon as control ones, insofar as they involved no anuria, only the usual clearance determination. In these three experiments the plasma concentrations of inulin were 12, 200 and 160, respectively, *i. e.* even that kidney was storing inulin which produced urine normally at a low plasma inulin concentration. It can be seen that the stored quantity was independent of the plasma inulin and PAH concentrations in the other experiments, and that there was no unequivocal correlation between diuresis and the measure of storage, either. For example, in experiment No. 8 the order of magnitude of inulin storage at a plasma inulin level of 54 mg per 100 ml was much lower

than in experiment No. 11, in which the plasma inulin level was 40 mg per 100 ml. Both animals had been anuric for 2 hours before transplantation. It is clear from Table I and Table II alike that, taking into consideration the low plasma PAH concentration, relatively more PAH was stored than inulin. This is in agreement with the classical view, according to which PAH is excreted by tubular secretion; such substances have been known to be stored intracellularly.

Our investigations thus confirmed GAYER's observation [11] that renal tissue does in fact store inulin. It remains to be examined whether the quantity stored suffices to explain the difference in inulin clearance demonstrable between the results of the RPF $\times$ E_{in} formula, the constant infusion and the classical urinary techniques. The data in Table I indicate that there was such a great difference between the amounts infused and excreted per minute that the total amount deposited in the kidney could cover it only for a period lasting a few minutes. The same follows from the data of Table II. Considering that in the classical clearance test urines containing 5000 mg of inulin and 500 mg of PAH per 100 ml are no rarity, it will be clear that the total amount stored is not more than what we may find in a few ml of urine. Thus the storage of inulin and PAH undoubtedly demonstrable in the kidney cannot explain the difference between the results obtained by the various clearance techniques nor why the value of the classical urinary clearance test is low in oliguric states. Our experiments indicate therefore that there exists an extrarenal deposition and/or excretion and/or breakdown of inulin and PAH. It is also possible that inulin and PAH leave the kidney not only in urine and venous blood, but by some other route, for example, the lymphatics.

GAYER [11], as well as FREY [12], regard the storage of inulin as a proof of the view that inulin is secreted by the tubules. We, on the other hand, do not think that either the data in the literature referred to or our own experiments would justify that claim. In our opinion, the correlation demonstrated by JANCSÓ [13] to exist between the oliguric and anuric states and the quantity of the material stored in the kidney gives the appropriate interpretation of the phenomenon. The concentration of primary urine in the tubules is less marked with normal diuresis and more marked in oliguria, the solvent is absorbed, inulin is precipitated first in the tubular urine, then in the preformed intracellular canals of the tubular cells and, finally, in the cells themselves. The inulin thus stored is carried away partly *via* the urinary ducts and partly with the blood flowing through the kidney. The only proof of secretion were if the tubular cells would contain substantial amounts of inulin following low pressure perfusion of the kidney with blood containing inulin. In other words, if it were unequivocally demonstrable in the experimental design of RICHARDS, WESTFALL and BOTT [10] that inulin is in fact stored, and it could be ruled out that inulin returned to the cells from the filtered primary urine.

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DATA ON THE SPECIFIC FUNCTIONAL ADAPTATION OF THE ADRENAL CORTEX

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A general survey has been given of experiments concerning the hormone production of the adrenal cortex. Certain aspects of the interglandular regulation and of the specific homeostatic function of the adrenal cortex have been elucidated. The results of the experiments may be summarized as follows.

(i) A total of 19 corticoids were observed to be biosynthesized by adrenal homogenates *in vitro*. These compounds proved to be Δ^4-3-ketosteroids with an *a* ketolic side chain at the position 17. Six of these derivates were more polar than hydrocortisone ; three of them lay between hydrocortisone and cortisone ; further three between cortisone and corticosterone ; four were less polar than corticosterone.

(ii) Among the compounds more polar than hydrocortisone, two derivates (III and IV) were found to exert in adrenalectomized rats and mice a lympholytic activity more intensive than that of hydrocortisone and cortisone. The ability of the two compounds to promote glycogen deposition in the liver was, however, slight.

(iii) The corticoids yielded by biosynthesis were found to be present in the venous blood of the adrenals of various animal species. Similarly, they appeared in the course of the so-called specific adaptation.

Adaptative changes of specific character in adrenal secretion

a) Sexual steroids induced qualitative changes in the corticoid composition of venous adrenal blood. In the dog, oestrone benzoate markedly decreased hydrocortisone secretion and gave rise to the appearance of 17-hydroxyprogesterone. In the cat, however, oestrone made to appear two new compounds, two Δ^4-keto corticoids with a polarity grade less than that of corticosterone.

b) In hyperthyroidism, five new compounds were found in the venous blood of the adrenals of the cat. Two of these compounds inhibited the secretion of thyreotrophine to a greater extent than any other corticoid so far known. These compounds were also present in the urine of subjects with hyperthyroidism. In the dog, both thyroidectomy and methylthiouracil treatment resulted in an increased corticoid secretion and in the augmentation of the ratio hydrocortisone : corticosterone.

c) In certain species, such as the wild and the domesticated rat and the fox, such corticoids occurred in the resting secretion of the adrenals, which do not appear in the dog or in the cat.

d) Stimuli falling within the frames of the general adaptation syndrome (such as tranqillizing drugs) evoked a reaction of specific character, in which only hydrocortisone secretion was extremely elevated. Since polar corticoids greatly increase the excitability of the central nervous system with concomitant tonization of the sympathetic nervous system, it has been suggested that the above increase in corticoid secretion was reflecting the augmented corticoid demand of the organism. Accordingly, under treatment with tranquillizing drugs, this effect would play the role of compenzatory homeostatic mechanism. A similar phenomenon was observed to occur in the course of behavioural changes following certain types of surgical intervention in the central nervous system (removal of the amygdale nucleus, as well as frontal leucotomy).

As a final summary of the phenomena investigated, it has been concluded that, in addition to the general adaptation syndrome, the adrenal cortex participates in the maintenance of homeostasis also by means of specific reactions. This specific adaptation is modified by species differences, due to alterations in the tissular metabolism of the adrenal cortex.

In order to maintain the equilibrium with the external environment, living organisms are forced to permanent adaptation controlled mainly by complex neuroendocrine mechanisms. CANNON was the first to recognize the sympatho-adrenal system to be one of the fundamental regulatory mechanisms of homeostasis in higher animals and in humans. SELYE, studying the role of the pituitary-adrenal system, concluded that, in addition to, and in functional connection with, the sympatho-adrenal system, adrenal corticoids were also involved in the formation of the "general adaptation syndrome". The pituitary-adrenocortical system is now widely known to play a decisive part in the maintenance of homeostasis. Our present knowledge of this question is, however, not sufficiently advanced to allow a correct and complete interpretation or appreciation of the role played by the above system. Various findings in the past decade pointed to the fact that the "stress" concept, with its generalizing outlines, reflects only part of the real homeostatic function of the adrenal cortex. Greater attention has, therefore, to be paid to events which occur under certain specific adaptation conditions.

Without discussing the problems of the "specific adaptation syndrome" at length, we have to point out certain aspects of pituitary-adrenocortical function. Numerous observations have demonstrated that stressor stimuli initiate *via* the diencephalon an increased secretion of adrenocorticotrophic hormone. This leads to an increased corticoid output by the adrenals, which then evokes complex changes in the metabolic processes and the structure of the thymus and lymphoid organs. Is the intensity of a stimulus sufficiently strong, so the changes are identical, of whatever kind the stress (thermal, psychic, toxic, etc.). From the above phenomena it has been concluded that the pituitary-adrenocortical system plays a fundamental part in the general adaptative processes.

In the last year we made some observations which, together with the pertaining literary data, have led us to conclude that the role ascribed to the adrenal cortex in the general adaptation syndrome means, in reality, only a general activity, while the specific cortical functions — though similarly representing adaptation — fall out of the frames of the proper "stress" concept.

I. Methods

The experiments were performed on dogs, cats and rats. Corticoids were estimated by paper chromatography, according to the method of BURTON, ZAFFARONI and KEUTMANN [6]; and of BUSH [8]. Venous blood from the adrenals of animals under Dial anaesthesia (2 ml/kg of a 2 per cent solution) and pretreated with heparine (5 mg/kg intravenously) was collected through a plastic tube with glass tip inserted into the vein. Blood collection took 20 to 90 minutes, depending on the experiment. The plasma was extracted with a 4 to 1 mixture of ether and ethyl acetate. Partition was made in equal volumes of 70 per cent ethanol-benzene-petrolether. Identification and determination of the corticoids were performed by the ultraviolet alkaline fluorescence technique, as well as by tetrazolium and Ag reduction. 17-ketosteroids were identified with the m-dinitrobenzene reaction. Details of the evalu-

ation have been published recently (ENDRŐCZI, BATA and MARTIN [12]; ENDRŐCZI, BATA and LISSÁK [13]; LISSÁK, ENDRŐCZI and MEDGYESI [37]; ENDRŐCZI, TELEGDY and MARTIN [17]).

a) *Experiments in vitro*

Adrenal glands of oxes and calves from the slaughter house were homogenized within 60 minutes after the death of the animals. The homogenate was incubated at 38 C° in a mixture of Tyrode's solution and of blood (1 : 1). Care was taken for the continuous oxygen supply of the reaction mixture. In this system the metabolism *in vitro* of cristalline hydrocortisone, cortisone and progesterone was investigated during an incubation period of three hours. The activation of the enzyme system was assured by continuous drop infusion of ACTH (0.1 I. U./min) dissolved in Tyrode's solution. Corticoids were added to the homogenates in form of suspended microcrystals, while progesterone (25 mg/100 g tissue) was administered in a mixture of propylene glycol and Tyrode's solution. Following the incubation the homogenates were deproteinized with two volumes of acetone. This latter was then removed under reduced pressure and the corticoids were extracted in the usual way.

b) *Standard compounds used in the analysis and other methodical details*

The method used for the quantitative determination of corticoids is based on the presence of a Δ^4-3-keto group in the molecule, and can be regarded as an absolutely specific one. In our hands the limit of error did not exceed 15 per cent, provided that the following conditions were fulfilled.

(i) The amount of the hormone under investigation should lie between 0.5—5 μg, and (ii) always two standard compounds with different polarity (hydrocortisone, cortisone) should be run simultaneously. For the identification of unknown compounds the following standard derivates were used: hydrocortisone, cortisone, corticosterone, 11-desoxycorticosterone and their acetates, resp., androstene [3, 11]-dione, androstene [3,11,17]-trione, 17-hydroxyprogesterone, progesterone, testosterone, cis-androsterone, dehydro-iso-androsterone and Δ^5-pregnenolone.

The compounds under investigation were acetylated in a medium containing anhydrous acetic acid and pyridine. Identification was made on the basis of the R_f values of the formed acetates (CONSTANCE DE COURCY *et al.* [10]).

The corticoid content was calculated partly as μg/g adrenal weight/kg body weight/hour. In some cases, however, when both body weight and adrenal weight had changed, the data were given as μg/g adrenal weight/hour, and also as μg/kg body weight/hour.

II. Biosynthesis of corticoids of different polarity in adrenal tissue homogenates

It is well-known that, between 1930 and 1938, REICHSTEIN, KENDALL, WINTERSTEINER and PFIFFNER isolated from the adrenal tissue about 30 steroids, parts of which were, naturally, biologically ineffective. The inactive compounds were either saturated ones, or contained reduced ketolic groups at the positions 3, 11, 20.

Most of the inactive compounds were characterized by the presence in the molecule of both a reduced carbonyl and a reduced Δ^4-3-keto group. It is the general view that the 3 and 20 keto groups, as well as the presence of the Δ^4 bond, are necessary for the biological activity. This activity can be modified by additional oxo groups at the positions 11, 17, or at the other carbon atoms. As a fundamental characteristic of our method in the detection of the Δ^4-3-keto group, it is only natural that mainly those compounds were involved in our investigations which possess the biological active structure.

The adrenal gland homogenates were incubated with hydrocortisone, cortisone and progesterone. Among these hormones, hydrocortisone produced corticoids in the greatest amount and variety. With regard to the fact that the initial precursor gave rise not only to compounds of very polar character, but also to less polar ones, two different systems were necessary for paper chromatography.

The extract from the homogenate was first run in a formamide-benzene system. This process resulted in the separation of compounds less polar than hydrocortisone. Following this separation, the space between the starting line and the hydrocortisone spot was eluated with methanol, then run again in benzene-methanol-water (100: 40: 50). In the latter case the descending method was carried out at 32°C for 16 hours. During this time the migration of the hydrocortisone enabled the separation of the more polar compounds.

After the incubation of both pig and ox adrenals with hydrocortisone, a total of 21 compounds with the Δ^4-3-keto structure were observed to occur. Among these compounds two derivates (XX and XXI) were "gestagens", since they proved to be 17-hydroxyprogesterone, resp. progesterone. The other compounds possessed an α-ketolic side chain at the position 17, as well as a Δ_4-3-keto group, as shown by the tetrazolium reduction and alkaline fluorescence. None of the compounds gave a positive reaction with m-dinitrobenzene.

Six of the above compounds (I to VI) showed a greater polarity than did hydrocortisone. Among these compounds, components III, IV, V and VI appeared in the greatest amount. Three derivatives were found to lie between hydrocortisone and cortisone, as far as their polarity was concerned. Compound X was most probably identical with aldosterone, although no biological identification was made. Three corticoids were found to lie between cortisone and corticosterone, and four components were less polar than corticosterone.

The biosynthesis of compounds I to VI occurred in considerable amounts only when the adrenals had been incubated with hydrocortisone. Incubation with progesterone yielded these materials only in minute quantities, very probably through the step progesterone-hydrocortisone as, in the above system, progesterone yielded hydrocortisone in considerable amounts. Incubation with cortisone did not lead to the formation of more polar compounds. By altering the pH of the medium, the direction of biosynthesis could be somewhat influenced. Thus, the elevation of the pH to 7.6 increased mainly the synthesis of compounds of weaker polarity, while a diminution of the pH value to 7.0—7.2 augmented the production of compounds of stronger polarity. Table I shows the amount of corticoids of different polarity synthesized from hydrocortisone. Hormone transformation did not occur in adrenals inactivated by heat at 100°C. The corticoid content of the adrenal without

added precursor was restricted to a minute amount of hydrocortisone and cortisone (20—40 μg [100 g tissue weight]).

Table I

Percentual distribution of corticoids in the biosynthetical process

Substances :	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
	0.5	0.5	3—5	3—5	1.0	1.0	100.0	0.5	0.5	2.0	20—25
Substances :	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI	
	4—6	2—4	1.0	35—40	2—4	4—6	3—5	2—3	2—4	0.5–1.0	

Corticoids produced from hydrocortisone by adrenal cortex homogenates. 100 g pig adrenal incubated in Tyrode : blood (1 : 1) at 30°C for 180 minutes under continuous oxygen supply. 25 mg hydrocortisone added. The amount of hydrocortisone present after incubation was taken for 100 per cent. Quantitative evaluation with 0.5 per cent accuracy

Most of the corticoids which had appeared during biosynthesis were not identified chemically, since their amount was too small. However, the administration of different precursors gave an opportunity to study both the metabolizing and synthesizing capacity of the adrenal enzyme system. In addition, investigations could be made also into the biological action of compounds obtained by biosynthesis.

Only five of the corticoids observed in the course of biosynthesis were chemically identified (hydrocortisone, cortisone, corticosterone, 17-hydroxyprogesterone and progesterone). As far as the ketosteroid series is concerned, we succeeded in identifying two of the compounds, namely Δ^4-androstene-3,11-dione and Δ^4-androstene-3, 11, 17-trione.

Compounds I to VI were not identical with tetrahydrocorticosterone, as they failed to show alkaline fluorescence and so did not contain a Δ^4-3-keto group. They were, however, of highly polar character. The origin of this polarity is not quite clear, but we have to draw attention to the assumption of HAYNES, SAVARD and DORFMAN [23], according to which new double bonds and keto groups may be taken up by the carbon. 6. and 16. of these compounds. Compound XVII was probably identical with 11-dehydrocorticosterone, as it showed both tetrazolium reduction and alkaline fluorescence, but no Ag reduction.

a) *Transformation by CrO_3 of compounds less polar than hydrocortisone*

The CrO_3 oxidation method was first introduced into the microanalysis of corticoids by ZAFFARONI and BURTON [69]. This reaction produces from hydrocortisone androstene-(3, 11, 17)-trione. Using a medium containing CrO_3

and glacial acetic acid we observed that, under certain conditions, hydrocortisone might be transformed into four new compounds, all possessing a Δ^4-3-keto, 17-ketolic side chain. Three of these derivates proved to be identical with the compounds XII, XIII and XVII found in the course of biosynthesis. The fourth compound was, however, a completely new one showing only slight polarity. In addition to these compounds, Δ^4- androstene- dione and trione, as well as a highly polar 17-ketosteroid, were also observed after treating the chromatogram with m-dinitrobenzene (Fig. 2).

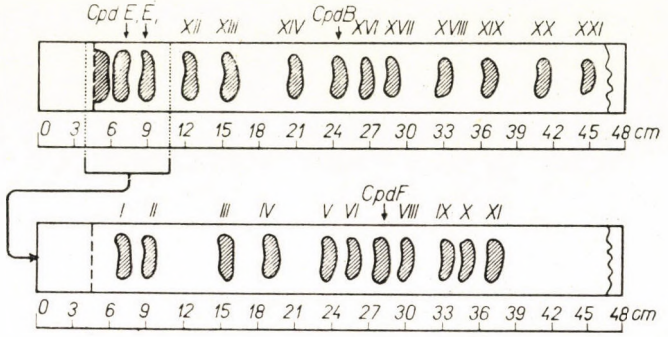

Fig. 1. Corticoid synthesis from hydrocortisone in adrenal homogenates. The upper chromatogram was run in formamide-benzene, the lower one in benzene-methanol-water. Spots Cpd F and Cpd E in the upper chromatogram were eluated with methanol and run again in benzene-methanol-water. This procedure allowed the separation of compounds more polar than hydrocortisone (lower chromatogram). Cpd F = hydrocortisone ; Cpd E = cortisone ; Cpd B = corticosterone

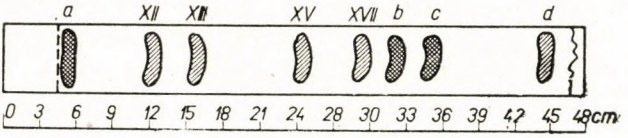

Fig. 2. Corticoids (XII, XIII, XV, XVII and *d*) and 17-ketosteroids *(a, b, c)* formed in the presence of CrO_3

Compound *a* is unknown ; *b* and *c* correspond to Δ^4-androstene-dione and -trione, respectively. Compound XV is corticosterone

b) *General remarks on the experiments performed by the method of biosynthesis*

As it was shown by a variety of earlier experiments on adrenal homogenates or adrenals perfused *in situ*, adrenal tissue possesses a specific enzyme system capable of producing both hydroxyl groups at the position 3, 11, 17, 21 and unsaturated bonds. According to our experience, other tissues fail to synthesize corticoids of higher polarity from either of the precursors. Even among the slightly polar corticoids only corticosterone is produced by extra-adrenal tissue (ENDRŐCZI and BOHUS [15]; ENDRŐCZI and MARTIN [16]). Of

the other organs the placenta alone is known to contain an enzyme system similar in action to that of the adrenal glands (for details, see ENDRŐCZI, TELEGDY and MARTIN [17]).

As regards the results of biosynthesis, the question arises whether the corticoids yielded by that method actually play a role in the normal function of the adrenal cortex. Since several compounds synthesized by adrenal tissue *in vitro* are regularly met with in the venous blood of the adrenals, the question about the physiological role of these hormones may be answered in the affirmative.

III. Individual and species differences in the hormone secretion of the adrenal cortex

It is well-known that different laboratory animals (dog, cat, rabbit, rat, guinea pig), as well as man, show differences in the corticoid composition of the venous blood of the adrenals. (BUSH, [8]; HECHTER et al., [25]; ZAFFARONI and BURTON, [69]; BUSH and SANDBERG, [9]; JUDAEV, PANKOV and DRUZHINA [30/a]; ROMANOFF, HUDSON and PINCUS, [45]; WEICHSELBAUM and MARGRAF, [65]; GRANT, FORREST and SYMINGTON, [20]; ENDRŐCZI, BATA and MARTIN, [12].) In general, the bulk of the corticoids in the adrenal blood is made up by hydrocortisone and cortisone. In addition to these, some unidentified corticoids were observed by various authors. Table II shows the corticoid composition of the venous blood of the adrenals in various species. The ratio hydrocortisone: corticosterone is also shown in Table II. As it may be seen, marked variations in this ratio may occur within the same species.

In this connection it is worth recalling that marked differences were found in the adrenocortical hormone pattern between wild and domesticated rats. In these investigations the adrenals of 6 to 8 rats were pooled, in order to have sufficient material for corticoid extraction. The main component in domesticated rat was corticosterone. In addition, two other compounds were found, one of them showing a weaker polarity than corticosterone, while the other was of more polar character. Both components proved to be Δ^4-3-keto, 17-ketol corticoids, as judged from the tetrazolium reduction and the alkaline fluorescence. Unlike in the domesticated rat, the main component in the wild rat was a compound more polar than corticosterone. In addition, two other corticoids of weaker polarity than corticosterone, as well as corticosterone itself were found.

One of those components, which commonly occurred in both wild and domesticated rats (compound XIV), was found also in the fox, but not in the dog or the cat.

The four components observed in wild rats corresponded to compounds XII, XIII, XIV and XVII of our biosynthetic series. The question arose whether

Table II

a)

Corticoid composition, as well as the ratio hydrocortisone : corticosterone in the venous adrenal blood of different animal species.

Unknown components appearing under the effect of interglandular influences have not been investigated

Species	*Substances*	*Ratio*	*Author (s)*
Man	Cpd F, Cpd B, 2 unkowns more polar than Cpd F, 2 unknowns of polarity between Cpd F and B, 1 unknown less polar than Cpd B	10 : 1	PINCUS & ROMANOFF, 1953
			ROMANOFF, HUDSON & PINCUS, 1953
		4—11 : 1	SWEAT, 1955
		2—11 : 1	HUDSON, LOMBARDO, 1955
		2.3 : 1	GRANT, FORREST & SYMINGTON, 1957
Monkey	Cpd F, Cpd E(?), Cpd B	20 : 1	BUSH, 1953
Dog	Cpd F, Cpd B, Cpd A, 2 unknowns more polar than hydrocortisone	5 : 1	BUSH, 1953
	Cpd F, Cpd B, 17—OH—desoxycorticosterone	2.3 : 1	FARRELL, 1953, FARRELL & LAMUS, 1953
	Cpd F, Cpd B, 3 unknowns more polar than Cpd F, 2 unknowns of polarity between Cpd F and B, 2 unknowns less polar than Cpd B	1.5—2.3 : 1	ZAFFARONI & BURTON, 1953
	Cpd F, Cpd B, 17—OH—desoxycorticosterone, 2 unknowns more polar than Cpd F	1.2—20 : 1	HECHTER *et al.*, 1954
	Cpd F, Cpd B, 1 unknown more polar than Cpd B, 1 unknown less polar than Cpd B	1.4—14 : 1	ENDRŐCZI, BATA, MARTIN, 1958 LISSAK, ENDRŐCZI, MEDGYESI, 1957
Cat	Cpd F, Cpd B, Cpd A, 2 unknowns more polar than Cpd F	4—6 : 1	BUSH, 1953
	Cpd F, Cpd B, occasionally 1 unknown more polar than Cpd B and 1 unknown less polar than Cpd B	1.2—7 : 1	ENDRŐCZI, BATA & MARTIN, 1958 ENDRŐCZI, BATA & LISSÁK, 1957
Rabbit	Cpd B, Cpd F	0.05 : 1	BUSH, 1953
		0.05 : 1	KASS, HECHTER, MACCHI, MUO, 1954
Fox	Cpd F, Cpd B, 1 unknown more polar than Cpd B	7 : 1	ENDRŐCZI, 1958
Rat	Cpd B, 2 unknowns of polarity less and more than Cpd B	0.05 : 1	ENDRŐCZI, 1957
	Cpd B	0.05 : 1	BUSH, 1953
	Cpd B, 2 unknowns		WEISZ *et al.*, 1958
Rat (wild)	Cpd B, 3 unknowns more polar than Cpd B	0.05 : 1	ENDRŐCZI 1958

these same corticoids were figuring also in the secretion (HOLZBAUER and VOGT [29]), working with the same problem, pointed out that the corticoid pattern of the venous adrenal blood was identical with that of adrenal tissue. Even the quantitative changes were parallel, and increased output of corticoids was associated with the elevation of the level of these materials in the adrenals. However, this observation was made in cases when secretion was increased only for a short time.

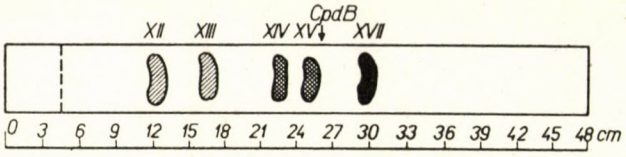

Fig. 3. Corticoid content in the adrenals of wild and domesticated rats. Compounds XII, XIII and XIV were found in wild animals, while XIV, XV and XVII in domesticated rats. Compound XV is corticosterone, a trace of which was present also in the wild rat

a) *Some aspects of the ratio of hydrocortisone : cortisone*

Table II already revealed the ratio hydrocortisone : cortisone to undergo marked variations within the same species. HECHTER and PINCUS [26], as well as LISSÁK, ENDRŐCZI and MEDGYESI [37], observed in the dog that the ratio may vary between 1.2—14:1, even in great experimental material. Extremely wide margins (3—11:1) were recorded also in man (GRANT *et al.*, [20]).

Our findings revealed the ratio hydrocortisone : cortisone to be characteristic of the individual, though under certain conditions it may change considerably. ACTH infusion failed to cause a measurable change in the ratio. The administration for 2 to 3 days of ACTH in a daily dose of 10 I. U./kg body weight to dogs and cats resulted, however, in an increase of the ratio. This increase indicates that under the effect of strong and long-lasting stimuli the enzyme system of the adrenals shifts in the direction of hydrocortisone synthesis. Increased ACTH secretion by the anterior pituitary activates more intensively the enzyme system carrying out the formation of the 17-hydroxyl-group than that responsible for the 11-keto-group. In other words, the enzymes of the adrenal cortex are activated mainly to synthesize polar corticoids. This phenomenon has been confirmed by other authors both in experimental animals and in man (HAYANOS *et al.*, [22]; SWEAT, [59]; GRANT *et al.*, [20]).

b) *Corticoids present at low concentration in the venous blood of the adrenals of different species*

As mentioned above, the venous blood of the adrenals contains not only hydrocortisone and cortisone, but also some other compounds. These compounds occur in small quantities and under certain conditions only. Fig. 4 shows that two each of these so-called trace compounds were found in the

venous blood of the adrenals in the cat and the dog. The amount of these compounds hardly exceeded two to three per cent of the total secretion, but under certain conditions they may attain the level of the main component. Thus, in a series of 30 untreated healthy cats there were two animals the adrenal blood of which contained compound XIV in an amount corresponding to nearly 70 per cent of the total secretion. These cats exhibited an unusually wild behaviour against both their cage associates and the keeper. It is worth recalling that the same compound XIV was commonly occurring in both wild

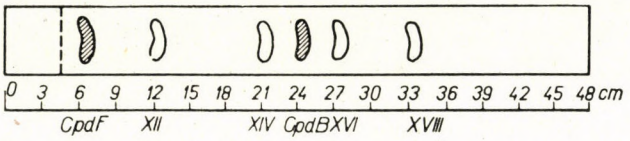

Fig. 4. "Trace compounds" in the venous adrenal blood of the dog and the cat. Compounds XII and XVII occur, under certain conditions, in the dog, while compounds XIV and XVI in the cat

and domesticated rats and also in foxes. However, the data at our disposal do not yet allow to infer to the relationship between aggressive behaviour and corticoid secretion (Fig. 4).

V. Influence of interglandular connections on the function of the adrenal cortex

a) "*Feed-back*" *effect of corticoids upon the hormone secretion in the adrenal gland*

Hormones are generally known to release reactions in the organs producing them. INGLE, HIGGINS and KENDALL [31], as well as SAYERS and SAYERS [47], were the first to demonstrate that administration of adrenocortical extracts decreased ACTH secretion in the anterior pituitary and the response of this gland to stressor stimuli. This effect of the corticoids was interpreted as a reaction of the processes regulating ACTH secretion at the hypothalamus-pituitary level. There are, however, some observations which postulate a direct action upon the adrenal cortex itself. Thus, exogenous corticoids were found to enhance the adrenal atrophy evoked by hypophysectomy, an effect best explained by a direct action of the hormone upon the adrenal tissue (KULENKAMPFF, [34]).

In recent experiments the effect of cortisone on adrenal weight and on the secretion of cortical hormones has been investigated in dogs and cats (ENDRŐCZI, KORÁNYI, FENDLER, TEKERES [18]). Cortisone acetate administered for seven days in a daily dose of 10 mg/kg body weight was found to decrease the weight of the adrenals to a much greater extent in the cat than in the dog.

Table III

Effect of 10 mg/kg cortisone acetate on adrenal weight.

a = Changes in the index, adrenal weight : body weight of puppies four months of age of two litters.

b = Changes in the index, adrenal weight : body weight after cortisone treatment of various duration, in the cat.

a

No	Adr. gland* / Body weight	Period of the treatment in days
1. untreated	200	—
2. ,,	218	—
3. ,,	180	—
4. ,,	260	—
5. ,,	245	—
6. cortisone	140	8
7. ,,	138	8
8. ,,	124	8
9. ,,	118	8
10. ,,	131	8

b

No	Adr. gland* / Body weight	Period of the treatment in days
1. untreated	176	—
2. ,,	180	—
3. ,,	167	—
4. ,,	202	—
5. ,,	158	—
6. ,,	160	—
7. cortisone	172	2
8. ,,	150	3
9. ,,	104	6
10. ,,	60	6
11. ,,	84	8
12. ,,	72	8
13. ,,	64	11
14. ,,	56	12
15. ,,	68	12
16. ,,	57	14

* Weight in mg / Weight in kg

As an effect of cortisone treatment, both hormone secretion and weight of the adrenals were changed, but these changes were of opposite direction. Thus, in the cat, hormone secretion was hardly influenced by marked cortical atrophy, while in the dog secretion was greatly inhibited even when the reduction in weight was slight. These observations point again to the fact that species differences can never be left out of account, even in the case of the most brutal experimental interventions.

The question arises, what is the mechanism responsible for the difference in the changes in the dog and the cat (opposite behaviour of synthetic processes and tissue weight). Two possibilities have to be considered. (i) The anterior lobe of the pituitary contains two factors, one regulating proliferation and another regulating secretion (STACK—DUNNE and YOUNG, [55]; STACK—DUNNE, [56]). (ii) Cortisone acts not only at the pituitary level, but also on adrenal tissue, and the two reactions differ according to species. The latter possibility is supported by the observation that cortisone decreases mainly the secretion of hydrocortisone, a hormone of similar polarity as cortisone itself, while, at the same time, corticosterone secretion is less diminished.

b) *Effect of sex hormones on the function of the adrenal cortex*

I. Effect of oestrone

VOGT and HOLZBAUER [64], [29] were the first to demonstrate that stilboestrol inhibits the synthesis and secretion of corticoids in the rat. This observation somewhat contradicted the earlier experimental finding that oestrogens induce marked cortical hypertrophy and a histologically demonstrable hyperfunction of the adrenals. The effect of oestrone on the functional activity of the adrenal cortex in the dog and the cat has been detailedly analysed in a previous paper (ENDRŐCZI, TELEGDY and BATA, [14]). In those experiments 3000 I. U. of oestrone (Oestrone benzoate, RICHTER) in oil was administered to animals of both sexes for 7—14 days. In the dog, marked adrenal hypertrophy resulted, together with considerable changes in the corticoid composition of the venous blood of the adrenals. Total secretion slightly diminished or was unchanged. The ratio hydrocortisone: corticosterone underwent, however, a marked decrease as a result of the considerable diminution in absolute hydrocortisone secretion. In addition, a qualitative change also occurred, as far as a new Δ^4-3-keto compound appeared. This compound exhibited alkaline fluorescence but gave no tetrazolium reduction. Its R_f values in both formamide-benzene and benzene-methanol-water revealed it to be identical with 17-hydroxyprogesterone.The secretion of this compound was sometimes very considerable, amounting even to 7.5 mg/24 hours.

In the cat, the weight of the adrenals remained unchanged after oestrone treatment. Corticoid secretion was increased and specific alterations occurred

Table IV

Corticoid content of venous adrenal blood, during treatment with 10 mg/kg cortisone acetate

a = Corticoid content in cortisone-treated dogs expressed in μg/g adrenal weight/kg body weight/hour and μg/g adrenal weight/hour, respectively.

b = Same data for the cat. R = ratio hydrocortisone: corticosterone.

a)

No	Corticoid secretion in μg/g/kg/h and in μg/g/h		R
1. untreated	24.0	19.0	6 : 1
2. ,,	30.0	21.0	2.5 : 1
3. ,,	60.0	24.0	3 : 1
4. ,,	71.0	19.0	2.5 : 1
5. ,,	38.0	22.0	2 : 1
6. cortisone	27.0	6.0	1 : 1
7. ,,	18.0	5.8	3 : 1
8. ,,	22.0	7.5	0.66 : 1
9. ,,	11.0	6.2	1.5 : 1

b)

No	Corticoid-secretion in μg/g/kg/h and in μg/g/h		R
1. untreated	40.2	22.6	2 : 1
2. ,,	48.4	26.0	1.8 : 1
3. ,,	52.0	20.0	2.0 : 1
4. ,,	38.2	24.0	2.6 : 1
5. ,,	41.6	19.0	4.2 : 1
6. ,,	62.0	29.0	3.0 : 1
7. ,,	36.0	17.0	6.0 : 1
8. cortisone	62.0	27.0	2.0 : 1
9. ,,	74.0	32.0	2.8 : 1
10. ,,	96.0	22.0	1.6 : 1
11. ,,	128.0	28.0	1.0 : 1
12. ,,	132.0	16.0	1.0 : 1
13. ,,	148.0	32.0	0.8 : 1
14. ,,	196.0	17.0	0.75 : 1
15. ,,	154.0	12.0	1.0 : 1
16. ,,	142.0	14.0	0.75 : 1

also in the composition of the secretion. In addition to the increased secretion of hydrocortisone and corticosterone, two new compounds appeared, both less polar than corticosterone. Unfortunately, we did not succeed in the chemical identification of these compounds. In any case, both contained Δ^4-3-keto group, but only one of them reduced tetrazolium. The less polar one of these compounds (b) might have been identical with 11-hydroxyprogesterone. The other compound (a) corresponded to the compound XVI, as judged from the R_f value of both its acetate and the free substance. The exact chemical identification of these components requires further experiments.

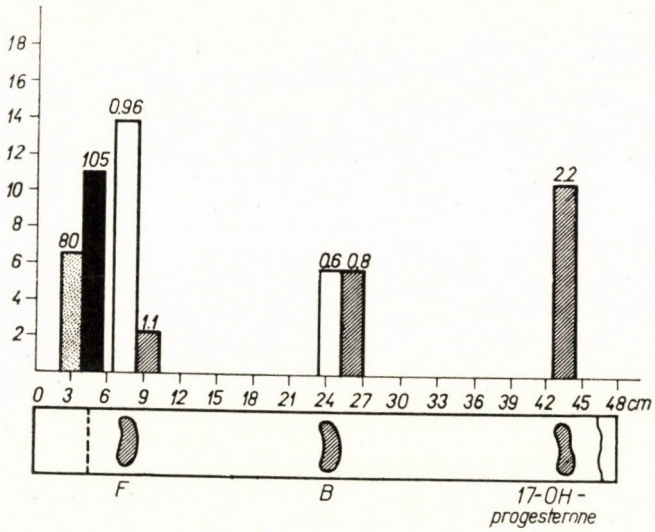

Fig. 5. Effect of oestrone treatment on the hormone secretion of the adrenals. Changes in the adrenal weight of 12 normal (dotted columns) and of 10 oestrone-treated animals (black columns). In the first and second columns the number on the ordinate refer to 100 mg. The other columns represent the amount of corticoids in μg/g adrenal weight/kg body weight/hour. Blank columns = untreated animals. Shaded columns = oestrone-treated animals. Number above columns show deviations from the mean values

The findings in oestrone-treated cats and dogs are another proof of the fact that the adrenals are capable of altering their hormone secretion in a very specific fashion. In addition, they show species differences. These differences cannot be accounted for by quantitative factors, since in the cat even 6000 I. U./kg body weight of oestrone had failed to initiate changes similar to those observed in the dog.

The mechanism of the oestrone action cannot be unequivocally explained by functional alterations of the anterior pituitary. The very same factor cannot, namely, produce changes of opposite direction. In agreement with VOGT and HOLZBAUER, we had to conclude that the oestrone effect may be supposed acting directly on the enzyme system of the adrenal cortex. The partially

decreased hydrocortisone secretion in the adrenals of the dog leads to increased ACTH mobilization from the anterior pituitary. This results in a considerably increased proliferation of the adrenal glands, and thus in an increase of adrenal weight. At the same time, the direction of the synthesis is also changed, an event leading to the appearance of new hormones. The existence of interconnections between adrenal cortex and gonads was shown also by HECHTER's observation [24], according to which the corticoid secretion of perfused adrenal glands of castrated oxes differed from that of normal animals.

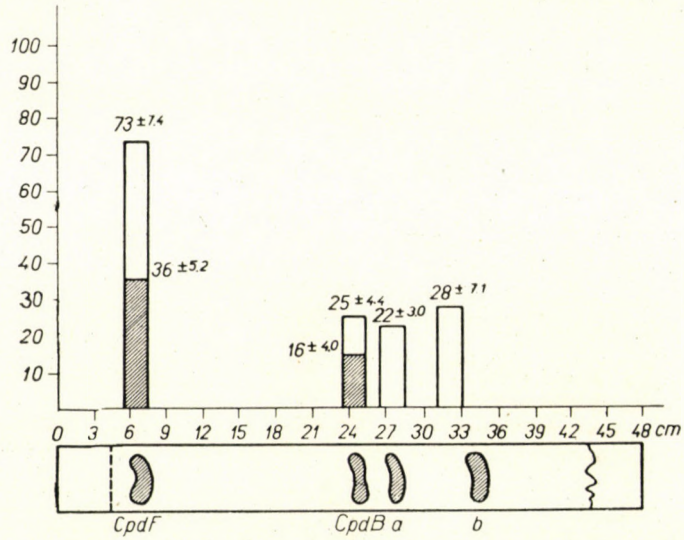

Fig. 6. Effect of oestrone treatment on the hormone secretion of the adrenal cortex. Shaded columns = mean of 12 control cats. Blank columns = mean of 10 treated cats. Numbers on the ordinate refer to µg/g adrenal weight/kg body weight/hour. Compounds *a* and *b* are new derivates

II. Effect of progesterone

In both the cat and the dog, progesterone treatment (0.5—2.5 mg/kg) for 5—8 days led to a considerable increase of the hydrocortisone secretion with the consecutive augmentation of the hydrocortisone : corticosterone ratio. No new compound appeared in the venous blood of the adrenals after progesterone treatment. The increased hydrocortisone secretion was similar as after prolonged treatment with ACTH. It is therefore possible that the same mechanism figured in the progesterone effect which did in the case of ACTH.

III. Effect of chioriongonadotrophine

Chioriongonadotrophine when given for 7—11 days in a daily dose of 200 I. U. evokes a considerable increase of the secretion in female dogs and cats. The marked augmentation in the ratio of hydrocortisone : corticosterone

indicates that the phenomenon was produced by the mechanism, figuring in the progesterone effect. Although the details of this mechanism require further analysis, some preliminary observations already indicated that the effect was an indirect one and was mediated through the ovary (TELEGDY and ENDRŐCZI, [60]).

Our experiments concerning sexual steroids could naturally not reveal all the possible mutual interconnections between adrenal system and gonads.

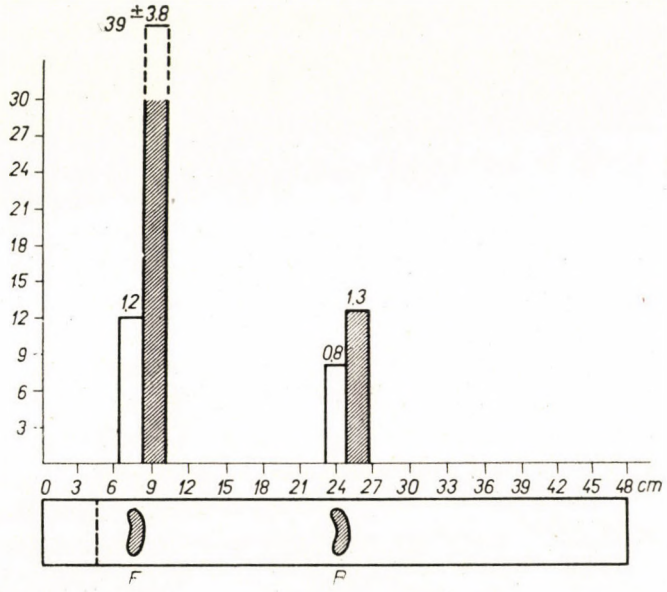

Fig. 7. Effect of progesterone on the hydrocortisone and corticosterone content of the venous blood of the adrenals in the dog. Blank columns = 10 control dogs. Shaded columns = = 8 treated dogs. Numbers above columns show deviations from the mean values. Ordinate shows the amount of corticoids in μg/g adrenal weight/kg body weight/hour

In any case, however, they illustrate that the interconnections between the two endocrine organs are not restricted to evoking simple quantitative changes but they could give rise to complex qualitative alterations, a fact, which cannot be left out of account consideration. The latter phenomenon should be regarded as a specific one.

c) *Functional connections between the thyroid and adrenal glands*

I. *The effect of hyperthyroidism induced by thyroxine*

More than 30 years ago MARINE and BAUMAN [40], as well as MARINE [1941], had called attention to the fact that in the pathomechanism of GRAVES' disease a role must be ascribed to the decreased function of the adrenal

glands. Numerous authors have since pointed out that thyroid hyperfunction, while constituting a stress to the organism, was not followed by increased corticoid secretion or by the characteristic signs of this condition.

On the other hand, adrenocortical hormones were recognized in the past decades to inhibit the function of the pituitary-thyroid system. This inhibition sets in not only after ACTH, hydrocortisone or cortisone administration, but also following stressor stimuli which mobilize ACTH, (WILLIAMS, JAFFE and KEMP, [67]; SOFFER, GABRILOVE and JAILER, [53]; VIDOVIC and VERZÁR, [63]; BROWN—GRANT, HARRIS and REICHLIN, [7]).

This mutual antagonism seems to be of great importance, mainly as regards the pathomechanism of hyperthyroidism. According to HARRIS and WOODS [21], it is the altered equilibrium of the hypothalamus-pituitary system, which accounts for the increased secretion of ACTH and TSH. In other words, the disorganization of this antagonistic relation would figure in the induction of hyperthyroidism. Without discussing this question in detail, it seems necessary to draw attention to some experimental proofs of the direct interglandular connections between thyroid gland and adrenal cortex.

We induced hyperthyroidism in cats by injecting thyroxine in a daily dose of 1.0 mg/kg for 7—14 days. The treatment evoked marked changes in the function of the adrenal cortex. The cats with hyperthyroidism responded to ACTH infusion with a 30—40 per cent increase of the secretion, while in normal animals the increase was always over 100 per cent. As an effect of the thyroxine treatment, total secretion at rest was slightly augmented, in spite of the fact that hydrocortisone secretion was markedly diminished. In addition to the increase in total secretion, new compounds also appeared. Two new components were found when hyperthyroidism was recent, and five new components in grave cases. None of these compounds occurs in the normal, unanaesthesized cat. The derivates found in venous adrenal blood during grave hyperthyroidism corresponded to compounds XII, XIII, XIV, XVI, and XVII of the biosynthetic series, and they proved to be Δ^4-3-keto, 17-ketol-corticoids, as judged by both the tetrazolium reduction and the alkaline fluorescence method (ENDRŐCZI, LISSÁK and BATA [13]).

The changes induced by hyperthyroidism in the function of the adrenals in the cat indicate that the underlying causal relationship must be sought for in the enzyme system of the adrenal gland and not in the regulatory processes of the hypothalamus-anterior pituitary system. The fact that following ACTH infusion the hormone production of the adrenals increased much less in cats with hyperthyroidism than in normal animals, shows that under the given conditions the secretory capacity of the adrenals was markedly decreased. This reduction, however, concerned the synthesis of certain corticoids only, as was revealed by the simultaneous appearance of some new compounds. As far as the mechanism of this action is concerned, there is a possibility that

hyperthyroidism induced by thyroxine evokes a marked decrease in the cholesterol content of the adrenals. This decrease is much more marked than that induced by stressor stimuli, a decrease always associated with adrenal hyperfunction (LONG and FRY, [38]; LONG, [39]). The marked decrease in question, however, is probably a sign of the general disturbance of the cholesterol household of the organism with hyperthyroidism. On the other hand, cholesterol is one of the starting materials of corticoid synthesis, an alternative pathway of the synthesis from acetate and C_4 dicarbonic acids. Using labelled

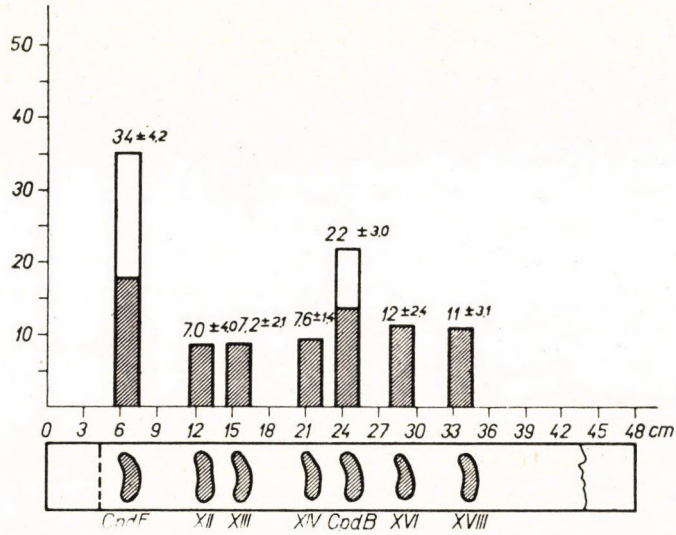

Fig. 8. Effect of thyroxine on the corticoid content of the venous blood of the adrenals in the cat. Blank columns = 10 untreated cats. Shaded columns = 8 thyroxine-treated cats. Marked secretion of compounds XII, XIII, XIV, XVI and XVIII in treated animals. Ordinate shows amount of corticoids in $\mu g/g$ adrenal weight/kg body weight/hour

precursors, STONE and HECHTER [57] observed that ACTH increased hydrocortisone and corticosterone synthesis by 1800 per cent, when this had started from cholesterol, but only by 140 per cent when acetate had been the starting point. The synthesis of corticoids from cholesterol being disturbed in hyperthyroidism, synthesis from compounds with a short carbon chain may come in the foreground. This process is, however, only slightly activated by ACTH, but at the same time thus enhances the production of metabolites which during synthesis from cholesterol occur only in traces or not at all.

All these lead to the conclusion that in hyperthyroidism the changes induced in the metabolic activity and corticoid synthesis of the adrenals are the result of complex functional alterations. However, the above investigations were performed in cats and so they are not directly valid in human pathology, considering the differences in the specific adaptation of various animal spe-

cies. The question also arises, whether the above secretory pattern of the adrenals should be considered a simple metabolic disturbance or to represent a certain type of adaptation of the adrenal gland?

The biological effect of the compounds found in the venous blood of the adrenals of animals with hyperthyroidism was analyzed mainly by procedures which had some connection with specific adaptation. Thus, the two compounds less polar than corticosterone were studied in methylthiouracil-treated mice and rats as to their effect on the secretion of thyreotrophic hormone. The compounds were eluted from the paper chromatogram by methanol, which was then removed under reduced pressure and the residue was dissolved in ethanol. After the determination of the hormone concentration (Porter-Silber method), the ethanol was similarly removed and a dose amounting to 10 μg/kg body weight was dissolved in 0.5 ml physiological saline. This procedure allowed the compounds to be administred in an alcohol free water medium without added organic solvents. Standard amounts of hydrocortisone, cortisone and corticosterone were treated in the same way. Mice and rats of the same bred and of about the same body weight (mouse, 22—25 g; rat, 75—90 g) were treated with methylthiouracil, in a daily dose of 0.5 mg/25 g body weight, intraperitoneally. Corticoids were injected intramuscularly. Both treatments lasted for 14 days in the mouse and for 16 days in the rat. At the end of the experiments the animals were killed by lighting gas and the thyroids weighed with an accuracy of 0.5 mg. The thyroids of the rats were controlled histologically.

Fig. 9 shows that all the corticoids used were inhibiting the methylthiouracil-induced increase in the secretion of thyreotrophic hormone, as judged by the changes in thyroid weight. Of the known corticoids, corticosterone was the most effective, the least effective having been hydrocortisone. According to the observations, inhibition of TSH secretion was inversely related to the polarity of the compound. Most marked effects were exerted by two unknown compounds, the relative potency of which was around 150—160 (hydrocortisone 100). This means that in hyperthyroidism such corticoids are produced which inhibit the output of thyreotrophic hormone to a greater extent than does any other corticoid secreted under normal conditions. No measurable inhibition of TSH secretion was caused by the other three compounds present in the venous blood of the adrenals of animals with hyperthyroidism (compounds XII, XIII, XIV). As far as their polarity is concerned, these compounds lay between hydrocortisone and corticosterone.

The appearance of the above corticoids in hyperthyroidism is not characteristic of the cat alone. Both compounds less polar than corticosterone were found also in the urine of certain subjects with clinically controlled hyperthyroidism (Bata, Endrőczi and Horváth, [5]). In these cases urine analysis was performed as follows. The urine collected during 24 hours was subjected to acid hydrolysis at pH 1. Following extraction with ether chloroform the

components were partitioned in 70% ethanol-benzene and then analyzed as described in the introduction of this report. We succeeded in identifying the above compounds in six out of fourteen cases. The amounts of these components reached sometimes 35 to 40 per cent of the total excretion.

Our findings in animals and humans showed hyperthyroidism to produce a complex disturbance in the function of the adrenal cortex. This trouble manifests itself with a decreased secretory capacity of the glands, as well as

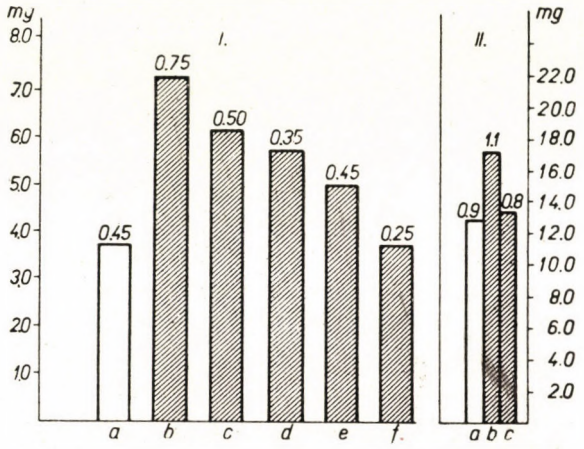

Fig. 9. Effect of corticoids on the methylthiouracil-induced increase in the thyroid weight in mice and rats. Part I shows experiments on the mouse, Part II those on the rat. Columns represent mean values of 10 animals

Part I.: a = untreated; b = methylthiouracil; c = 10 μg hydrocortisone + methylthiouracil; d = 10 μg cortisone + methylthiouracil; e = 10 μg corticosterone + methylthiouracil; f = 10 μg compound XVI (unknown)
Part II..: a = untreated; b = methylthiouracil; c = 10 μg compound XVII + methylthiouracil

with the appearance of certain new compounds. As far as the antagonism between the two endocrine organs is concerned, these new compounds are connected with specific adaptation. They were detected in the urine of certain subjects with hyperthyroidism whose Δ^4-3-keto-corticoid excretion was normal or subnormal (six control cases, 60 to 90 μg, 24 hours; subjects with hyperthyroidism, 25 to 80 μg, 24 hours). Data concerning the concentration of these compounds in the venous blood of the adrenals are, however, still lacking.

II. *Effect of thyroidectomy and methylthiouracil treatment on the secretion pattern of the adrenals*

Eik—Ness and Brizzeé [11] observed in the dog that one week after thyroidectomy the corticoid content of the venous blood of the adrenals was

markedly decreased. The level returned to normal on thyroxine administration. Earlier findings of other authors had, however, showed that in hypothyroidism the response to stressor stimuli of the pituitary-adrenocortical system was enhanced. Thus, HESS, SLADE *et al.*, as well as HESS and FINERTY [27, 28], demonstrated that the stress-induced decrease in the ascorbic acid content of the adrenals was more marked in thyroidectomized rats than in normal ones. As the ACTH secretion and ACTH content of the pituitary was found unchanged, it was concluded that the organism with hypothyroidism is more sensitive to ACTH than the normal one.

The problem was analysed in detail by BATA, ENDRŐCZI and JONEC [4]. Young and adult dogs were thyroidectomized. Four months (young animals), resp. 2 to 6 weeks (adult dogs) later the corticoid content of the venous adrenal blood was determined. Other dogs were treated with 300 to 700 mg/kg methylthiouracil by mouth and examined 2 to 4 weeks later. The findings unequivocally showed that after thyroidectomy the corticoid secretion increased parallel to the time elapsed between operation and experiment. The increase involved mainly hydrocortisone, as was shown also by the great augmentation of the ratio hydrocortisone : cortisone.

In animals with hypothyroidism there was no decrease in the corticoid content of the venous blood of the adrenals. Thus, the earlier findings of EIK—NESS and BRIZZEÉ could not be confirmed. The suggestion of HESS *et al.* that

Table V

Changes in adrenal weight and corticoid content of the venous adrenal blood, in the dog

No	Adrenal weight Body weight	Total amount of corticoids in μg/kg/h	and in μg/g/h	R :	*
1.	9.7	29.0	308	2.8 : 1	—
2.	6.3	23.8	288	2.6 : 1	—
3.	11.8	32.8	273	3 : 1	—
4.	10.8	26.8	250	2 : 1	—
5.	11.0	27.9	130	7 : 1	—
6.	12.0	24.4	216	3 : 1	—
7.	10.0	24.1	269	4 : 1	—
8.	14.9	35.6	238	3 : 1	14 days
9.	16.1	52.0	226	3 : 1	27 days
10.	13.0	59.2	452	2.6 : 1	27 days
11.	13.5	61.0	449	3 : 1	38 days
12.	13.0	107.3	820	3 : 1	38 days
13.	16.4	136.0	746	3.6 : 1	40 days

* indicates the day after operation

Table VI

Changes in adrenal weight and corticoid content of venous adrenal blood, in dogs treated with methylthiouracil. * indicates the amount of methylthiouracil in mg, administered orally

No	Adrenal weight Body weight	Total amount of corticoids in μg/kg/h	and in μg/g/h	R	*
1.	9.7	29.0	308	2.8 : 1	—
2.	6.3	23.8	288	2.6 : 1	—
3.	11.8	32.8	273	3 : 1	—
4.	10.8	26.8	250	2 : 1	—
5.	11.0	27.9	130	7 : 1	—
6.	12.0	24.4	216	3 : 1	—
7.	10.0	24.1	268	4 : 1	—
8.	9.7	25.4	264	2 : 1	200 mg/10 days
9.	11.8	59.7	504	6 : 1	300 mg/16 days
10.	12.2	39.0	320	3 : 1	400 mg/12 days
11.	8.7	22.0	252	4 : 1	200 mg/13 days
12.	8.4	34.7	410	9 : 1	500 mg/13 days
13.	12.7	69.5	551	3 : 1	700 mg/15 days
14.	13.1	71.0	536	6 : 1	700 mg/15 days

the response of the pituitary-adrenocortical system to stressor stimuli is greater in thyroidectomized animals than in normal ones is most probably correct. Yet, a direct proof of the assumption would require an analysis in unanaesthesized animals of the enhancing effect of stressor stimuli on adrenal secretion.

V. Observations concerning the biological effects of corticoids more polar than hydrocortisone

Six out of the compounds obtained by biosynthesis were more polar than hydrocortisone (compounds I to VI). All these components showed a glucocorticoid-type biological action. The effect of the compounds eluated from the paper chromatogram was studied in adrenalectomized rats and mice, partly with the method of glycogen deposition, partly with that of thymus involution and of lymphopenia.

In the present reports only compounds III and IV will be dealt with, two compounds characterized by a high lympholytic activity and a slight effect on glycogen deposition in the liver. As demonstrated in Fig. 11, the lymphopenic action of these compounds, as well as their effect inducing thymus involution, were stronger than those of any other corticoid so far known. Both

compounds evoked a fifty per cent lymphopenia in adrenalectomized rats, in a dose of 5 μg/100 g body weight, an effect not produced by less hydrocortison ethan 10 to 12 μg/100 g. Their effect on the of involution of the thymus of

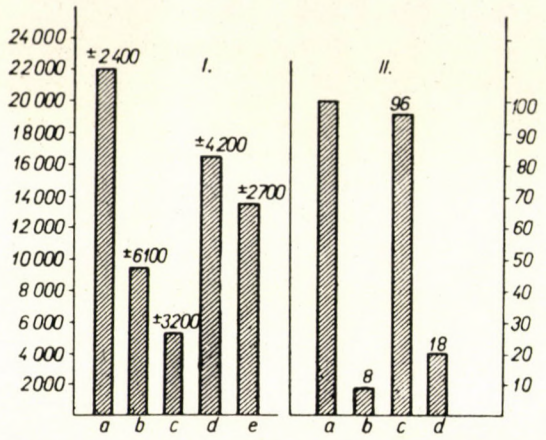

Fig. 10. Lympholytic and glycogen-depositing effect of corticoids in rats and mice. Ordinate on the right shows the liver glycogen content of adrenalectomized mice, in per cent. Ordinate on the left demonstrates the absolute number of lymphocytes in the blood of adrenalectomized rats. In Group I each column represents the mean value of six animals, while in Group II the columns correspond to eight animals. Part I: *a* = untreated; *b* = 5 μg/100 g compound IV; *c* = 10 μg/100 g compound IV; *d* = 5,0 μg/100 g hydrocortisone; *e* = 10 μg/100 g hydrocortisone
Part II: *a* = control without adrenalectomy); *b* = adrenalectomy; *c* = adrenalectomy 50 μg/100 g hydrocortisone; *d* = adrenalectomy + 100 μg/100 g compound IV

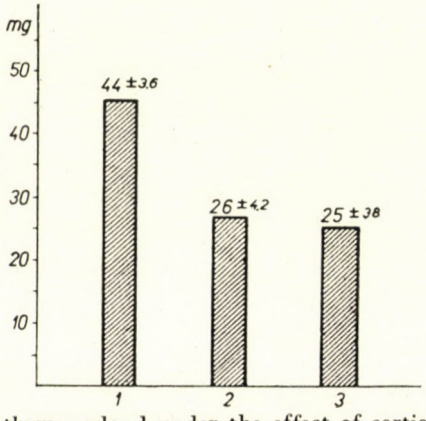

Fig. 11. Involution of the thymus gland under the effect of corticoids, in rats weighing 35 to 40 g. Each column represents the mean value of eight animals. 1 = control; 2 = 50 μg/100 g cortisone; 3 = 50 μg/100 g compound IV. Columns show the decrease in thymus weight evoked by three days' treatment with the above materials

rats weighing 35 to 40 g was similarly marked. The ability of these compounds to promote glycogen deposition in the liver was only slight in comparison with hydrocortisone and cortisone. These experiments were perform-

ed in adrenalectomized mice kept alive with 50 μg DOCA. 100 μg/100 g of compounds III and IV given one week after the operation did not normalize the glycogen content of the liver to more than 15 to 20 per cent. At the same time, 50 μg/100 g hydrocortisone or cortisone completely restored the ability of the liver to accumulate glycogen. Thus the biological activity of compounds III and IV is characterized by a strong lympholytic and a weak glycogen-depositing effect.

The stability of the compounds more polar in structure than hydrocortisone is weak. Their alkaline-fluorescence, due to the Δ^4-3-keto group, is lost in a methanol—ethyl acetate medium within four to five days. At the same time, they became biologically inactive. This lability, which does not occur with the corticoids hitherto known, makes the chemical identification of the compounds somewhat problematic. In any case some additional questions must also be cleared, namely, whether these compounds are really secreted in the adrenals and at what concentration, and whether they play a part in the pathomechanism of connective tissue disease.

VI. Neural aspects of specific adaptation

Next, some findigs obtained in animals with altered function of the central nervous system will be dealt with. As it has been pointed out above, the site of action of the interglandular endocrine effects partly may be represented by the enzyme system of the adrenals. However, the ACTH secreted by the pituitary may be also involved in these effects, namely, in the qualitative changes of the secretion pattern. Functional alterations in the central nervous system influence the secretion of cortical hormones primarily *via* ACTH secretion. Accordingly, the changes in the adrenal hormone pattern are of quantitative character only, corresponding to the outlines of the general adaptation syndrome. There are, however, some observations indicating that nervous processes may evoke also complex qualitative changes in the secretion pattern of the adrenals. These nervous processes, which are mediated partly through direct mechanisms and partly through indirect ones, are still scarcely known.

I. *Effect of tranquillizing drugs*

Two widely known tranquillizing drugs, reserpine and chlorpromazine, were investigated in dogs; the changes in the corticoid content of the venous adrenal blood were recorded (MEDGYESY and ENDRŐCZI, [43]). Both reserpine (*Rausedyl*) and chlorpromazine (*Largactil, Specia*) when given for five to ten days resulted in a 12 to 15 fold increase of the corticoid secretion. As to the qualitative composition of the corticoids secreted, this was characterized by a very great augmentation of the ratio hydrocortisone: corticosterone, even to 12—16:1. In this effect, it is mainly the unusually high output of corticoids

that deserves attention. 40 *I. U.* ACTH given to dogs weighing 15 to 20 kg for a similar period of time as the tranquillizing drugs induced only a three to fivefold increase in the secretion, a fact indicating that extremely great amounts of ACTH must be secreted under the effect of reserpine or chlorpromazine. Without discussing the site of action of these drugs within the central nervous system, it is necessary to recall the fact that inhibition of both the diffuse activating system and the archicortical structures very mark-

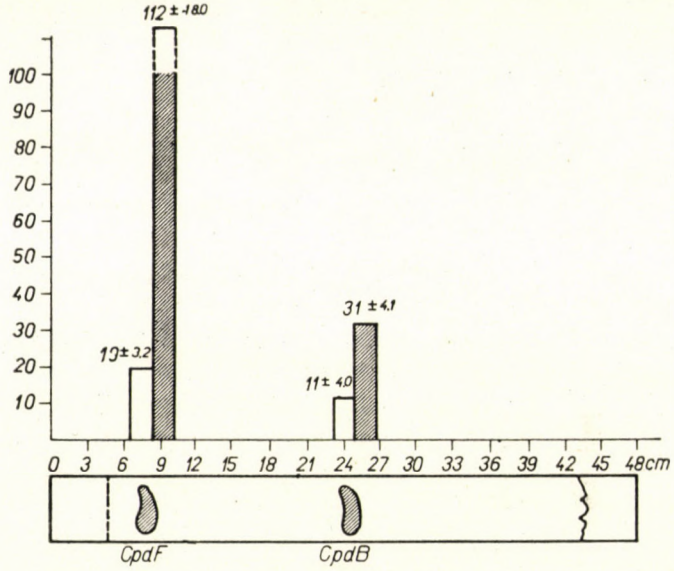

Fig. 12. Changes in the corticoid content of the venous adrenal blood in dogs treated with 250 μg/kg reserpine for six days. Shaded columns = reserpine (eight animals); blank columns = = control (eight animals)

edly activates the ACTH secretion of the anterior pituitary. Central excitants, as for instance benzedrine, also increase ACTH secretion, but this increase is by far not so great as that induced by the tranquillizing drugs. This difference in action is not merely quantitative, even convulsive doses of benzedrine did not enhance corticoid secretion, but very slightly (Bata and Endrőczi, [3]).

II. *Function of the adrenal cortex during behavioural changes induced by lesions of the central nervous system*

Next, let us consider the changes in adrenal secretion brought about by two types of surgical intervention in the central nervous system. In these cases a certain relation could be established between the changes evoked by the operation in the general behaviour, resp., in the secretion pattern of the adrenals. It is known that the removal of the amygdale nucleus and of the

temporal cortex is followed by permanent and characteristic changes in the behaviour (BARD and MOUNTCASTLE, [2]; SCHREINER and KLING, [48]; KAADA, [32]; KLÜVER and BUCY, [33]; and others). In earlier experiments on amygdalectomized cats (ENDRŐCZI, LISSÁK, TIGYI and SZÉP, [19]; MARTIN, ENDRŐCZI and BATA, [42]) an extreme increase in corticoid secretion was found, which 3 to 4 weeks following the operation still did not show a tendency to decline. The behavioural changes described by various authors under the above conditions are rather conflicting. However, recent data generally agree, and this has been confirmed also by our own experience (MARTIN, ENDRŐCZI and BATA, [42]) that the removal of the amygdale nucleus and of the periamygdaloid cortex leads to a quiet, so-called placid behaviour, the animals fail to respond to emotional stimuli and their spontaneous activity is decreased. SCHREINER and KLING, [48] observed certain changes in sexual behaviour some weeks after the operation. Changes of this kind did not consistently appear in our experiments. At the second week following operation only two female cats exhibited an extreme sexual behaviour characterized by spontaneous lordosis, copulative activity and orgasm. The adrenal secretion of these animals showed a pattern qualitatively similar to that observed after treatment with oestrone. Compounds less polar than corticosterone (XVI, XVII) were, however, found only in the above two cats. All other animals with placid behaviour showed a marked increase in hydrocortisone and corticosterone secretion. The ratio of hydrocortisone : corticosterone was similarly elevated.

Another characteristic change in the adrenal secretion pattern was observed in female dogs after frontal leucotomy. The operation, which involved the lesion of the orbito-frontal pathways, led in certain animals to a very marked sexual reaction with copulative body posture and extreme priapism. In three animals in which these phenomena were most marked, the venous blood of the adrenals contained, in addition to the known hormones, two new compounds both of which were less polar than corticosterone. One of these compounds exhibited alkaline fluorescence but no tetrazolium reduction. On the basis of the R_f value, the compound was identified as 17-hydroxyprogesterone. The other compound showed similar chemical characteristics but was more polar than 17-hydroxyprogesterone. Its chemical structure has not yet been ascertained.

Dogs exhibiting a normal sexual behaviour after leucotomy, failed to show any characteristic change in the corticoid secretion, except a slight increase in the total amount secreted. The specific changes in the secretion pattern appearing in both the dog and the cat after surgical intervention in the central nervous system, were very probably not directly related to the altered ACTH secretion of the pituitary, but evoked by interglandular mechanisms (pituitary-gonad system).

VII. General discussion of the specific adaptation

The changes observed in the adrenal function under specific stress, point to the existence of a specific adaptation, in addition to the general one. Corticoids, which under normal conditions are not present, appear in the blood, as a sign of specific though not completely explored process.

As far as the significance of the method of biosynthesis is concerned, two points should be mentioned. (i) The method allows the synthesis of corticoids which are appearing in the blood only under specific conditions; and (ii) it yields information about the corticoid metabolizing capacity of the enzyme systems of the adrenals.

ad (i). The method of biosynthesis has made it possible to isolate 19 new corticoids with the Δ^4-3-keto, 17-ketol side chain. Three of the compounds I to VI had been unknown. Unfortunately, the report of DORFMAN *et al.* [22, 23] does not contain detailed data concerning the three compounds observed in their experiments.

As pointed out above, some of the compounds I to VI possess a marked biological activity and are highly lympholytic in character. It is still a question whether or not these compounds are secreted by the adrenal cortex, in view of the very low concentration at which some of these corticoids occur. Their biological activity is high, but amounts obtained during the usual time of blood collection (20 to 90 minutes) do not suffice for chemical identification. Similar methodical difficulties are met with when determining aldosterone in adrenal blood. Although a decisive part is ascribed to adrenocortical hormones in the pathomechanism of the various connective tissue diseases, yet we must confess that the mechanism of the therapeutical successes obtained by ACTH and cortisone is still quite obscure. The urinary excretion of hydrocortisone and cortisone in subjects with asthma or rheumatic arthritis is not at all unsatisfactory; it may sometimes even lie above the normal range. Further investigations will have to decide whether or not these compounds contribute to the antiinflammatory actions of the adrenal cortex.

Nearly all of the compounds obtained in the course of biosynthesis (corticoids less polar than cortisone) were found to occur in the venous blood of the adrenals, but they were not present in normal animals not subjected to previous adaptation. The question arises whether a discrimination of two distinct lines in adrenal function, *i.e.* a specific and a general one, is necessary, and whether this interpretation is at all correct.

(i) The corticoids appearing during specific adaptation do not occur in the venous adrenal blood during resting secretion.

(ii) Chronic administration of ACTH, while increasing the ratio hydrocortisone : cortisone, fails to give rise to the appearance of any new compound.

(iii) Stressor stimuli acting within the frames of the general adaptation syndrome produce effects similar to those observed after ACTH treatment.

(iv) Certain corticoids do not appear but under specific conditions.

(v) Corticoids appearing under specific adaptation strengthen the homeostatic unity of the organism and influence the interglandular connections in a specific way.

However, it is only to a certain extent that the above observations justify the existence of a specific adaptation. This makes a brief survey of some additional data necessary.

(i) Within the ranges of specific adaptation, species differences, too, are to be taken into account. This statement is based on the observation that oestrone treatment leads to 17-hydroxyprogesterone secretion in the dog, but to two new Δ^4-3-keto corticoids in the cat. Moreover, in addition to species differences, there are also strain differences. This was demonstrated by WESTBERG, BERN and BARNEWELL, [66], who on administering oestrogen to various strains of mice observed great differences in the function of the pituitary-adrenocortical system. The two above-mentioned compounds are under analysis as to their biological activity. Of them 17-hydroxyprogesterone is gestagen in action. This compound deserves some further interest, since its biological action is subject to species differences. Its action is weak in man and the rabbit, while in the mouse it is sixty times stronger than that of progesterone (SALHANICK, HOLMSTROM and ZARROW, [46]). As already mentioned, the other compound found in the cat after oestrone treatment is probably 11-hydroxyprogesterone, although its final identification is still lacking. All these observations demonstrate that oestrone treatment leads to the appearance of compounds, which have gestagen properties and are probably involved into the maintenance of homeostasis.

(ii) Progesterone treatment did not give rise to new corticoids. Yet, the elevation of the ratio hydrocortisone : corticosterone, as well as of the total secretion are worth mentioning, especially considering the general biological effects of increased hydrocortisone secretion. Numerous literary data revealed progesterone markedly to alter the excitability of the vegetative nervous system. In high doses it can even produce signs of narcosis (SELYE, [50]). Hydrocortisone, on the other hand, markedly enhances both the excitability of the central nervous system and the reactivity of vegetative processes (WOODBURY *et al.*, [68]). As to the interconnections of the adrenal cortex and the sexual glands, the fact cannot be left out of consideration that corticoids decrease the metrotrophic and decidual effects of the oestrogens and progesterone (SZEGO and ROBERTS, [58]; VELARDO, [61]). This antagonism is known also to figure in certain pathological states. Thus, hyperadrenalism or corticoid therapy with high doses may lead to amenorrhoea, even when the secretion of gonadotrophine and sexual corticoids is normal or elevated (SPRAGUE, MASON and POWER, [54]). At present, we have no definite data on this antagonism between sexual steroids and corticoids asserting itself also in other

extragonic structures and thus in the central nervous system. In any case, the antagonistic influence of hydrocortisone and oestrone on the maternal aggressivity of domesticated rats has been observed by us (ENDRŐCZI, LISSÁK and TELEGDY, [13, 14].)

(iii) Accordingly, the progesterone-induced increase in the ratio hydrocortisone : corticosterone may be considered one of the specific adaptative reactions. This is further supported by the fact that progesterone treatment alone does not lead to such metabolic alterations and thereby to increased demand for corticoids, which would explain this increased secretion. On the other hand, the specificity of the phenomenon is demonstrated by the relation between the sexual behaviour following central nervous lesions and the corticoid pattern after progesterone treatment.

(iv) In thyroxine-induced hyperthyroidism there appeared in the venous blood of the adrenals compounds inhibiting the pituitary secretion of thyreotrophic hormone. These compounds were also found in the urine of subjects with hyperthyroidism. A further characteristic of this pathological state is a decrease in the secretion of hydrocortisone and, in general, of more polar corticoids. This can be accounted for mainly by the above-discussed changes in the enzyme system of the adrenal cortex. In hyperthyroidism there is a relative insufficiency of the adrenal cortex, the manifestations of which are observable not only in the condition of certain tissues, but also in various metabolic processes (ALTERMAN, [1]). On the other hand, hypothyroidism results in a considerable increase in the secretion of corticoids, mainly of the polar ones. The secretion of aldosterone has not been investigated in our experiments, but it would be of interest to know, if similar changes occurred also in the synthesis of this mineralo-corticoid, especially with respect to the water household in hypothyroidism. The increase in corticoid secretion may constitute an adaptation mainly in the sense that it activates suppressed metabolic processes (glycogenolysis, gluconeogenesis, tonization of the sympathetic nervous system, etc.).

(v) The enhancement of hydrocortisone and cortisone secretion by tranquillizing drugs can be regarded primarily as a compensatory process in the sense that the inhibition of central activating processes initiate an increased production of those hormones by which the excitability of the central nervous system is considerably augmented. WOODBURRY *et al.* [68] found namely hydrocortisone and cortisone markedly to increase, and corticoids of low polarity to decrease, the excitability of th e central nervous system. Without going into the details of this question, it is necessary to draw attention to the fact that highly polar corticoids greatly enhance the manifestations of certain behavioural changes in complex emotional and neurotic states (ENDRŐCZI, TELEGDY and BATA, [14]; LISSÁK, MEDGYESI, TÉNYI and ZÖRÉNYI, [35]; LISSÁK, ENDRŐCZI and MEDGYESI, [37]). In our opinion, the in-

creased corticoid output induced by tranquillizing drugs represents a compensatory mechanism with the role to maintain homeostasis. A similar phenomenon is the placid state observable after removal of the amygdalous complex, a state for which naturally the altered equilibrium of the organizatory mechanisms of complex neural processes is responsible.

It is a question of decisive importance if the corticoids yielded by biosynthesis were involved into some pathological processes of man. Adrenal homogenates synthesize from hydrocortisone, cortisone and progesterone various corticoids, which under certain conditions were found in the venous adrenal blood of different animals. The question arises why these compounds do not appear in the resting secretion and whether they are produced also in the course of physiological corticoid synthesis. Since even high doses of ACTH failed to give rise to these compounds, and they were not found to appear during chronically increased corticoid secretion, it seems probable that, in certain species, these derivates are not synthesized. In some species, their synthesis is initiated exclusively by specific interglandular endocrine effects, while in others they constitute a constant component of the corticoid pattern. All these findings lead to the conclusion that the method of biosynthesis is capable of yielding corticoids which otherwise occur only under specific conditions or in certain species. In this respect we have found no essential difference between ox and pig adrenals. This does not yet exclude the possibility that other species are also capable of producing these derivates.

The term specific adaptation, as used in the present report, requires some explanation, since a general adaptation syndrome related to the function of the adrenal cortex has been introduced (SELYE, [50], [51]). The two concepts cannot be sharply separated, since the term specific homeostatic reaction must be applied also to that case, when the exclusive amount of those corticoids is augmented which occur in the resting secretion. The differences between these two types of adaptation can be defined as follows.

a) In general adaptation the secretory pattern of the adrenals changes only quantitatively. For this change the pituitary ACTH secretion is solely responsible.

b) Specific adaptation depends mainly on interglandular connections. It is brought about by stimuli from the environment, which affect primarily other endocrine systems.

c) During specific adaptation the enzyme system of the adrenals undergoes a change, the quality of which depends on the species.

d) Under certain conditions (hypothyroidism, administration of tranquillizing drugs, etc.), general adaptation may take a specific character, without any qualitative change in the secretory pattern.

For the time being, only the outlines of the specific adaptation have been explored. In our opinion, the syndrome plays an important role in the

pathomechanism of certain systematic diseases, as well as in the maintenance of interglandular regulation. The above observations have made it possible to analyze some aspects of this great complex. The complete elucidation of the problems involved will require further extensive investigations.

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CORTICOSTEROID SECRETION OF THE ACCESSORY ADRENALS

By

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Corticosterone determinations have been carried out in the venous blood extracted from the adrenals of adrenalectomized rats, postoperatively, at different intervals. When gaining venous blood from the adrenals, we intended to collect the blood of the accessory adrenocortical tissue located in the periadrenal adipose tissue. According to our results, already in the early stage following adrenalectomy, a certain amount of corticosterone secretion was traceable which increased further on. Thus we cannot speak about hormone outfall, rather about hypofunction.

The function of the accessory adrenals was the cause of many a problem in the studies on the adrenal cortex, especially in experiments with adrenalectomy. It has been known since long that rats, especially mature ones, often survive bilateral adrenalectomy, obviously owing to the compensatory hypertrophy of an extra-adrenal, so-called accessory adrenal tissue and to the hormonal substitution brought about by the function of the same. The problem has been investigated by numerous authors, but unfortunately only with morphological methods [1,2]. By these investigations the presence of an accessory adrenal tissue located in its major part in the periadrenal and perirenal adipose tissue and in the retroperitoneum has been demonstrated histologically. However, no functional analysis of the problem has been carried out, though when evaluating experiments with adrenalectomy, it is important to know the time, the amount and level of hormone secretion of the accessory adrenals.

We started to study the problem assuming that when collecting venous blood from the adrenals (after placing a ligature on the renal hilus and at the emptying into the inferior vena cava, respectively, and after cannulation of the renal vein), the venous blood of the accessory adrenals located periadrenally and near the retroperitoneum should also be collected.

The experiments were performed on mature male rats of the Wistar-K strain weighing between 200 g and 300 g each. Adrenalectomy was effected through lumbal exploration, with great caution and taking care that the surrounding tissue should suffer the least possible damage. Postoperatively, until termination of the experiment, the animals were offered physiological saline to drink *ad libitum*.

To examine corticosteroid excretion, the renal vein was cannulated in a manner similar to that when obtaining venous blood from the adrenal. The

blood was collected through 60 minutes under urethane anaesthesia. The plasma to which heparin had been added, was extracted with ethyl acetate [3] and after purification was chromatographed, in toluol /methanol/ water (4:3:1) [3]. The chromatograms were developed in tetrazolium blue. The formazon spots were eluated and estimated by photometry as to their content of corticosterone, the main corticosteroid secreted by rats.

In addition to the tetrazolium blue reaction, in some cases the soda-fluorescence test was also examined, which always agreed with the results of the tetrazolium blue reaction.

Table I shows the result of corticosterone analyses performed at different points of time.

Table I

Amount of corticosterone secreted by the accessory adrenals of bilaterally adrenalectomized rats

	Number of animals	Secreted corticosterone μg/hour/kg body weight
Controls	15	16.7 ± 0.11*
2 days following adrenalectomy	8	3.9 ± 0.34
5—7 days following adrenalectomy	15	4.7 ± 0.67
10 days following adrenalectomy	10	6.3 ± 0.14
19—20 days following adrenalectomy	6	7.8 ± 0.88
25—46 days following adrenalectomy	9	8.7 ± 0.14

* Standard deviation

Note. About 75% of the animals died in 10 days after operation

In 5 further animals the so-called zero value was also determined in the following way. Before beginning to collect blood, the small venule coming from the adrenal was ligated (this ligature plausibly failing to influence the venous supply of the periadrenal adipose tissue) and the adrenal was removed. The blood thus obtained contained no measurable amount of adrenal hormone by the tetrazolium blue method.

As early as 2 days after bilateral adrenalectomy, a certain amount of secreted hormone was demonstrable. After 25—40 days this amount increased to about 50 per cent of the control value. That this value did not reach the amount of hormone secreted by the controls, may be explained by the fact that a part of the blood of the accessory adrenals could not be collected due to the anatomical conditions (part of the accessory adrenals were located in the renal substance).

According to our results, soon after adrenalectomy the hormone secretion of the accessory adrenals must be taken into consideration. This means

that numerous experiments carried out in adrenalectomized rats had elucidated the consequences of hypofunction rather than those of total hormone outfall. ZENKER and BERNSTEIN [4] have reported on the method of fluorometrically determining the corticosterone hormone content of rat blood. To control their method, they carried out determinations from the peripheral blood at various intervals after adrenalectomy and the comparison of the values they obtained at different points of time seems to support our own conclusions.

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CHANGES IN THE CARBOHYDRATE METABOLISM OF THE RAT BRAIN IN RESPONSE TO WORK

By

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The glycogen, glucose, lactic acid and pyruvic acid content of the brain has been examined in 79 rats after 3 hours of swimming.

The glycogen level was significantly increased; the other levels did not change significantly.

It has been concluded that the generally accepted view concerning the stability of glycogen should be revised. At least under certain conditions, there is a parallelism between the functional exposure of the central nervous system and the changes in the glycogen content of the brain.

In 1924, TAKAHASI [1] found measurable amounts of glycogen in the brain of experimental animals and postulated that the central nervous system had an autonomic carbohydrate metabolism. Since then, many authors have investigated under highly variable conditions the changes in the glycogen [2—18], glucose [9, 19], lactic acid [9, 19—24] and pyruvic acid [26] content of the nervous system. The results, however, are far from being unequivocal, even though the experimental conditions employed were closely comparable. This applies first of all to the changes in the glycogen level. Very few workers have investigated the above-mentioned metabolites of the carbohydrate within the framework of one study. Moreover, the metabolism conditions employed by many were often far from what may occur physiologically.

In the present investigations we have studied the changes in the glycogen, glucose, lactic acid and pyruvic acid levels of the brain in response to work under identical experimental conditions, in order to decide whether there existed any correlation between functional exposure and the quantitative changes in these metabolites.

Materials and methods

A total of 79 infantile albino rats of both sexes was used in the experiments.

The animals came from the same stock and were collected from big litters. They were fed a mixed diet. Care was taken to keep all the factors of some influence (time of feeding, size of cage, litter, etc.) as constant as possible.

Swimming was chosen as the functional exposure, an activity which may occur in the normal life of the rat. The animals were made to swim without loading 3 hours in a vessel 40 cm in diameter, containing water of 37 to 38° C to a height of 40 cm. While the experimental animals were swimming, the controls were kept in a dark incubator at 37° C, where they were usually sleeping.

The animals were killed by immersing them into liquid air for two minutes. This was performed with the least delay possible, in order to reduce excitation to a minimum. The animals showing marked convulsions during freezing were excluded from the study. (Convulsions do not alter the results unequivocally. They cause either a positive or a negative maximum deviation from the group mean, or correspond to the group mean.)

The brain was weighed in a dish enclosed in a wooden box, by analytical scales. The dish was later remeasured because of the moisture that had frozen to the brain and melted later. Subsequently, it was placed into the tube of the POTTER—ELVEHJEM homogenator, containing about 5 ml of ethanolic potassium hydroxide as described by KERR for the estimation of glycogen [9], and was homogenized for 3 minutes. The plunger was washed with a sufficient volume of ethanolic potassium hydroxide and the volume was adjusted to 10 ml. This was followed by boiling in a water bath, with shaking, until the brain was dissolved. After cooling, it was centrifuged, decanted and washed three times in warm methanolic chloroform, according to KERR [9]. After expelling the traces of solvent, the dried residue was dissolved in 10 ml of luke-warm distilled water and glycogen was estimated colorimetrically by the MOLISH test modified by SZÁRA and BAGDY (personal communication).

The modification was the following. To 1 ml of the glycogen dissolved in water were added a layer of 8 ml of 86 per cent sulphuric acid, 0.5 ml of water and 0.5 ml of a 10 per cent aqueous solution of 1 — alpha 2-naphtolsulphonic acid. After 20 minutes in a water bath of 100° C and cooling, the violet colour was read in a PULFRICH photometer with an S_{57} filter. The calibration curve was plotted with MERCK's glycogen.

The procedure for glucose estimation was the following. To block glycolysis, an about 0.1 *N* solution of barium hydroxide was prepared with 0.1 *M* NaF. With phenolphtalein as an indicator, the $Ba(OH)_2$ was titrated with 2 per cent $ZnSO_4$, which was so diluted that exactly the same volumes of the two solutions be equivalent. The weighed brain was dropped into 5 ml of the $Ba(OH)_2$ solution, and was homogenized for 4 minutes. To the homogenate 5 ml of the $ZnSO_4$ solution was added. After centrifuging, 2 ml aliquots were tested for sugar, according to SOMOGYI [27].

To determine lactic and pyruvic acids, the brain was for 4 minutes homogenized in 5 ml of recently prepared 10 per cent trichloracetic acid and the volume was adjusted to 10 ml. One ml aliquots were tested for lactic acid, according to BARKER and SUMMERSON [28], and 3 ml aliquots for pyruvic acid, according to FRIEDEMANN and HAUGEN [29]. The calibration curves were plotted with Zn—lactate and recently distilled pyruvic acid, respectively.

Results

The values (expressed in mg/100 g of wet brain weight) for glycogen are shown in Table I, those for glucose in Table II, for lactic acid in Table III and for pyruvic acid in Table IV. The glycogen content was significantly higher in the "swimmers" group ($P \ll 0.01$), whereas the levels of glucose ($P > 0.8$), lactic acid ($P > 0.6$) and pyruvic acid ($P > 0.7$) did not change.

Table I

Brain glycogen values

	Control group	Experimental group
Number of experiments	13	11
Mean (mg/100 g wet tissue)	53.4 ± 11.6	75.4 ± 12.5
$P \ll 0.01$		

Table II

Brain glucose values

	Control group	Experimental group
Number of experiments	20	20
Mean (mg/100 g wet tissue)	20.8 ± 8.2	20.6 ± 9.9

$P > 0.8$

Table III

Brain lactic acid values

	Control group	Experimental group
Number of experiments	10	5
Mean (mg/100 g wet tissue)	14.7 ± 2.5	15.3 ± 1.6

$P > 0.6$

Table IV

Brain pyruvic acid values

	Control group	Experimental group
Number of experiments	10	5
Mean (mg/100 g wet tissue)	0.8 ± 1.1	0.8 ± 1.0

$P > 0.7$

Discussion

Most of the data in the pertaining literature refer to changes in the glycogen and lactic acid content of the brain.

An increase in the glycogen level in the brain has been observed in response to convulsant stimuli [5], after the righting reaction during climbing, falling, after active fear reaction [6], resuscitation from lethal acute bleeding [7], and in response to hyperglycaemia of various origin [8].

The glycogen level was found to decrease after prolonged swimming [13, 14], in the cerebral cortex during clinical death caused by acute bleeding [7].

The glycogen content of the nervous system did not change after glucose infusion, glucose infusion + insulin, pancreatectomy, overfeeding,

fasting + phloridzin injection [9], convulsions caused by picrotoxin metrazole, strichnine (18), during sleep, running, walking, in passive fear reaction [6].

Among the works on the behaviour of the brain glycogen level, that by CHESLER and HIMWICH [33] merits special attention. These authors studied the changes in the cerebral glycogen content not in response to external effects, but during individual evolution, in the different parts of the brain. They found that in the philogenetically newer parts of the brain the glycogen content increased with age, whereas in the more ancient parts it decreased gradually. The maximal oxygen consumption increased and decreased, respectively. with the glycogen content [34].

The changes in the lactic acid content of the brain in various conditions have been widely investigated, with conforming results. According to STONE [34], the cerebral lactic acid level appears to depend first of all on intracerebral metabolism. This was indicated by the fact that the lactic acid content of the brain was found to increase in the following order: sleep, rest, training, emotional excitation, convulsions. Low levels were found in the brain of animals treated with barbiturate or ether [23]. Higher values were found at rest [25, 35] and in emotional states [23].

PALLADIN's investigations are particularly interesting [24]. He found, among others, that parallel with the decrease in the lactic acid level, the level of glycogen increases during sleep, whereas the opposite takes place in excitation produced by benzedrine and convulsants.

Our own results concerning unchanged lactic acid values agree with those reported by other authors as occurring in similar conditions.

The results for the glucose level do not permit further conclusions because in addition to sugars the method employed determines also other reducing agents. As far as the elevated glycogen level is concerned, our findings agree with those published mainly in the past few years [6, 13, 14], observing changes in the glycogen content of the brain in response to various circumstances. The studies by CHESLER and HIMWICH indicate that a parallelism may exist between the changes in the activity of the brain and the glycogen content. All these results, including ours, are in contradiction with the view concerning the behaviour of glycogen that is still universally accepted. This has been based first of all on the results of KERR *et al.* [9] worded in 1938 by PAGE in that the glycogen content was stable irrespective of the variants in the physiological processes of the organism.

Thus, this view does not take into consideration the correlation between the glycogen content of the brain and the functional exposure of the nervous system.

We are certainly not justified to consider the brain glycogen immobile. Even KERR *et al.* did not go further than to claim that they had been unable

to demonstrate a storage of excess glycogen, whereas in hypoglycaemia they found a decrease in the glycogen content of the brain.

KLEIN and OLSEN [31] pointed out that KERR *et al.* removed the brain of the experimental animal under amytal anaesthesia. Anaesthesia lowers not only the glucose and lactate content, but also the glycogen level of the brain. This may therefore lessen or obscure a rise which might have resulted from the experimental treatment.

It should be borne in mind, too, that the experimental animals used by KERR had not originated from the same stock. KULENKAMPFF has emphasized the importance of the same litter [31].

We should now examine the changes in the blood sugar level in response to muscle activity. This is necessary, because KLEIN, HURWITZ and OLSEN [36], CHANCE and WALKER [8], reported that the significant elevation of the blood sugar level was always associated with a significant rise in the glycogen content of the brain.

According to the data in the literature, the changes in the blood sugar level in response to muscle work depend on the following factors.

(i) The condition of the nervous system. CANNON [37] has emphasized the importance of psychic excitation during exercise, but even at rest. Excitement causes a rise in the blood sugar level.

(ii) The measure of exposure. Moderate muscle activity does not alter the blood sugar level [39]. Total exhaustion results in a fall [40].

(iii) Training. [41]. The blood sugar of the untrained animal decreases after exercise, that of the trained animal remains unchanged.

In our experiments, swimming for 3 hours did not mean too much of a strain, the albino rat being able to swim 6 to 10 hours. On the basis of the literary data, the blood sugar level should rather have decreased in our animals, they having been untrained. In fact, VRBA [14] found a decrease after 4 hours of swimming.

Excitation would have an opposite effect. Our animals, which were unaccustomed to water, were in all likelihood excited, but excitement was probably over in about 3 hours. Excitation increases namely the lactic acid level of the brain [31].

As our investigations revealed no significant change in the lactic acid level of the brain after 3 hours of swimming, the cause of the elevation of the brain glycogen level should be sought for elsewhere, in some other mechanism.

Coming back to the investigations in which functional exposure involved swimming or running, *i. e.* a complex biological stimulation as under our own experimental conditions, the following may be mentioned.

TAKAHASI [1] made rats run for 1½ hours, but gave them also thyroid tablets and phloridzin. For this reason his results cannot be compared with ours.

CHANCE [6] made mice to run at a speed of 8.8 yards/min for 13 minutes, during which the animals covered a total of 120 yards. He found no change in the glycogen content of the brain.

LESSKEVITCH [13] found a significant decrease in the brain glycogen level after making rats swim for 1½ to 2 hours.

VRBA [14] forced one group of rats to swim for 2½ hours and another for 4 hours. After 2½ hours of swimming the glycogen level decreased significantly in the brain. The animals which had swum 4 hours showed a further but not significantly greater decrease.

Thus, the three last named authors have not obtained the same results as we did. The cause of the difference is not clear and requires further elucidation. *Nevertheless*, provided the technique employed is reliable, it occurs as if the unchanged, increased and decreased brain glycogen levels were single steps in the response to physical work and should one subject animals of the same species, strain, grade of nutrition and condition to kinetic experiments, their brain glycogen values would go through the above sequence of changes.

This view is in harmony with the opinion of GERALD to whom the law of "all or nothing" does not seem to apply to central nervous metabolism. It appears that between the metabolic states characteristic of rest, hyperactivity and total exhaustion there may be a whole series of intermediate grades.

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EFFECT OF HYPOTHERMIA ON THE LIBERATION OF HISTAMINE

By

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It has been demonstrated by Jancsó's test that the storage of dyes diminished parallel to the decrease of body temperature, an effect which may be accounted for by a diminution in the rate of histamine liberation. Large doses of cortisone, and especially of chlorpromazine, also inhibited dye storage, but these effects were not related to the blocking of histamine liberation.

At the 1954 meeting of the Hungarian Physiological Society we reported that hypothermia was able to counteract death due to active anaphylactic shock [1]. Our further experiments [2] revealed that hypothermia prevented the anaphylactic syndrome also in guinea pigs sensitized passively with the usual dose of ovalbumine. However, animals sensitized more vigorously still died, even at 23°—25° body temperature. It was also stated [3] that the sensitivity to histamine did not substantially decrease at low temperatures. Thus, the protective effect could not be accounted for by an eventual diminuation of the sensitivity to histamine. We had, therefore, to conclude that the main factor in the protective effect of hypothermia was a marked blockade of histamine liberation. The present experiments were carried out in order to prove this assumption. It was also investigated whether previous administration of chlorpromazine or cortisone had any influence on the liberation of histamine.

Methods

The back and side of rabbits weighing 2 to 2.5 kg were shaved and, after three days, a skin area 3 cm in diameter was rubbed under slight pressure with 4 per cent formic acid for 20 seconds. (Jancsó's test, [4]). Some minutes later one group of the rabbits was given intravenously 5 ml one per cent trypan blue dissolved in physiological saline. The other group was treated with 3 ml Indian ink intravenously (10 per cent phenol-free Indian ink dissolved in physiological saline containing 1 per cent gelatine). The results were evaluated one hour after the injection and were characterized as follows.

— no colouring
\+ pale blue colouring
\++ dark blue colouring with small pale spots
\+++ uniform dark blue colouring.

The skin areas of animals treated with Indian ink were examined histologically after embedding in paraffine and staining according to Gollego.

Treatment with cortisone *(Adreson, sec. Organon)* was performed as follows. 20 mg cortisone were injected intramuscularly for four days, and, the fifth day, three hours before the experiment, further 40 mg were introduced intramuscularly. Chlorpromazine *(Largactil sec. Specia)* was administered in doses of 30 mg/kg for two days. Lowering of the body temperature was made by ice packs without previous drug treatment. Rectal temperature was measured with a thermocouple.

Results

Table I shows the results of the experiments made with the "indicator" trypan blue. As seen, storage of the dye decreased parallel to the reduction of body temperature. Storage was less marked at 26°—28° C than under normal conditions, and it ceased completely between 22° and 24° C. No colouring was observed in these animals after several days.

Table I

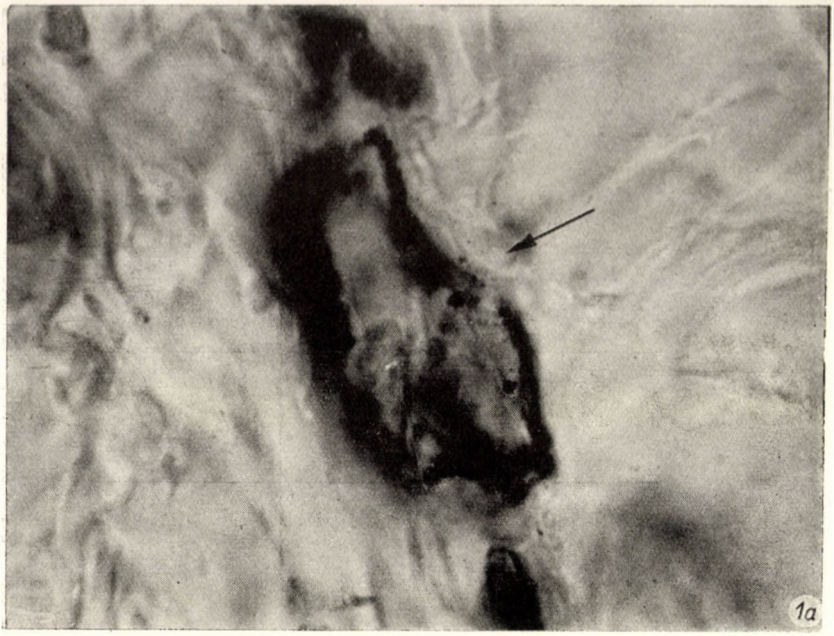
Storage of trypan blue at different body temperature

Number of rabbits	Rectal temperature °C	Intensity of the reaction*
1	38	+++
2	37.5	+++
3	37	+++
4	37.5	+++
5	37.5	+++
6	37	+++
7	28	+++
8	26.2	++
9	26.5	++
10	26	++
11	27.5	++
12	25	+
13	22.5	—
14	23	—
15	23	—
16	22	—
17	22	—
18	22.5	—
19	22.5	—
20	23	—

* For explanation, see Methods

Next, three rabbits were cooled to 23° C and the skin at the back was rubbed — instead of formic acid — with a two per cent solution of histamine. After fifteen minutes, a ++ grade reaction was observed in two animals and + grade in one animal. After the rabbits had regained their normal body temperature (in about four hours) all the reactions were intensified to the +++ grade.

Fig. 1 shows the histological picture of the skin area rubbed with formic acid. The storage of Indian ink in the endothelial cells of the capillaries



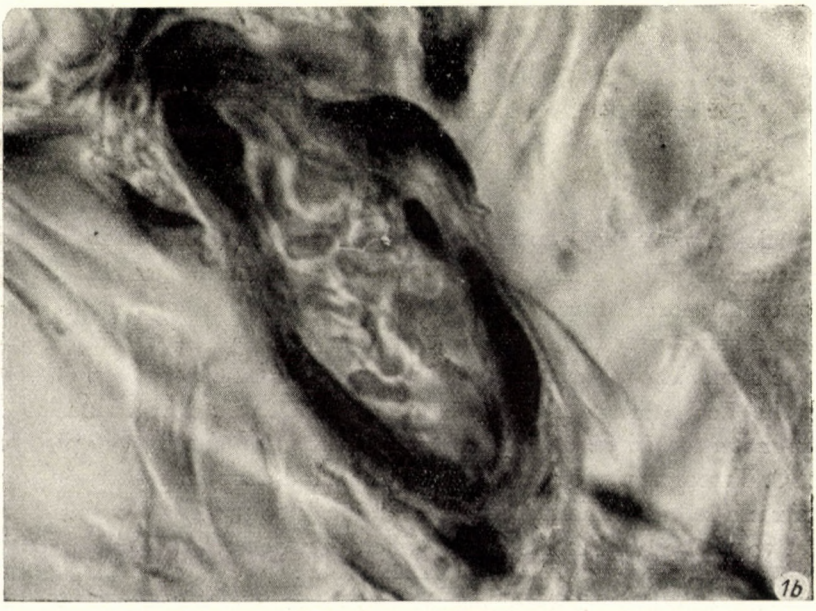


Fig. 1a. Normothermic rabbit (37.8° C). Storage of Indian ink in the endothelial cells of the capillaries. *1b.* Hypothermic rabbit (23°C). No storage in the endothelial cells of the capillaries. × 1600

was considerable in normothermic animals, but no Indian ink was found in animals whose body temperature had been lowered to 23° C.

The results of our experiments with chlorpromazine resp. cortisone treatment are summarized in Table II. Chlorpromazine was found to inhibit storage of the dye to a greater extent than cortisone.

Table II

Effect of previous chlorpromazine or cortisone treatment on the storage of trypan blue

Number of animals	Pretreatment	Intensity of the reaction*
21	Cortisone	++
22		++
23		+
24	Largactil	—
25		+
26		++
27		+
28		—
29		+
30		—
31		+
32		—

* For explanation, see Methods

Discussion

In a previous paper [3] hypothermia was shown to be without any measurable effect either on the action of histamine on blood pressure and bronchial musculature, or on the toxicity of the drug. Thus, an eventually altered sensitivity to histamine could not account for the absence of anaphylactic signs. The present experiments revealed that hypothermia did not diminish the accelerating effect of histamine on dye storage. In 1939, SCHILD [5] demonstrated *in vitro* that histamine liberation nearly completely ceased at 17° C. VUKOBRATOVIČ and BATA [6] reported deep hypothermia to prevent the dextrane-induced liberation of histamine. Our present findings lend new support to the view that histamine liberation decreases parallel to the decrease of body temperature, and that liberation at 24° C is already negligible.

It is generally accepted that the storage and liberation of histamine occurs mainly in the mast cells [7]. JUNQUEIRA and BEIGUELMAN [8] reported that at a low body temperature even the potent histamine liberator 48/80 was

not capable of liberating histamine from the mast cells of rats. HÖGBERG and UVNÄS [9] found that the degranulation of mast cells induced by 48/80 considerably diminished under 20° C. As far as cortisone is concerned, LUDÁNY *et al.* found that pharmacological doses of the hormone (20 mg) significantly inhibited phagocytosis. Although our findings seemingly confirm the latter observation, in our opinion cortisone has no role whatever in the hypothermia-induced inhibition of dye storage, since moderate, physiological doses of ACTH [11] or cortisone [12] were found to increase rather than to decrease the activity of phagocytes.

Recently, we have published experiments indicating that the protective action of chlorpromazine against anaphylactic shock was due mainly to the antihistaminic properties of the drug [13]. In the present experiments, however, even large doses of chlorpromazine were not always capable of counteracting the activating effect of histamine on storage. DALE [14] supposes the existence of an "intrinsic" and of an "extrinsic" histamine, the former acting on the same cell from which it had been liberated, while the latter exerts its effect at some other site. According to ALBERTY and TAKKUNEN [15], the histamine liberated in experiments of the above type would be the "intrinsic" form, while PATON [16], mainly on grounds of the important role of the mast cells, considers this histamine to be extrinsic. However, PATON, too, accepts the view that the mast cells are in close relation with the effector organ. In our opinion, the results obtained with chlorpromazine should be interpreted in the sense of DALE's view [14], namely, that antihistamines are not as effective against intrinsic histamine as against the extrinsic one.

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ACTION OF INORGANIC IONS ON THE EFFECT OF EPINEPHRINE

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The adrenolytic effect of cadmium, manganous, stannous, nickelous and vanadium ions has been investigated. Of these ions, cadmium proved to be the most effective, inhibiting the epinephrine action on the frog heart, the Trendelenburg frog, the blood pressure and spleen volume of the dog. It has been shown that the inhibitory effect was not due to a blockage of sulphydryl groups, but the decisive role is ascribed to changes in cell permeability.

In previous experiments [1, 2, 3] ferrous ions were found to abolish or markedly to diminish the effect of epinephrine on smooth muscle. We observed a similar antagonism between cobaltous ion and epinephrine, which was, however, by far not so general as with ferrous ions [4]. Ando *et al.* [5] concluded from a study on 18 cations that manganous, cadmium, ferrous, cobaltous, nickelous and vanadium ions all had an epinephrine-antagonistic effect. Since in our earlier experiments some of these ions had given results contrasting to those obtained by the Japanese authors, a more detailed analysis was now made of the influence of manganous, stannous, nickelous, cadmium and vanadium ions on the reactions of epinephrine and, in some cases, on those of acetylcholine and histamine.

Materials and methods

Isolated frog hearts were used for studying the effect on heart function, and Trendelenburg frogs for studying the effect on blood vessels. The frog experiments were made between February and May, and between October and December. Excised rabbit intestine was prepared according to Magnus and suspended in 20 ml Tyrode solution. Volume changes of the spleen were measured by oncometry, and blood pressure in the carotid artery by means of a mercury manometer, in dogs under chloralose (0.11 g/kg) anaesthesia. The ions under investigation were infused into one saphenous vein, and the drugs were injected into the other.

The following salts were used: $SnCl_2 \cdot 2\,H_2O$; $MnCl_2 \cdot 4H_2O$; $NiSO_4 \cdot 7\,H_2O$; and $CdCl_2$ *(Merck)*. The vanadium compound was prepared by dissolving V_2O_5 in nitric acid and adjusting the solution to pH 7.0 with sodium hydroxide. Under these conditions, vanadium was almost exclusively present as vanadate.

Results and discussion

Isolated frog heart. 0.05 μg cadmium was found markedly to diminish the effect of 0.1 μg epinephrine (Fig. 1). This dose of cadmium had no effect upon the heart function, while 10—30 μg caused standstill. As Fig. 2 shows,

100 μg nickelous considerably depressed the heart function. After the nickelous infusion 0.05 μg epinephrine was far less effective than in the control experiments. Nickelous exerted an adrenolytic effect only in doses high enough to depress heart function. On the other hand, even high doses of manganous, stannous and vanadium ions had no epinephrine-antagonizing action.

The effect of acetylcholine on heart function was not influenced by any of the five ions investigated. In this respect identical results were obtained on both spring or autumn frogs.

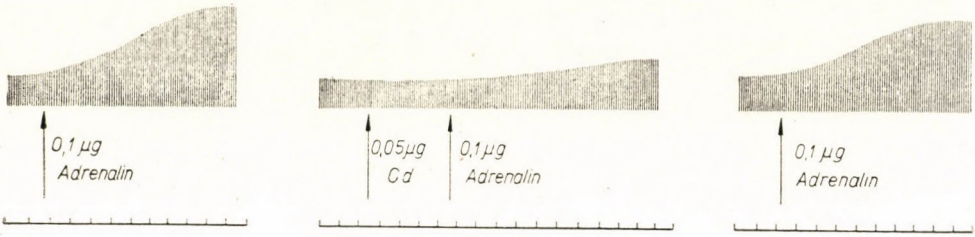

Fig. 1. Hypodynamic frog heart. Time signal, 6 secs

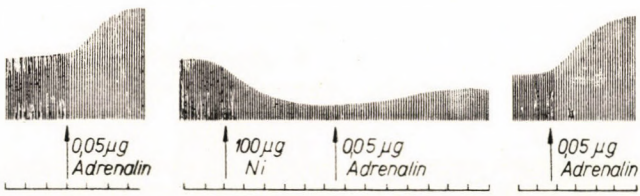

Fig. 2. Hypodynamic frog heart. Time signal, 6 secs

Trendelenburg frog. As seen in Fig. 3, 400 to 600 μg cadmium diminished, and 800 μg cadmium abolished the effect of 30 μg epinephrine. Perfusion with 200 μg/kg/min cadmium antagonized the vasoconstrictory effect of epinephrine. As to the other ions, 800 μg vanadate (Fig. 4) and 1000 μg nickelous or manganous ions were capable of abolishing the effect of 30 μg epinephrine, while stannous ions had no measurable adrenolytic effect, even when given in high doses.

Isolated rabbit intestine. Only stannous ions were weakly antagonistic to epinephrine, and not even in every experiment. All the ions investigated failed to influence effect in this test.

Blood pressure and changes of the spleen volume. Fig. 5 shows that, in the dog, infusion of 300 μg/kg/min manganous ions nearly abolished the effect of 20 μg epinephrine, 10 μg acetylcholine or 10 μg histamine. 90 minutes after the infusion 100 μg acetylcholine was still hardly effective. An almost similar inhibitory action was evoked by the infusion of 200 μg/kg/min manganous.

Fig. 6 demonstrates the effects of 20 μg epinephrine and of 10 μg acetylcholine. These doses were nearly ineffective during the infusion of 350 μg/kg/

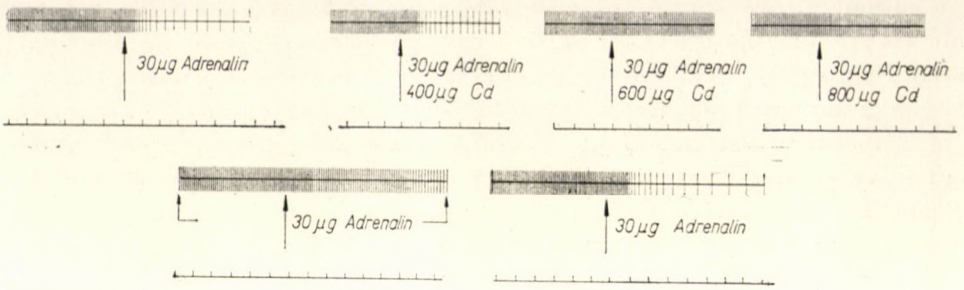

Fig. 3. Trendelenburg frog. Arrows indicate the administration of the drug. Between the ↑ ↑ arrows, perfusion of 200 µg/ml cadmium. Time signal, 6 secs

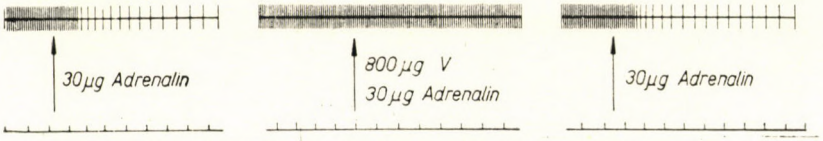

Fig. 4. Trendelenburg frog. Time signal, 6 secs

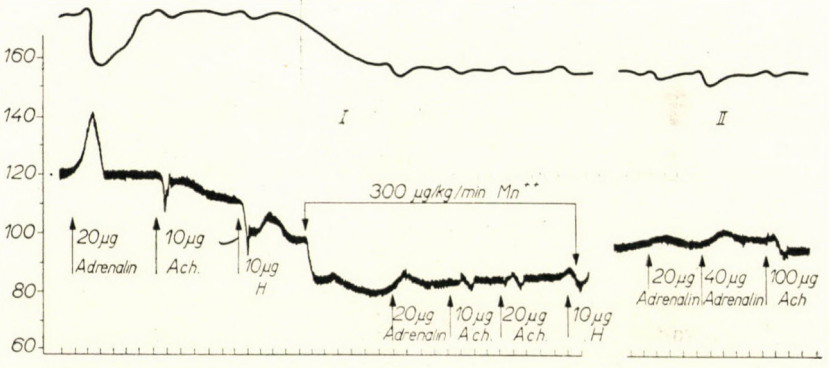

Fig. 5. Male dog weighing 12 kg. Upper line, spleen ; lower line, blood pressure. Time signal, 20 secs. Interval between I and II = 90 minutes

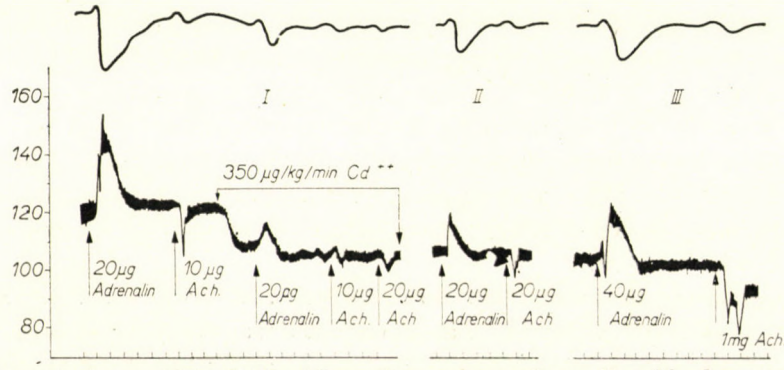

Fig. 6. Female dog weighing 12 kg. Upper line, spleen ; lower line, blood pressure. Time signal, 20 secs. Interval between I and II = 90 minutes ; between II and III = 30 minutes

min cadmium. 120 minutes after the infusion even 40 μg epinephrine was not able to produce the effect 20 μg had exerted before the infusion, and only about 1000 μg acetylcholine was as effective as had been 10 μg. The effect of 240 μg/kg/min cadmium was also marked, but transitory.

Infusion of 955 μg/kg/min nickelous made 20 μg epinephrine nearly ineffective (Fig. 7). This inhibition was invariably present after 30 minutes and began to disappear only 60 minutes after the infusion. 170 μg/kg/min nickelous too, had an adrenolytic effect which, however, did not persist for more than 10 minutes after the end of infusion.

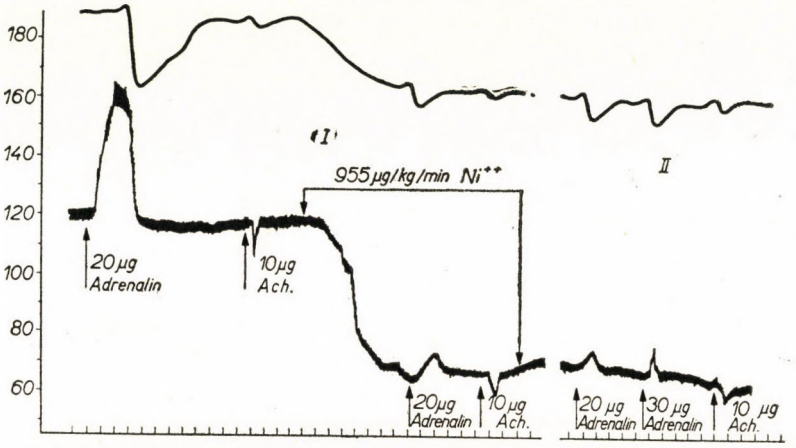

Fig. 7. Male dog weighing 15 kg. Upper line, spleen; lower line, blood pressure. Time signal, 20 secs. Interval between I and II = 30 minutes

Fig. 8 shows the influence of 160 μg/kg/min vanadium on the effect of 20 μg epinephrine, 10 μg acetylcholine and 20 μg histamine. During the infusion, histamine alone was effective. Acetylcholine sensitivity returned 120 minutes after the infusion, but epinephrine continued to act weaker than before the vanadium infusion.

During the infusion of 200 μg/kg/min stannous ions only the epinephrine action was weakened, until 30 minutes after the infusion.

As it can be seen from Fig. 5 to 8, the constricting effect of epinephrine on the spleen diminished considerably during the infusion of the inorganic ions. This inhibitory effect was especially marked and long-lasting with manganous: in this case epinephrine was still hardly effective 90 minutes after the infusion had ceased.

Summing up, the most marked adrenolytic action was exhibited by cadmium. Stannous ions evoked no change except a transitory weakening of the effect on blood pressure and spleen. As it can be seen from the Figures, blood pressure was markedly lowered by all the ions tested. This supports the view

that the paralysing effect on the musculature of the blood vessels is primarily responsible for the adrenolytic action of the ions. LAGNADO and SOURKES [6] observed that *in vivo* neither cadmium nor manganous ions influenced the amino-oxydase activity of the tissues, while *in vitro* both had an inhibitory effect. It is thus obvious that neither of these ions can enhance the inactivation of epinephrine. ANDO *et al.* [5] explained the adrenolytic effect of inorganic ions by the fact that, by virtue of their slight ionization, they react with the sulphydryl groups of the receptor proteins, which groups are supposed to play a

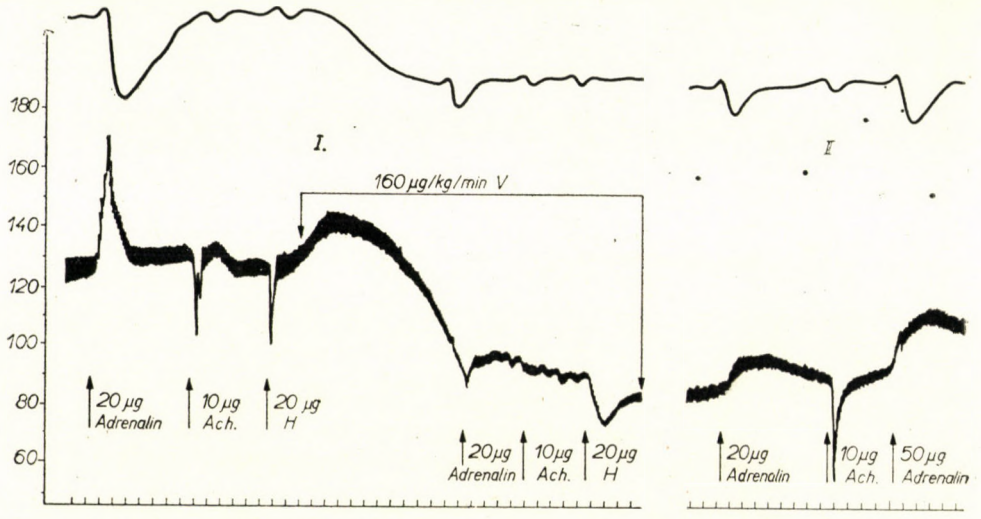

Fig. 8. Male dog weighing 14 kg. Upper line, spleen; lower line, blood pressure. Time signal, 20 secs. Interval between I and II = 120 minutes

decisive part in the epinephrine-induced vasoconstriction. This explanation seems to be most acceptable in the case of cadmium. In order to elucidate this point we made the following experiment. As shown in Fig. 9, the effect of 0.05 µg epinephrine on the isolated frog heart was completely abolished by 20 µg cadmium. This antagonism was not reversed by 1000 µg of cysteine. On the *Trendelenburg* frog 500 µg cadmium completely antagonized the effect of 30 µg epinephrine: here again, not even 2 mg cysteine altered the cadmium effect (Fig. 10). These findings show clearly that cysteine does not influence the antagonism between cadmium and epinephrine, a fact making obvious that [1] the adrenolytic action of cadmium is not due to its blocking the sulphydryl groups; and (2) sulphydryl groups do not play a decisive part in the manifestation of the epinephrine effect.

KOSHTOYANTS mentions in his monograph [7] that 1 µg acetylcholine injected into the isolated rabbit ear, which has only nervous connections, evokes a reflectory fall in blood pressure, which can be prevented by 1 mg (!)

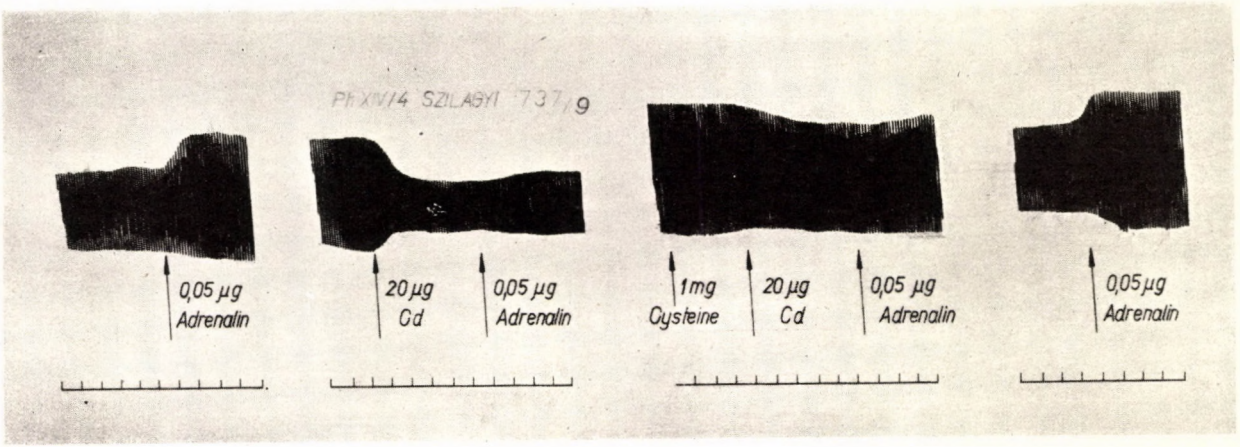

Fig. 9. Isolated frog heart. Time signal, 6 secs

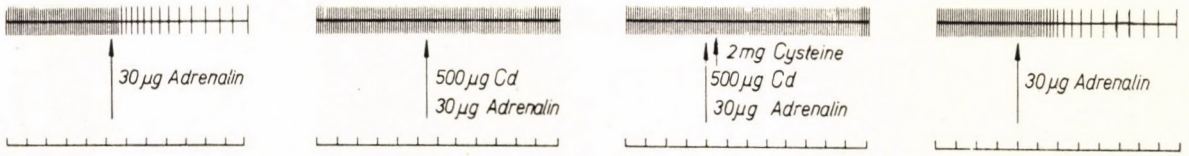

Fig. 10. Trendelenburg frog. Time signal, 6 secs

cadmium. This inhibitory effect is abolished by 100 μg cysteine. SMYRNOV, BYZOV and RAMPAN [8] stimulated the cervical sympathetic chain of the rabbit and registered the contractions of the nictitating membrane. They found that $2 \cdot 10^{-4}$ $CdCl_2$ introduced intraarterially was capable of blocking the transmission of the stimuli, meanwhile the effect of $5 \cdot 10^{-4}$ acetylcholine remained unchanged. According to DAMJANOVICH, HALÁSZ and MECHLER [9], the above dose of cadmium inhibited also the acetylcholine effect, while smaller doses blocked only the response to electrical stimulation. They also found that cadmium inhibited cholinesterase activity. According to our own findings, cadmium markedly diminishes the effect of acetylcholine on blood pressure. It must, however, be noted that in our experiments the low initial blood pressure made the evaluation of the results somewhat questionable.

A further assumption of ANDO *et al.* [5] is that the weakly ionizing cations attach themselves to the negative part of the receptor and so prevent epinephrine from attaining the receptor surface; they attribute less importance to the changes in the cell permeability. In our opinion, the latter phenomenon is the essential one, especially when the inorganic ions are administered in high doses, as in our experiments concerning blood pressure and spleen volume.

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We are indebted to the *Institute* of *Medical Chemistry* for supplying the vanadium.

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DIE WIRKUNG VON TRYPTOPHAN AUF DIE LEUKOZYTENZAHL

Von

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(Eingegangen am 16. Juni 1958)

Die Wirkung von Tryptophan auf die Leukozytenzahl und auf das qualitative Blutbild wurde an Ratten untersucht.

1. Tryptophanmangel verursacht Leukopenie, die nach parenteraler Verabreichung von 10 mg/100 g Tryptophan aufhört.

2. Bei normalen Tieren ruft 10 mg/100 g parenteral gegebenes Tryptophan in der 24. Stunde eine ausgeprägte Leukozytose hervor. Diese Erscheinung konnte durch andere 6 Aminosäuren nicht hervorgerufen werden, weshalb Verfasser der Ansicht sind, daß es sich wahrscheinlich um eine spezifische Wirkung des Tryptophans handelte.

3. Auf Wirkung von Tryptophan erscheinen in den ersten Stunden Lymphopenie und Granulozytose, die am ausgeprägtesten in der 2.—4. Stunde hervortreten. Diese Wirkung wird auch durch andere Aminosäuren hervorgerufen (Stressoreffekt).

4. Durch Nebennierenexstirpation konnte die infolge der Tryptophangabe auftretende frühe Veränderung des Blutbildes verhindert werden, doch die in der 24. Stunde sich manifestierende Leukozytose trat auch in diesem Fall unverändert auf.

Bekanntlich beeinflussen einige Aminosäuren die Leukozytenzahl und rufen auch im qualitativen Blutbild eine Änderung hervor. Juada [6] in 1936 beobachtet vorübergehende Leukopenie bei Kaninchen nach Verabreichung von Glykokoll, Alanin, Tyrosin, Cystin, Glutaminsäure oder Tryptophan. Harris und Lang [5] stellten fest, daß die über eine Dicarboxylgruppe verfügenden Aminosäuren Lymphopenie und Eosinopenie hervorrufen. Aschkenasy [1] beobachtete an Ratten, daß 14 Aminosäuren Eosinopenie auslösen und Bachromeff [2] Leukozytose bei Katzen nach Eiweißfütterung. Kurth [7] nahm wahr, daß bei Menschen nach Verabreichung von Eiweiß Eosinopenie und Lymphopenie entsteht. Dinning, Payne, und Day [3] haben an Ratten festgestellt, daß auf Wirkung einer methioninfreien Diät Leukopenie entsteht, die durch Beimischung von Methionin zum Futter verhindert werden kann.

In den gegenwärtigen Versuchen untersuchten wir die Wirkung von Tryptophan auf die Leukozytenzahl und auf das qualitative Blutbild. Als Ausgangspunkt diente die Beobachtung, daß Tryptophanmangel Leukopenie verursacht.

Die Untersuchungen führten wir an aus eigener Zucht stammenden weißen männlichen Ratten durch. Junge, 80—90 g schwere Tiere erhielten eine Mangeldiät, die übrigen Tiere die normale halbsynthetische Diät von Sós, u. zw. 18% Kasein, 3% getrocknete Hefe, 10% Fett (davon 2% Lebertran), 4% komplexe Salzmischung und 65% Stärke. Verabreichung der Amino-

säuren erfolgte subcutan, bzw. bei einer Gruppe intraperitoneal. Zu diesen Untersuchungen gelangten 120—150 g schwere Tiere, jeweils 14—18 Stunden nach der letzten Fütterung. Das Tryptophan wurde in n/l HCl gelöst und die Lösung mit n/10 NaOH neutralisiert. Die Konzentration betrug 10 mg/ml Tryptophan. Den Kontrolltieren wurde das neutralisierte Lösungsmittel eingespritzt.

Ergebnisse

In der ersten Versuchsserie haben wir 10 Tiere auf tryptophanfreier Diät gehalten. Am 7. und am 18. Tage erfolgte die Zählung der weißen Blutkörperchen. Bereits am Ende der ersten Woche konnte eine Verminderung der Leukozytenzahl beobachtet werden die immer ausgeprägter wurde (Abb. 1). Die Leukozytenzahl der normal ernährten Tiere zeigte in der gleichen Zeit

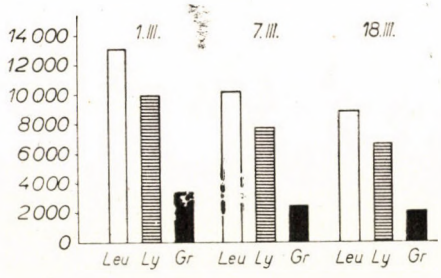

Abb. 1. Veränderung der Leukozytenzahl bei tryptophanfrei ernährten Ratten. 1. III. Zu Beginn der Versuche. 18. III. Bei Beendigung der Versuche

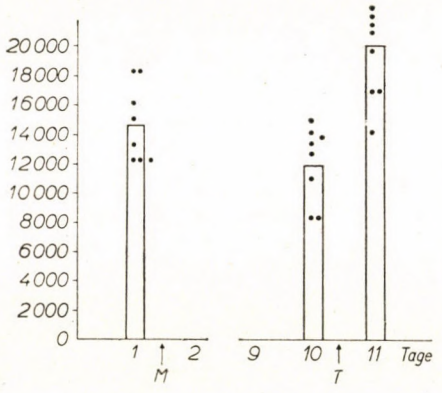

Abb. 2. Veränderung der Leukozytenzahl bei tryptophanfrei ernährten Ratten auf Wirkung von 10 mg/100 g subcutan injiziertem Tryptophan. M: Beginn der Diät, T: subcutane Einführung von Tryptophan

keine wesentliche Veränderung. Im qualitativen Blutbild von an tryptophanfreier Diät gehaltenen Tieren wurde keine wesentliche Veränderung beobachtet. Sowohl die Zahl der Granulozyten, als auch die der Lymphozyten zeigte eine proportionelle Verminderung.

Es wurde demnach die Frage aufgeworfen, ob die auf diese Weise erhaltene Leukopenie durch parenteral gegebenes Tryptophan aufgehoben werden bzw. ob die Normalisierung der Leukozytenzahl beschleunigt werden kann? Zu diesen Untersuchungen wurden 10 Tage hindurch 8 Tiere von je 80—90 g Gewicht an tryptophanfreier Diät gehalten. Die Leukozytenzahl verminderte sich. Dann wurde den Tieren 10 mg/100 g Tryptophan subcutan eingespritzt, wobei 24 Stunden später die Erhöhung der Leukozytenzahl über den Ausgangswert erfolgte (Abb. 2).

Hiernach untersuchten wir, welche Wirkung das parenteral verabreichte Tryptophan bei normalen Tieren auslöst. Selbstkontrollversuche wurden an 6 Tieren von je 120—160 g Gewicht durchgeführt. Die Resultate sind aus der Abbildung 3 zu ersehen. Nach der parenteralen Verabreichung von 10 mg/100 g Tryptophan zeigte die Leukozytenzahl eine bedeutende Erhöhung. Bei den einzelnen Tieren erhielten wir einheitliche Werte. Das Blutbild zeigte einen proportionalen Anstieg der Granulozyten und Lymphozyten.

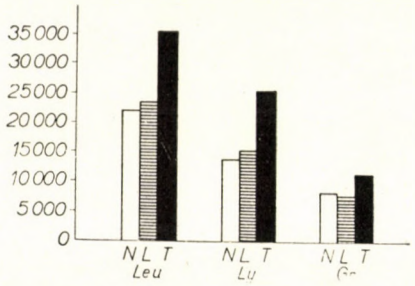

Abb. 3. Wirkung von 10 mg/100 g subcutan gegebenem Tryptophan auf das Leukozytenbild. *N:* Ausgangswert, *O:* 24 St. nach subcutaner Injektion des Lösungsmittels, *T:* 24 St. nach der Injektion von Tryptophan

Ferner wurde untersucht, ob diese nach 24 Stunden erscheinende leukozytosesteigernde Wirkung eine spezifische Eigenschaft des Tryptophans sei, oder ob auch mit anderen Aminosäuren eine ähnliche Erscheinung hervorgerufen werden könne. Zu diesem Zweck verabreichten wir aus je 3 Tieren bestehenden Gruppen subcutan Glykokoll, Isoleucin, Glutaminsäure, Threonin, Cystein und Methionin Dosen von 10 mg/100 g. Eine bedeutende Veränderung konnte in der 24. Stunde weder im qualitativen noch im quantitativen Blutbild festgestellt werden. Die Ergebnisse sind auf Tab. I. zusammengefaßt. Aus

Tabelle I

Die Wirkung von 10 mg/100 g subcutan injizierten Aminosäuren auf die Leukozytenzahl

Aminosäuren	Leukozytenzahl	
	vor	nach 24 Stunden
	Verabreichung der Aminosäuren	
Glykokoll	17400	17700
Isoleucin	13100	14000
Glutaminsäure	15500	13000
Threonin	13300	13400
Cystein	12700	15000
Methionin	14100	14000
Tryptophan	15800	23300

dieser Erscheinung folgerten wir, daß die nach 24 Stunden beobachtete leukozytosesteigernde Wirkung aller Wahrscheinlichkeit nach eine spezifische Eigenschaft des Tryptophans darstellt.

Nachdem bereits JUADA [6] beschrieben hatte, daß intravenös gegebenes Tryptophan anfänglich Leukopenie verursacht, untersuchten wir, welche Veränderung im Blutbild durch subcutan gegebenes Tryptophan in den ersten Stunden hervorgerufen wird. 7 Ratten wurden 10 mg/100 g Tryptophan verabreicht und die Veränderungen des Blutbildes in der 1., 2., 4., 6. und 24. Stunde untersucht. Auf Wirkung des parenteral eingeführten Tryptophans erfolgte während 24 Stunden eine Verminderung der Leukozytenzahl, doch in der

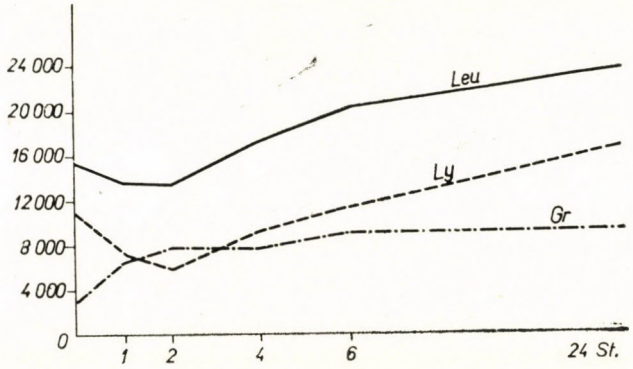

Abb. 4. Wirkung von 10 mg/100 g subcutan gegebenem Tryptophan auf das Leukozytenbild

4. Stunde war bereits Leukozytose zu beobachten, die sich im Laufe von 24 Stunden noch weiter erhöhte. Beachtenswert war das Verhalten des qualitativen Blutbildes. Zu der in der 1. und 2. Stunde auftretenden Leukopenie gesellten sich Lymphopenie und Granulozytose. Nach zwei Stunden erfolgte eine vollkommene Umstellung des Blutbildes. Die Zahl der Granulozyten erhöhte sich von 3800 auf 7200, während die Lymphozytenzahl von 11 000 auf 6300 sank. Diese Erscheinung ergab eine charakteristische Kreuzung in der Blutbildkurve. Die Ergebnisse werden auf Abb. 4 veranschaulicht.

Ähnliche Erscheinungen wurden von WACHHOLDER und Mitarbeitern [9] auf Wirkung von ACTH beschrieben. Auch in diesem Fall erfolgte die Umstellung des Blutbildes. Von der 4. Stunde an war hauptsächlich die Erhöhung der Lymphozytenzahl vorherrschend.

Die auf Wirkung von großen Dosen Tryptophan — 50 mg/100 g i. p. — eintretende Veränderung wurde an 10 Tieren untersucht. Kennzeichnend war — wie aus Abb. 5 ersichtlich — die mit der anfänglichen Lymphopenie einhergehende Granulozytose und die nachfolgende anhaltende Umstellung des Blutbildes (Abb. 5).

Die in den ersten Stunden auftretende Erscheinung kann jedoch nicht als spezifisch für Tryptophan angesehen werden, da einerseits auch nach Verabreichung von anderen Aminosäuren (Isoleucin, Methionin) ähnliche Erscheinungen beobachtet wurden, und HARRIS und LANG [5] auf Wirkung von

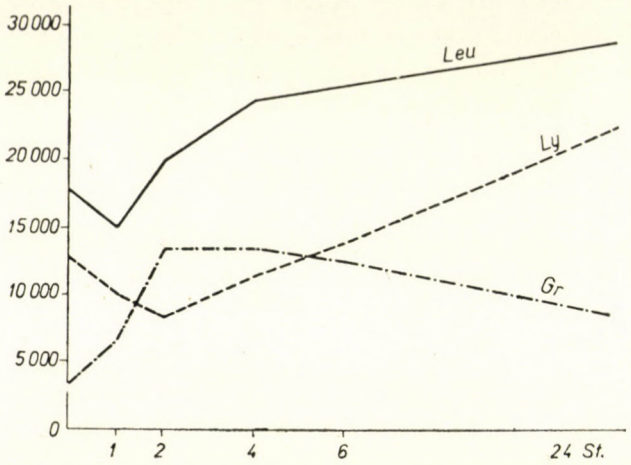

Abb. 5. Wirkung von 50 mg/100 g intraperitoneal gegebenem Tryptophan auf das Leukozytenbild

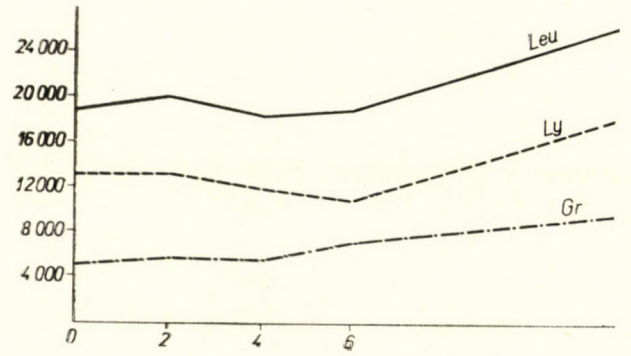

Abb. 6. Wirkung von 10 mg/100 g subcutan injiziertem Tryptophan auf das Leukozytenbild nach zweiseitiger Nebennierenexstirpation

Asparagin und Glutaminsäure Lymphopenie beobachten konnten. Die gleichzeitig mit der Lymphopenie auftretende Leukozytose kann als Stressoreffekt angesehen werden. Die durch ASCHKENASY [1] in der 4. Stunde nach Aminosäureverabreichung beobachtete Veränderung der Eosinophilenzahl kann gleichfalls in diesem Sinne aufgefaßt werden. An 7 Tieren von je 120—150 g Gewicht wurde in Äthernarkose zweiseitige Nebennierenexstirpation vorgenommen und den Ratten 24 Stunden nach der Operation 10 mg/100 g Tryptophan subcutan gegeben. Die auf Abb. 6. zusammengefaßten Ergebnisse

zeigen, daß — obwohl eine gewisse Lymphopenie auftrat — die Umstellung des Blutbildes unterblieb, doch konnte in der 24. Stunde ebenfalls Leukozytose beobachtet werden. Es scheint demnach, daß durch Nebennierenexstirpation die infolge der Tryptophangabe auftretende anfängliche Veränderung der Leukozytenzahl abgewehrt werden kann, doch die spätere — als spezifisch geltende — Veränderung indessen unbeeinflußt bleibt.

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Pathophysiologia

ÜBER DAS VERHALTEN DES UMSATZES DER RATTE NACH HYPOTHALAMUSLÄSIONEN IN DER WÄRME UND DESSEN BEZIEHUNGEN ZUR THERMOREGULATION IN KÜHLER UMGEBUNG

Von

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Bilaterale elektrolytische Läsionen des Hypothalamus führten in etwa einem Drittel der Ratten zu einem Ausbleiben der hyperthermischen Steigerung der Wärmeproduktion, die unter gleichen Versuchsbedingungen und gleicher Hyperthermie bei intakten Tieren niemals ausbleibt. Ähnliche Störungen können auch nach unilateralen Läsionen beobachtet werden. Die hyperthermische Umsatzerhöhung kann bei erhaltener chemischer Regulation ausbleiben, kann aber auch mit einem Fehlen derselben einhergehen; andererseits kann auch beim Ausbleiben der chemischen Regulation die hyperthermische Umsatzsteigerung voll erhalten bleiben. Lokalisation, sowie sich ergebende Folgerungen werden besprochen.

Im Zusammenhang mit Untersuchungen über den Mechanismus der hyperthermischen Umsatzsteigerung [1, 2, 3, 5, 6] wurde bereits kurz darauf hingewiesen, daß nach Läsionen des Hypothalamus diese vermißt werden kann [4]. Die bunte Vielfalt der Störungen des Energiewechsels und der Körpertemperatur in thermoneutraler [10] und in kühler Umgebung nach Läsionen des Hypothalamus [9], ließ es wünschenswert erscheinen, die Reaktion des Umsatzes auf eine, zu Hyperthermie führende warme Umgebung an einem größeren Versuchsmaterial etwas eingehender zu prüfen und an denselben Tieren auch das Verhalten bei Versetzung in eine kühle Umgebung zu beobachten.

Versuchsanordnung. Läsionen, Bestimmungen des O_2-Verbrauches und histologische Untersuchungen wurden auf dieselbe Weise ausgeführt, wie dies in früheren Mitteilungen bereits beschrieben wurde [9, 10]. Es sei jedoch nochmals bemerkt, daß die Luft der Stoffwechselkammer praktisch mit Wasserdampf gesättigt war, demzufolge sich bereits bei einer Temperatur von 35°C ausnahmslos eine so beträchtliche Hyperthermie entwickelte, die z. B. in einem gut durchlüfteten Thermostaten nur bei höheren Umgebungstemperaturen beobachtet wird.

Die einzelnen Säulen der Abbildungen wiedergeben auch in dieser Arbeit — wenn nicht anders angegeben — den Mittelwert dreier gut übereinstimmenden Perioden von je 15 min. Da etwa 20 min. zum Temperaturausgleich zwischen Wasserbad und Kammer benötigt werden, wurde die erste Bestimmung des O_2-Verbrauches erst nach diesem Zeitpunkt vorgenommen.

Die Ratten befanden sich daher meistens etwa 70—90 min. in der angegebenen Umgebungstemperatur als die Körpertemperatur gemessen wurde.

Versuchsergebnisse

Von dem 170 Ratten mit Hypothalamusläsionen umfassenden Gesamtmaterial wurden insgesamt 55 Tiere in 73 Versuchen einer zur Hyperthermie führenden warmen Umgebung ausgesetzt. Von diesen 55 Ratten reagierten 33 nach der Läsion normal: in der warmen Umgebung stieg der Umsatz in demselben Ausmaß wie in intakten Tieren. Die ausgeprägtere Hyperthermie zeigt, daß wenigstens in der Mehrzahl dieser Tiere doch eine thermoregulatorische Störung bestanden haben muß. Bei intakten Tieren kommt es unter ansonsten gleichen Bedingungen nur in einer Umgebung von 36—37°C zu einer Hyperthermie gleichen Grades.

Tabelle I

Körpertemperatur und O_2-Verbrauch intakter Ratten und Tieren mit bilateralen Läsionen des Hypothalamus mit normal erhaltener chemischer Regulation und hyperthermischer Umsatzerhöhung

$$M \pm \sqrt{\frac{\Sigma \Delta x^2}{n(n-1)}}$$

	O_2-Verbrauch		„t" Test	Körpertemperatur		„t" Test
	Intakt n=26	Lädiert n=33		Intakt n=26	Lädiert n=33	
29 C°	71±1	74±1	P>0.1	37.3±0.1	37.7±0.1	P>0.05
35 C°	92±1	96±1	P>0.1	39.2±0.1	40.5±0.1	P<0.001

Trotz Hyperthermie wurde bei 18 Ratten in 40 Versuchen eine Steigerung des Umsatzes vollkommen vermißt. Es sei bemerkt, daß sich die hyperthermische Umsatzsteigerung nach einigen Tagen oder Wochen auch bei diesen Tieren wieder auslösen ließ. Diese Störung ist also geradeso reversibel, wie dies bei Störungen in der Kälte bei genügend langer Beobachtungsdauer mehrfach festgestellt wurde [7, 9, 12, 13].

In allen 55 Tieren, die einer warmen Umgebung ausgesetzt worden waren, wurde auch die Reaktion auf eine kühle Umgebung untersucht. Von den 18 Ratten, bei denen eine hyperthermische Umsatzsteigerung vermißt wurde, kam es in 6 auch in einer kühlen Umgebung (20—22°C) zu keiner Erhöhung des O_2-Verbrauches; es fiel also gleichzeitig mit der hyperthermischen Umsatzsteigerung auch die chemische Regulation aus. In den übrigen 12 Ratten blieb die chemische Regulation bei Ausfall der hyperthermischen Stoffwechselsteigerung erhalten: der Umsatz erhöhte sich in einer Umgebung von 20—22°C

wie bei intakten Tieren. Bei 18 weiteren Ratten kam es bei voll erhaltener hyperthermischer Umsatzsteigerung in einer kühlen Umgebung zu keinem Anstieg des O_2-Verbrauches und bei 19 Tieren blieben nach der Läsion so die chemische Regulation wie die hyperthermische Umsatzsteigerung erhalten (Tab. II).

Tabelle II

Hyperthermische Umsatzsteigerung und chemische Regulation bei 55 Ratten mit Hypothalamusläsionen

Hyperthermische Umsatzsteigerung	Chemische Regulation	Anzahl der Tiere
erhalten	erhalten	19
fehlt	fehlt	6
fehlt	erhalten	12
erhalten	fehlt	18

Da eine ausführliche Mitteilung aller Versuche die gegebenen Rahmen überschreiten würde, werden bloß einige Versuche zur Erläuterung der Ergebnisse herangezogen.

Abb. 1 wiedergibt zwei Versuche. In dem einen trat nach bilateraler Läsion eine normale hyperthermische Umsatzerhöhung auf, in dem anderen blieb

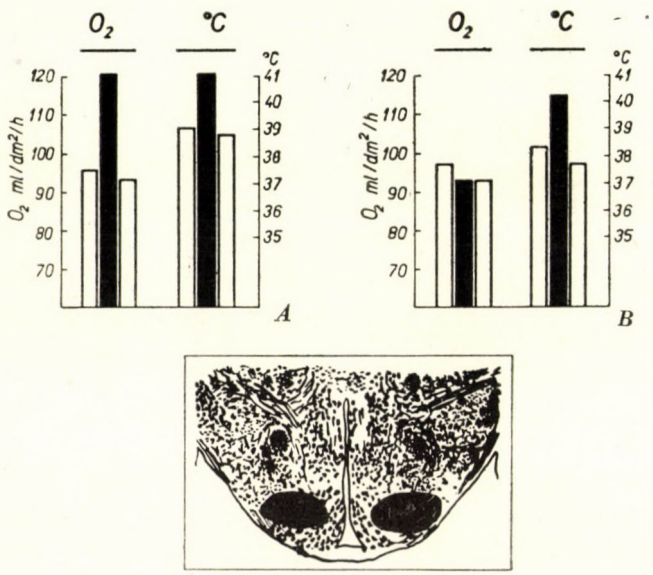

Abb. 1. O_2-Verbrauch und Körpertemperatur bei 29°C (weiß) und bei 35°C (schwarz)
A Ratte No 473. Läsion: 14. X. 1951; Versuch: 16. X. 1951; getötet: 6. XI. 1951. Lokalisation: Symmetrische Läsionen im mittleren vorderen Teil der Regio tuberalis, die bis an die Hirnbasis reichend die Nuclei ventromediales und die Areae hypothalamicae laterales erfassen (Diagramm: mittleres Niveau der Regio tuberalis)
B Ratte No. 501. Läsion: 20. II. 1952; Versuch: 21. II. 1952; weiteres siehe Abb. 6/A

dagegen nach einer praktisch unilateralen Läsion die Umsatzerhöhung völlig aus. In beiden war der Grundumsatz, wie dies nach Läsionen des Hypothalamus öfters beobachtet wurde [8, 10], um mehr als 30 Prozent erhöht. Diese Beobachtung zeigt, daß das Ausbleiben einer hyperthermischen Umsatzsteigerung nicht damit erklärt werden kann, daß der Umsatz bereits in

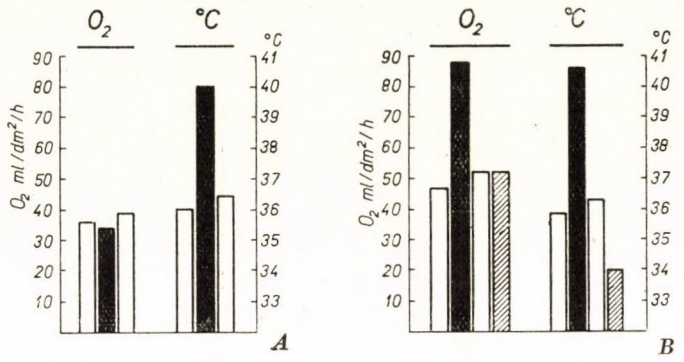

Abb. 2. O_2-Verbrauch und Körpertemperatur bei 29°C (weiß), bei 35° C (schwarz) und bei 20° C (gestrichelt)

A Ratte No. 289. Läsion: 15. VII. 1950; Versuch: 18. VII. 1950; getötet: Zeitpunkt nicht feststellbar. Lokalisation: bilaterale stecknadelkopfgroße Läsionen des Mesencephalon hinter den Kernen der Corpora mamillaria

B Ratte No. 500. Läsion: 11. II. 1952; Versuch: 16. II. 1952; weiteres siehe bei Abb. 4.

thermoneutraler Umgebung so hoch ist, wie im intakten Tier in Hyperthermie. Dasselbe geht aus Abb. 2 hervor: bei niedrigen Grundumsatz fehlt in dem einen Versuch bei gleicher Hyperthermie die Umsatzsteigerung vollkommen (außerhypothalamische Läsion), dagegen ist sie im anderen weit überdurchschnittlich ausgeprägt.

Abb. 3 wiedergibt einen Versuch in welchem sogar zwei Wochen nach der Läsion die hyperthermische Umsatzerhöhung noch vollkommen ausblieb, während in eine kühle Umgebung versetzt die Ratte mit normaler Erhöhung der Wärmeproduktion reagierte. Es fällt auf, daß bei Rückversetzung aus der Wärme in eine thermoneutrale Umgebung der Umsatz weit unter das Niveau des Grundumsatzes sinkt. Selbst eine sehr ausgeprägte „zweite chemische Regulation" ist also keineswegs an eine hyperthermische Umsatzsteigerung gebunden, wie dies auch auf Grund von Versuchen an hypophysektomierten Ratten schon vor einigen Jahren betont wurde [3]. Da der Frage der „zweiten chemischen Regulation" [11] in dieser Versuchsreihe keine besondere Aufmerksamkeit gewidmet wurde, und so manchmal auch 60—90 min. vergingen bis nach Rückversetzung in eine thermoneutrale Umgebung mit der Bestimmung des O_2-Verbrauches begonnen wurde, oder aber von einer solchen Rückversetzung auch abgesehen wurde, läßt sich über deren Verhalten nichts genaueres aussagen; immerhin wurde bei 5 Tieren neben vollkommenem Fehlen einer

hyperthermischen Umsatzsteigerung eine recht ausgeprägte „zweite chemische Regulation" beobachtet.

Abb. 4 wiedergibt Versuche an derselben Ratte deren Beobachtung auch Abb. 2/B zu Grunde lag. Am Tage nach der Läsion (A) fehlt bei stark erhöhtem Grundumsatz die hyperthermische Umsatzsteigerung, während bei Versetzung in eine kühle Umgebung die Wärmeproduktion ansteigt; die Körpertemperatur liegt über der Norm und wird auf diesem Niveau auch in der kühlen Umgebung unverändert erhalten. Zwei Tage später (B) kommt es bei

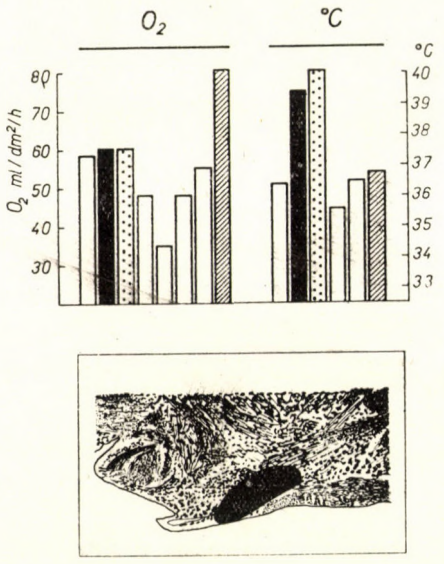

Abb. 3. O_2-Verbrauch und Körpertemperatur bei 29° C (weiß), bei 35° C (schwarz), bei 37° C (punktiert) und bei 20° C (gestrichelt). Säulen 4 bis 7 des O_2-Verbrauches wiedergeben Einzelperioden von 15 min. Die Körpertemperatur wurde nach je zwei dieser Perioden gemessen (Säule 4 und 5 der Körpertemperatur). Zwischen dem Ende der in Säule 5, und dem Beginn der in Säule 6 wiedergegebenen Bestimmung des O_2-Verbrauches vergingen etwa 25 min. Ratte No. 484. Läsion: 1. XI. 1951; Versuch: 13. XI. 1951; getötet: 2. I. 1952. Lokalisation: Laterale Läsionen, deren größere von dem hinteren Niveau der Regio supraoptica bis in das mittlere Niveau der Regio tuberalis reicht und den Nucleus supraopticus diffusus, sowie die Area hypothalamica lateralis erfaßt. Die gegenseitige, kleinere Läsion sitzt zwischen dem vorderen und mittleren Niveau der Regio tuberalis. Der Stichkanal verursachte beiderseitig im Niveau der Regio infundibuli, an der dorsalen Oberfläche des Thalamus lateral kleine Zerstörungsherde, deren größerer Stecknadelkopfgröße erreicht (Diagramm: paramedian-sagittale Ebene des Hypothalamus)

unverändert hohem Grundumsatz auch in kühler Umgebung zu keiner Erhöhung der Wärmeproduktion. Dasselbe sieht man drei Tage nach der Läsion bei etwas niedrigerem aber immer noch erhöhtem Grundumsatz (C). Am 4. Tage (D) liegen Körpertemperatur und Grundumsatz an der unteren Grenze des Normalbereiches, es kommt zu einer normalen hyperthermischen Umsatzerhöhung, in kühler Umgebung bleibt jedoch die Steigerung der Wärme-

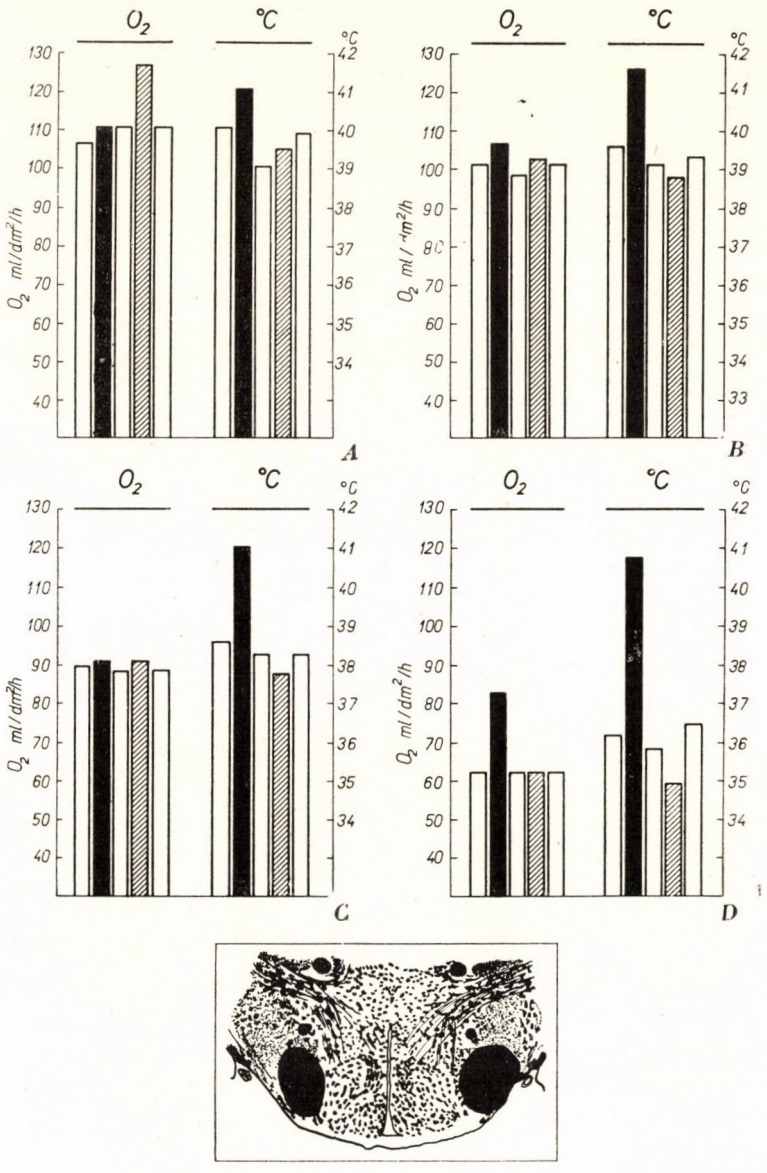

Abb. 4. O_2-Verbrauch und Körpertemperatur bei 29° C (weiß), bei 35° C (schwarz) und bei 20° C (gestrichelt)

Ratte No. 500. Läsion: 11. II. 1952; Versuche: 12—16. II. 1952; getötet: 22. II. 1952. *A* am Tage nach der Läsion, *B* 2 Tage, *C* 3 Tage, *D* 4 Tage nach der Läsion. Den Versuch 5 Tage nach der Läsion siehe in Abb. 2/B.

Lokalisation: Der Mittelpunkt beider Läsionen liegt im vorderen Niveau der Regio tuberalis und beide reichen bis an die Hirnbasis. Auf der einen Seite dehnt sich die Läsion nach vorne in die Regio supraoptica aus, auf der anderen zieht sie nach hinten bis an die Grenze des mittleren und hinteren Niveaus der Regio tuberalis. Die Area hypothalamica lateralis ist beiderseits erfaßt, hiezu gesellt sich auf der einen Seite die Läsion des Nucleus supraopticus (Diagramm: vorderes Niveau der Regio tuberalis)

produktion auch jetzt aus, und die Körpertemperatur fällt erstmalig auf ausgesprochen subnormale Werte. Noch ausgeprägter tritt dies bei noch stärker gesenktem Grundumsatz am nächsten Tage in Erscheinung (Abb. 2/B).

Abb. 5 zeigt den Ausfall und die Restitution der hyperthermischen Umsatzsteigerung bei praktisch normalem Grundumsatz. 2 Tage nach der Läsion (A) bleibt der Umsatz selbst in einer Umgebung von 37°C unverändert und

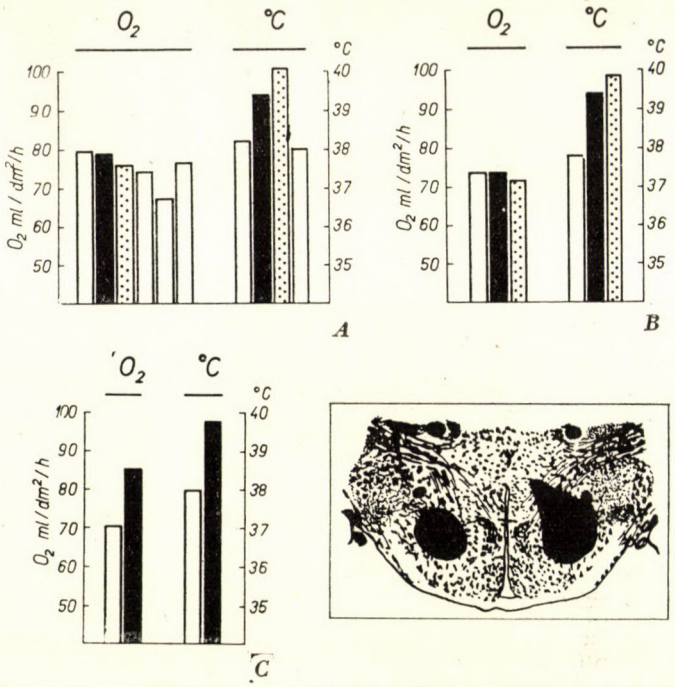

Abb. 5. O_2-Verbrauch und Körpertemperatur bei 29° C (weiß), bei 35° C (schwarz) und bei 37° C (punktiert). Die Säulen 4 bis 6 in *A* wiedergeben Einzelbestimmungen von je 15 min. Ratte No. 492. Läsion: 17. I. 1952; Versuche: 18. I.—15. II. 1952; getötet: 29. II. 1952. *A* Versuch 2 Tage nach der Läsion, *B* 18 Tage, *C* 29 Tage nach der Läsion. Lokalisation: Läsionen im mittlereren Niveau der Regio tuberalis; auf der einen Seite erfaßt diese die Area hypothalamica lateralis und den dorsomedialen Kern des Hypothalamus, auf der anderen Seite ist der dorsale Teil der Area hypothalamica lateralis zerstört. Blutung in die III. Kammer (Diagramm: vorderes Niveau der Regio tuberalis)

auch 18 Tage (B), sowie 21 Tage nach der Läsion kommt es trotz ausgeprägter Hyperthermie zu keiner Umsatzsteigerung, und erst am 29. Tage nach der Läsion wird eine solche beobachtet (C).

Anhaltspunkte für eine feinere Lokalisation lassen sich aus diesen Versuchen trotz des ziemlich großen Versuchsmaterials kaum gewinnen. Einerseits sind die Läsionen, wie darauf bereits hingewiesen wurde [9, 10], im Verhältnis zum Rattenhypothalamus doch ziemlich groß, andererseits können bei ganz ähnlichen Läsionen eine Störung der chemischen Regulation bei erhal-

tener hyperthermischer Umsatzsteigerung, oder eine normale chemische Regulation bei fehlender hyperthermischer Umsatzsteigerung beobachtet werden, auch können beide Reaktionen fehlen oder erhalten bleiben.

Eine eingehendere Besprechung erfordert die Beobachtung, daß auch unilaterale Läsionen des Hypothalamus zu ausgeprägten Störungen der Reak-

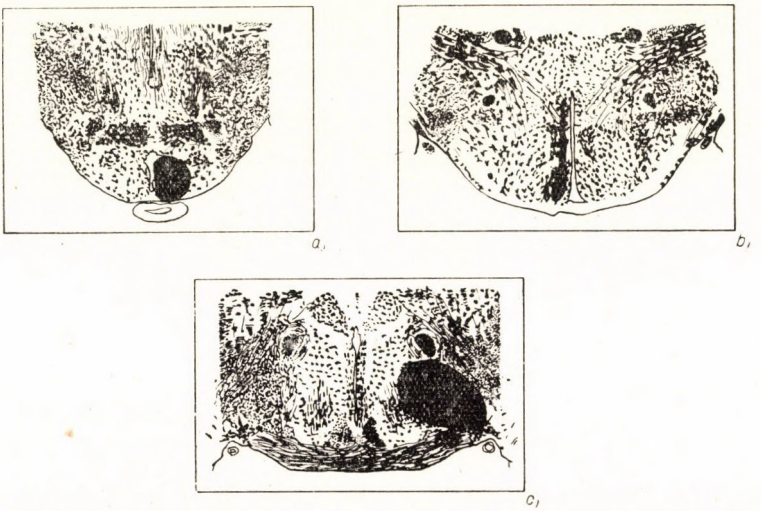

Abb.6.A Ratte No. 501. Läsion : 20. II. 1952 ; getötet 17. III. 1952. Funktionsstörung : Ausbleiben der Umsatzsteigerung in Hyperthermie. Lokalisation : Große Läsion mit Mittelpunkt an der Grenze der Regio tuberalis und der Regio mamillaris knapp an der Mittellinie. Nach vorne dehnt sich die Läsion bis in das vorderste Niveau der Regio tuberalis aus und trifft einseitig die III. Kammer, nach rückwärts läßt sich die, in ihrer ganzen Ausdehnung auf der Hirnbasis verlaufende Läsion bis zum hinteren Niveau der Corpora mamillaria verfolgen (Diagramm : hinteres Niveau der Regio tuberalis)

B Ratte No. 447. Läsion : 4. V. 1951 ; getötet : Zeitangabe fehlt. Funktionsstörung : Ausbleiben der chemischen Regulation bei 20° C und Fehlen der hyperthermischen Umsatzerhöhung. Lokalisation : Unilaterale Läsion die den Thalamus im Niveau der Regio supraoptica durchquerend den Nucleus paraventricularis erreicht und im hinteren Niveau lädiert. Der Stichkanal verläuft dann weiter im vorderem Niveau derselben Region neben der III. Kammer bis zur Hirnbasis. Eine gröbere Läsion ist nicht vorhanden, man findet bloß in der nächsten Umgebung des Stichkanals eine Infiltration in dem Nucleus dorsomedialis, dem Nucleus ventromedialis und dem Nucleus arcuatus (Diagramm : vorderes Niveau der Regio tuberalis)

C Ratte No. 497. Läsion : 5. II. 1952 ; getötet : 17. III. 1952. Funktionsstörung : Fehlen der hyperthermischen Umsatzsteigerung. Lokalisation : Unilaterale Läsion im Niveau der Regio supraoptica, die bis an die Hirnbasis reichend den Nucleus supraopticus, den Nucleus supraopticus diffusus, sowie den lateralen Teil der Area hypothalamica anterior erfasst und an das Chiasma opticum heranreicht (Diagramm : vorderes Niveau der Regio suprachiasmatica)

tionen des Umsatzes auf Änderungen der Umgebungstemperatur führen können. Nach insgesamt 9 einseitigen Läsionen wurde bei zwei Tieren in warmer Umgebung ein Anstieg des Umsatzes vermißt, in einem Tiere blieb die Wärmeproduktion so in kühler, wie in zu Hyperthermie führender warmer Umge-

bung gegenüber der bei thermoneutraler Temperatur beobachteten vollkommen unverändert. In einem Falle kam es bei erhaltener hyperthermischer Umsatzsteigerung in kühler Umgebung zu keiner Erhöhung der Wärmeproduktion, in einem anderen sank die Körpertemperatur trotz normalem Anstieg des Energiewechsels. Zwei weitere Ratten reagierten zwar so auf die Wärme wie auf Kälte mit einer Steigerung der Wärmeproduktion, hatten aber einen erhöhten Grundumsatz und eine hohe Körpertemperatur, ein anderes Tier mit ebenfalls einseitiger Läsion hatte bei normaler Körpertemperatur einen erhöhten Grundumsatz. Bei einer etwas eingehender Analyse zeigt sich also, daß nach einseitiger Läsion bloß in einem einzigen Tier keine Störungen des Energiewechsels und der Körpertemperatur beobachtet werden konnten.

Überblickt man die Lokalisation dieser unilateralen Läsionen, so muß wohl bei den an die Mittellinie heranreichenden die Möglichkeit in Betracht gezogen werden, daß funktionell auch die andere Seite für kürzere oder längere Zeit durch ein perifokales Ödem oder eine perifokale Infiltration geschädigt wurde (Abb. 6, A und B), jedoch kann es wie bei Ratte No. 497 (Abb. 6,C) auch bei weiter lateral gelegenen einseitigen Herden zu einem Ausbleiben der hyperthermischen Umsatzsteigerung kommen. Auch ist die durch unilaterale Läsionen hervorgerufene Störung nicht immer ganz flüchtig; in einem Falle war sie am 8. Tage nach der Läsion noch nachweisbar.

Besprechung

Überblickt man das ganze Versuchsmaterial von Hypothalamusläsionen, so läßt sich feststellen, daß sich das Verhalten des Energiewechsels und der Körpertemperatur auch in einer zu Hyperthermie führenden warmen Umgebung dem intakten Tier gegenüber verändert. Die hyperthermische Erhöhung des Umsatzes blieb zwar bloß in 18 von 55 Ratten aus, doch der statistisch hochsignifikant höhere Anstieg der Körpertemperatur zeugt auch in den übrigen Versuchen für eine veränderte Regulation in der Wärme. Das Ausbleiben der erhöhten Wärmeproduktion in lädierten Tieren bei einer Hyperthermie, die in der intakten Ratte ohne Ausnahme mit einer ganz bedeutenden Steigerung des Umsatzes einhergeht, beweist ausdrücklich — wie darauf bereits kurz hingewiesen wurde [4] —, daß der Anstieg nicht, wie ziemlich allgemein angenommen wird, einfach eine durch die VAN'T HOFFsche Regel bedingte Erscheinung ist, sondern einer zentralnervösen Steuerung unterliegt.

Von besonderem Interesse ist, daß der Ausfall der hyperthermischen Umsatzsteigerung sowohl mit einer erhaltenen, wie auch mit einer fehlenden chemischen Regulation einhergehen kann, und daß andererseits bei fehlender chemischer Regulation die hyperthermische Umsatzsteigerung voll erhalten sein kann, sowie, daß bei dem Ausfall beider Reaktionen die Restitution der

chemischen Regulation und der hyperthermischen Reaktion getrennt erfolgen kann. Diese Beobachtungen beweisen, daß die Steigerung der Wärmeproduktion in Hyperthermie und in der Kälte durch unabhängige zentrale Mechanismen vermittelt werden, und sprechen auch gegen die Existenz eines einfachen (efferenten) thermogenetischen Zentrums im Hypothalamus, und für die Annahme, daß es sich in allen Fällen um Läsionen handelt, die den zentralen Steuerungsapparat des Energieumsatzes vor den eigentlichen zentralen Effektormechanismen treffen. In dieser Hinsicht bestärken die Versuche in der Wärme die Folgerung, zu welcher schon das Verhalten der chemischen Regulation in kühler Umgebung zwang [9], nämlich, daß auch eine zentrale Störung der Analyse der Afferenzen in Betracht gezogen werden muß.

In einer kühlen Umgebung war das Ausbleiben einer erhöhten Wärmeproduktion keinesweges zwangsmäßig mit einer Störung der Regulation der Körpertemperatur verbunden [9], und auch das Ausbleiben einer Steigerung des Umsatzes in Hyperthermie bei thyreoidektomierten oder mit Methylthiouracil behandelten, sowie bei hypophysektomierten Ratten [2, 6] war mit keiner Änderung im Verhalten der Körpertemperatur verbunden [5]. Die Frage, ob Hypothalamusläsionen, nach welchen keine hyperthermische Umsatzsteigerung auftritt, unbedingt mit anderen thermoregulatorischen Störungen in der Wärme einhergehen, wird im Zusammenhang mit Läsionen epithalamischer Gebiete in einer anderen Arbeit besprochen.

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ÜBER DIE HERSTELLUNG DES PHOSPHOGLUKOMUTASE-ANTISERUMS UND SEINE EIGENSCHAFTEN

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Es wurde aus Hahnenmuskel ein hochgradig gereinigtes Phosphoglukomutase-Präparat hergestellt und mit diesem — unter Anwendung eines Adjuvans — die Immunisierung von Kaninchen vorgenommen.

Das Serum der behandelten Kaninchen zeigte am 16. Tage geringe, am 28. Tage starke phosphoglukomutasehemmende Wirkung. Zur quantitativen Bestimmung des Antimutasegehaltes der Sera wurde eine Methode ausgearbeitet. Die Antimutase hemmt die Mutaseaktivität bei Geflügelarten (Hahn, Taube, Ente) nicht nur im Muskel-, sondern auch im Herz- und Leberextrakt, so daß also die Phosphoglukomutase nicht über Organspezifität verfügt. Die Mutaseaktivität von Säugetieren und Fröschen wurde vom Antiserum nicht gehemmt, so daß die Phosphoglukomutase der Hähne Geflügel- (= Klassen-) Spezifität aufweist.

Neuestens hat man die immunologischen Eigenschaften mehrerer am Kohlenhydratstoffwechsel teilnehmender Enzyme untersucht. So berichteten Henion und Sutherland [1] über die Herstellung und Eigenschaften des Phosphorylase-Antiserums aus Leber und Herz, Jókay, Bot und Szilágyi [2] über die Herstellung und immunologische Spezifität des Muskelphosphorylase-Antiserums, Lipzett, Reisberg und Bodansky [3] über den Antigencharakter der aus Leber gewonnenen Phosphohexoisomerase, Bueding [4] über die Spezifität der aus Schistosoma Mansoni hergestellten Isomerase sowie Bozsóki und Elődi [5] über die immunologischen Eigenschaften der Phosphoglyzerinaldehyd-Dehydrogenase. Mit Hilfe von Phosphorylase-Antisera konnte nachgewiesen werden, daß sich die aus verschiedenen Geweben desselben Tieres stammenden Phosphorylasen voneinander unterscheiden [1, 2], was ihrem abweichenden physiologischen und chemischen Verhalten entspricht.

In den hier besprochenen Untersuchungen wünschten wir vor allem zu klären, ob ebenso wie bei den Phosphorylasen auch zwischen den Phosphoglukomutasen in den einzelnen Geweben desselben Tieres und den Phosphoglukomutasen verschiedener Arten immunologische Unterschiede bestehen.

Die Phosphoglukomutase katalysiert die Umwandlung G-1-P$\rightleftharpoons$G-6-P und spielt im Glykogenstoffwechsel eine große Rolle. Es schien daher nicht unwichtig zu untersuchen, ob zwischen den Phosphoglukomutasen der einzelnen Gewebe und Spezies immunologische Differenzen bzw. damit zusammenhängende physiologische und chemische Abweichungen vorhanden sind.

Methoden

Wir immunisierten drei 3 kg schwere Kaninchenmännchen mit einem aus der Brustmuskulatur eines Hahnes extrahierten und hochgradig gereinigten Phosphoglukomutase-Präparat. Die Präparation und Reinigung der PGM* erfolgte nach der für Kaninchenmuskel ausgearbeiteten NAJJARschen Methode [6]. Den Tieren gaben wir in Abständen von 3 Tagen insgesamt 8mal (3mal i. v. und 5mal i. m. nach der FREUNDschen Adjuvanstechnik) 16 ml PGM-Präparat, insgesamt 300 PGM-Einheiten (d. h. etwa 16 mg PGM-Protein). Am 16. Tage wurden Blutproben entnommen, am 30. Tage ließen wir die Tiere verbluten und gewannen sterile Sera, die 30 Minuten bei 56° C inaktiviert wurden. Als Kontrollserum wurden die ebenso gewonnenen Sera unbehandelter Kaninchen verwendet.

Die Hemmungswirkung der Sera untersuchten wir folgendermaßen: 0,2 ml Serum (oder physiologische NaCl-Lösung) wurden mit 0,3 ml entsprechend verdünntem Enzym 2 Minuten bei 30° C inkubiert, 0,5 ml Substrat zum Gemisch gegeben und die PGM-Aktivität bestimmt, die wir nach dem teilweise modifizierten [7] NAJJARschen Verfahren [6] auf Grund der Senkung des säurelabilen P-Gehaltes im G-1-P bestimmten. Das zur Aktivitätsmessung verwendete Inkubat hatte im Endvolumen folgende Zusammensetzung: 0,005 *M* G-1-P, 0,0015 *M* $MgSO_4$, 0,002 *M* 8-Hydroxychinolin, 0,02 *M* Na-glyzerophosphat (pH = 7,4). Inkubation bei 30° C. Die Aktivität geben wir in Einheiten an, wobei wir diejenige PGM-Menge als Einheit annehmen, welche 1 mg säurelabiles P in 5 Minuten zu stabilem P umgestaltet.

Die Zubereitung der rohen Gewebsextrakte (Muskel, Herz, Leber) erfolgte aus den mit Kohlensäureschnee gefrorenen Geweben durch Zerreiben mit Quarzsand und kaltem destilliertem Wasser bei 0° C.

Ergebnisse und Besprechung

Quantitative Bestimmung des PGM-hemmenden Effektes der Antisera

Zur quantitativen Bestimmung der PGM-hemmenden Wirkung der Antisera haben wir eine Methode ausgearbeitet. Wir geben nicht die Verdünnung des als Antigen benutzten Präparates und die prozentuale Konzentration des Antiserums sowie den Prozentsatz der auftretenden Hemmung an, weil dieses Verhältnis relativ ist und von der Antigenkonzentration abhängt: die in Prozent ausgedrückte Hemmungswirkung desselben Antiserums ist bei konzentrierter Enzymlösung geringer als bei stärker verdünnter. Durch Bezeichnung der Enzymhemmungsfähigkeit des Antiserums in PGM-Einheiten läßt sich dieser Fehler vermeiden. Wenn wir die in Anwesenheit von Kontrollserum und Antiserum gemessenen Aktivitäten in PGM-Einheiten ausdrücken, so ergibt die Differenz der beiden Messungen die Hemmungswirkung des angewandten Antiserums in PGM-Einheiten. Wird der Hemmungseffekt auf 1 ml Serum errechnet, so charakterisiert dieser Wert quantitativ die enzymhemmende Fähigkeit der Antisera.

Tabelle I enthält die in Anwesenheit von physiologischer NaCl-Lösung, Kontrollserum und Antiserum gemessene PGM-Aktivität des Hahnenmuskelextraktes.

* Es werden folgende Abkürzungen benutzt:
PGM = Phosphoglukomutase
G-1-P = Glukose-1-phosphat
G-6-P = Glukose-6-phosphat

Tabelle I

PGM-Aktivität des Hahnenmuskelextraktes in Anwesenheit von NaCl, Kontroll- und Antiserum

	PGM Einheiten $\times 10^{-3}$/ml
Physiologische NaCl-Lösung	75
1. Kontrollserum	74
2. „	77
3. „	75
Antiserum A	20
„ B	26
„ C	29

0,2 ml NaCl (bzw. Kontroll- oder Antiserum) + 0,3 ml Hahnenmuskelextrakt, Inkubation 2 Minuten bei 30° C, + 0,5 ml Substrat und Aktivitätsmessung.

Wie Tabelle I zeigt, wird die PGM-Aktivität vom 1., 2. und 3. Kontrollserum nicht gehemmt, während die Antisera A, B und C beträchtliche PGM-Hemmung herbeiführen. Das Ausmaß der Hemmung ergibt sich aus der Verschiedenheit der Messungen. Die einzelnen Antisera bewirken folgende Hemmungen:

0,2 ml Antiserum A $75 - 20 = 55$ Einheiten $\times 10^{-3}$
0,2 ml „ B $75 - 26 = 49$ „ $\times 10^{-3}$
0,2 ml „ C $75 - 29 = 46$ „ $\times 10^{-3}$

Wie aus diesen Angaben errechnet werden kann, übt 1 ml Antiserum 275, 245 und 230 Einheiten $\times 10^{-3}$ PGM-Hemmung aus. Tabelle I zeigt ferner, daß z. B. 0,2 ml Antiserum A auf die angewandte Enzymmenge 75%ige Hemmung ausüben. Wenn wir nur 50 statt 75 PGM-Enzymeinheiten benutzen würden, so müßte dieselbe Serummenge 100%ige Hemmung bewirken. Zur Bestätigung dieser Annahme untersuchten wir im weiteren, wie das Hemmungsausmaß durch Veränderung der Enzym- und Antiserummenge beeinflußt wird (Tabelle II).

Wie sich aus Tabelle II ergibt, wird die Hemmungswirkung des Antiserums durch Veränderung der Enzymmenge nicht wesentlich verändert. Dagegen erhöht sich das Ausmaß der Hemmung im geraden Verhältnis zur Vermehrung der Antiserummenge (Abb. 1).

Auf Grund der in Abb. 1 angegebenen Gesetzmäßigkeit läßt sich, wenn die angewandten Antisera noch nicht 100%ige Hemmung verursachen, die Hemmungswirkung von 1 ml Antiserum aus der in Anwesenheit von Kontroll- und Antiserum (oder von zwei verschiedenen Antiserummengen) gemessenen Aktivitätsdifferenz errechnen. Nach den Angaben der Tabelle II ruft 1 ml Antiserum A 260 PGM-Einheiten $\times 10^{-3}$ Hemmung hervor.

Tabelle II

Effekt der Veränderung der Enzym- und Antiserummenge auf das Ausmaß der Hemmung

Zur Anwendung gelangten		
PGM Einheiten $\times 10^{-3}$	Antiserum ml	Hemmung PGM-Einheiten $\times 10^{-3}$
20	0,06	15
20	0,08	17
20	0,10	20
20	0,11	20
20	0,12	20
25	0,10	25
37	0,10	30
50	0,06	19
50	0,08	22
50	0,10	30
50	0,15	41
50	0,20	50
50	0,30	50
70	0,10	23
70	0,15	34
70	0,20	48
75	0,20	55

Die Zusammensetzung der Proben ist dieselbe wie in Tabelle I, in 1 ml Gesamtvolumen sind die Enzym- und Antiserummengen auf die angegebene Weise variiert.

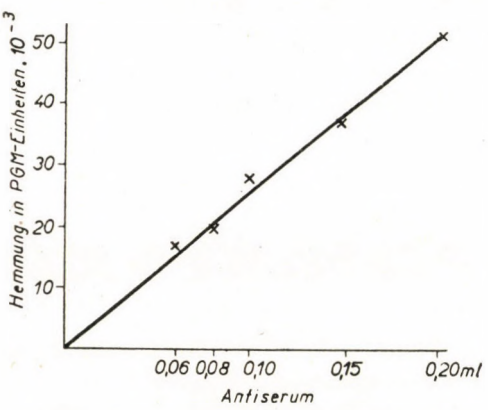

Abb. 1. PGM-hemmende Wirkung verschiedener Mengen von Antiserum. Die Angaben stellen die Mittelwerte der Tab. II. dar.

Der verhältnismäßig große Antienzymgehalt des Antiserums ergibt sich daraus, daß die Hemmung in Anwesenheit von 0,06 ml Antiserum noch gut meßbar war (d. h. bei 6%iger Antiserumkonzentration) und ebenso in Anwesenheit von 0,03 ml Antiserum (3%ige Konzentration). Mit Hilfe dieser quantitativen Methode läßt sich die Hemmungsfähigkeit der am 16. und 30. Tage gewonnenen Antisera vergleichen:

	A	B	C			
16. Tag	100	125	75	Einheiten	$\times 10^{-3}$	Hemmung
30. „	275	245	230	„	$\times 10^{-3}$	„

Die Spezifität des aus dem Hahnenmuskel stammenden PGM-Antiserums

Zur Nachprüfung der Spezifität des Antiserums untersuchten wir, ob die Phosphoglukomutase sog. Organspezifität aufweist. Das Muskelphosphorylase-Antiserum übt bekanntlich auf die Leberphosphorylase keine Wirkung aus und hemmt die Herzmuskelphosphorylase nur sehr wenig [2], ferner wird die Muskelphosphorylase vom Leberphosphorylase-Antiserum nicht und die Herzmuskelphosphorylase nur in geringem Ausmaß gehemmt [1].

Die Ergebnisse der mit PGM-Antiserum durchgeführten Spezifitätsversuche sind in Tabelle III enthalten.

Tabelle III

Hemmungswirkung des aus Hahnenmuskel stammenden PGM-Antiserums auf die Aktivität der Herz- und Leber-PGM des Hahnes

	Zur Anwendung gelangten			
	PGM Einheiten $\times 10^{-3}$	Antiserum ml	Hemmung PGM-Einh. $\times 10^{-3}$	Hemmungsfähigkeit von 1 ml Antiserum PGM-Einh. $\times 10^{-3}$
Hahnenherz	54	0,025	15	600
	54	0,05	24	480
	53	0,05	23	460
	57	0,10	47	470
	57	0,20	57	—
Hahnenleber	53	0,025	9	360
	53	0,05	19	380
	53	0,10	39	390
	69	0,10	51	510
	69	0,20	69	—
Hahnenmuskel (Tab. II)	70	0,10	23	230
	70	0,20	48	240

Aus den Resultaten geht hervor, daß das Muskel-PGM-Antiserum auch auf die Aktivität der Herz- und Leber-PGM ausgesprochene Hemmungswirkung ausübt.

Eine interessante quantitative Differenz zeigt sich im Ausmaß der Hemmungsfähigkeit, indem die Herz- und Leber-PGM vom Muskel-PGM-Antiserum stärker gehemmt wird als die Muskel-PGM. Diese Resultate lassen den Schluß zu, daß die PGM — im Gegensatz zu den Phosphorylasen — keine grundlegenden organspezifischen immunologischen Unterschiede aufweist.

Die Hemmungswirkung des Hahnen-PGM-Antiserums untersuchten wir auch an anderen Geflügel-, Säuger- und Froschgewebsextrakten. Die Ergebnisse sind in Tabelle IV wiedergegeben.

Tabelle IV

Spezifität des Hahnenmuskel-PGM-Antiserums

Extrakt	Ausmaß der Hemmung in %
Hahnenmuskel	100
Entenmuskel	100
Entenleber	100
Taubenmuskel	100
Hundemuskel	∅
Rattenmuskel	1,5
Froschmuskel	∅
Froschleber	∅

Wie die Ergebnisse zeigen, ist das Hahnenmuskel-PGM-Antiserum imstande, die Mutaseaktivität des Muskels und der Leber auch anderer Geflügelarten zu hemmen. Dagegen hemmt sie nicht die PGM-Aktivität des Muskels bzw. der Leber von Säugern und Fröschen. Die Hahnen-PGM verfügt demnach über Geflügel- (= Klassen-) Spezifität.

Schließlich untersuchten wir, ob PGM-Antiserum auf andere Enzyme des Hahnenmuskelextraktes hemmend wirkt. Eine Antiserummenge, welche die PGM-Aktivität völlig hemmte, übte auf die Phosphorylase-Aktivität des Muskelextraktes überhaupt keine Hemmwirkung aus. Die Phosphohexoisomerase-Aktivität des Hahnenmuskelextraktes wurde vom Antiserum ebenfalls nicht gehemmt. Diese Angaben beweisen, daß das PGM-Antiserum weder Antiphosphorylase noch Antiphosphohexoisomerase enthält. Zu gleicher Zeit bestätigen sie den enzymspezifischen Charakter des Hemmungseffektes der Antimutase.

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Pharmacologia

THE ANALGESIC, HYPERGLYCAEMIC AND MOTOR EXCITATORY EFFECTS OF D-2,2-DIPHENYL-3-METHYL-4-MORPHOLINO-BUTYRIL-PYRROLIDINE (R-875, PALFIUM) AND THE TOLERANCE TO THESE EFFECTS

By

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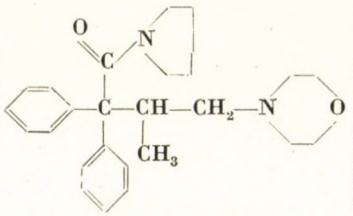
The analgesic, hyperglycaemic and hypermotility-inducing effects and the tolerance to these effects of D-2,2-diphenyl-3-methyl-4-morpholino-butyril-pyrrolidine (Palfium, R-875), a new analgesic have been investigated in comparison with morphine.

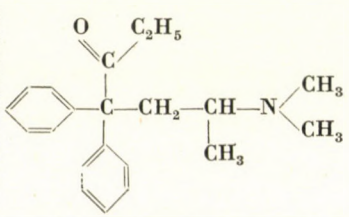
As a conclusion, it can be stated that of all the analgesics known R-875 is the most potent concerning the analgesic, hyperglycaemic and motor activity enhancing effects.

Tolerance develops fastest to the hyperglycaemic effect (which is hypothalamic in origin) of morphine and R-875 alike, with the analgesic effect of morphine coming next. No tolerance develops in either case to the cortical hypermotility and to the spinal HERMANN—STRAUB reaction.

P. A. J. JANSSEN *et al.* [1, 2, 3] have prepared a synthetic analgesic, D-2,2-diphenyl-3-methyl-4-morpholino-butyril-pyrrolidine (R-875, Palfium), which is far more active than the formerly known analgesic agents, including the numerous butyramide derivatives. The significance of the discovery is increased by the fact that, according to JANSSEN *et al.* [2], the rat would develop hardly any tolerance to the analgesic effect of the compound. Similar observations have been made on chronic administration in man by SOUPAULT *et al.* [4], as well as by DÁVID *et al.* [5]. The chemical structure of the compound is



D-2,2-diphenyl-3-methyl-4-morpholino-butyril-pyrrolidine (R-875, Palfium)



L-4,4-diphenyl-6-dimethyl-amino-3-heptanon HCl (Methadone)

The compound is chemically similar to Methadone, differing from the latter in the presence of a morpholine group instead of the dimethylamino one and in the acid amide type of the oxo group, which in Methadone is keton-type. The presence of pyrrolidine, a cyclic amine in acid amide bond, is

characteristic of R-875. Furthermore, the D-isomer of R-875 is active, whereas in the case of Methadone the l-isomer is the active one.

We have investigated the analgesic effect of R-875 in mice and rats, the hyperglycaemic effect in rabbits and the hypermotility-inducing effect in mice. Another problem to be investigated was the tolerance to these effects on chronic administration. All these effects were compared with those of morphine.

Methods

Analgesic effect and the tolerance to it. The analgesic effect was measured both in the mouse and the rat by the contact heat method developed by PÓRSZÁSZ and HERR, JANSSEN, and modified by us [2, 6, 7]. The modification was that after determining twice the normal response time we injected the drug to be tested subcutaneously, then the pain response time was again determined 15, 30, 45 and 60 minutes later. Applying the principle of "all or nothing", the response was considered positive only when the animal's normal response time is prolonged by 150 per cent. Did this not take place, the response was considered negative. At least 4 doses of each compound were tested, using for each dose 25 albino mice weighing 16 to 24 g or 15 white Wistar rats weighing 120 to 180 g. The results were analysed statistically by the probit method of LITCHFIELD and WILCOXON [8].

The development of tolerance was studied in rats. The ED_{98} dose (the dose exerting a 98 per cent analgesic effect) was administered; of morphine also the eightfold ED_{50} was tested in some cases. Groups of 15 rats weighing 120 to 180 g each were treated with these doses subcutaneously, between 8 and 10 o'clock in the morning, over a period of 30 days. Every other day the response time to pain was determined and the analgesic effect was measured as described above.

Hyperglycaemic effect and the tolerance to it. The hyperglycaemic effect was studied after 18 hours of fasting in 30 rabbits weighing between 1.8 and 2.5 kg. After drawing a control sample of blood, the drug was injected subcutaneously and 1 and 2 hours later a second blood sample was taken. Blood sugar was determined by the method of SOMOGYI and NELSON [9, 10].

The tolerance developing to that effect was investigated in groups of 6 rabbits, each animal weighing 1.5 to 2.2 kg. The animals were treated subcutaneously with equally effective doses of morphine hydrochloride and R-875, over a period of 28 days. Once every week (after 18 hours of fasting) a control blood sample was drawn. Subsequently, the usual dose was injected and the blood sugar level was determined 1 and 2 hours later.

The effect causing central nervous motor excitation (hypermotility) and the tolerance to it. The excitatory action was studied in mice, by the method of DEWS [11]. Groups of 5 mice each were used. The activity of the controls and of the experimental animals was determined at 15-minute intervals. In the 30th minute physiological saline or the drug to be tested was injected intraperitoneally and the changes in excitation were measured for 60 minutes. A total of 90 albino mice were involved in these experiments.

The tolerance was studied in 6 groups of 5 mice each. Three groups were treated intraperitoneally with morphine hydrochloride and 3 groups with R-875, over a period of 28 days. The degree of excitation was estimated once every week, by the method outlined above.

Results

Analgesic effect in the mouse and the rat. The data for the analgesic effects of morphine and R-875 in the rat are presented in Table I.

As it can be seen in Table I, the ED_{50} of morphine was found to be 3.1 mg/kg in the mouse (19/20 confidence limits, 2.21—4.34), whereas the ED_{50} of R-875 was 0.15 mg/kg (19/20 confidence limits, 0.133—0.169). R-875 proved to be 20.7 times more potent than morphine (19/20 confidence limits, 14.4—29.8).

Table I

Analgesic action of morphine-HCl and R-875 in mice and rats

Compound	Species	Number of animals	ED_{50} mg/kg s. c.	19/20 confid limits		S^{x}	$f^{xx}_{S.}$	$P.R.^{+}$	19/20 confid. limits		$S.R.^{++}$	$f^{+++}_{S.R.}$
				lower	upper				lower	upper		
Morphine-HCl	mouse	100	3.1	2.21	4.34	2.63	1.9	1.0	—	—	—	—
R-875	mouse	125	0.15	0.133	0.169	1.4	1.19	20.7	14.4	29.8	1.88	2.46
Morphine-HCl	rat	60	2.9	2.21	3.8	1.47	1.24	1.0	—	—	—	—
R-875	rat	85	0.15	0.103	0.217	2.75	1.51	19.4	12.3	30.6	1.87	1.6

S^{x} = slope
f^{xx}_{S} = slope factor
P. $R.^{+}$ = potency ratio
S. $R.^{++}$ = slope ratio
$f^{+++}_{S.R.}$ = factor slope ratio

In the rat, the ED_{50} for morphine was 2.9 mg/kg (19/20 confidence limits, 2.21—3.8), that for R-875, 0.15 mg/kg (19/20 confidence limits, 0.103—0.217), and its relative potency as compared to morphine, 19.4 (19/20 confidence limits, 12.3—30.6).

A comparison of the values for the ED_{50} showed R-875 to be nearly 20 times more active than morphine in mice and rats. As regards the ED_{98}, for R-875 this was in mice 0.3 mg/kg and for morphine, 8 mg/kg, *i.e.* the potency ratio was 26.6. In contrast with this, in the rat the ED_{98} for morphine was found to be 6 mg/kg and for R-875 1.2 mg/kg. This means that in the rat the potency ratio was only fivefold.

The time of the maximum effect is shown in Table II.

Table II

Duration of analgesic action of morphine-HCl and R-875 in the mouse

Compound	mg/kg s. c.	Number of animals	Percentage of positive reactions, minutes after treatment			
			15	30	45	60
Morphine-HCl	8.0	25	20	78.5	64	32
R-875	0.2	20	60	25	10	10

As seen in Table II, the maximum analgesic effect of 8 mg/kg morphine fell to 30 minutes and by the 60 th minute the effect was considerably reduced. With R-875 most of the positive reactions occurred in 15 minutes and in 30 minutes the effect was much weakened.

Tolerance to the analgesic effect. To study this effect, animals were subjected to chronic treatment with the respective ED_{98} of the drugs. The ED_{98} was

computed from the dose-response curves, and was 6 mg/kg for morphine and 1.2 mg/kg for R-875. The latter dose being 8-fold the ED_{50}, a similar dose of morphine, *i. e.* 24 mg/kg was also tested.

The data for these experiments are shown in Fig. 1. It is clear that during the 30 days of treatment the animals gradually became accustomed to morphine and after 27 days the ED_{98} had no analgesic effect in any of the animals.

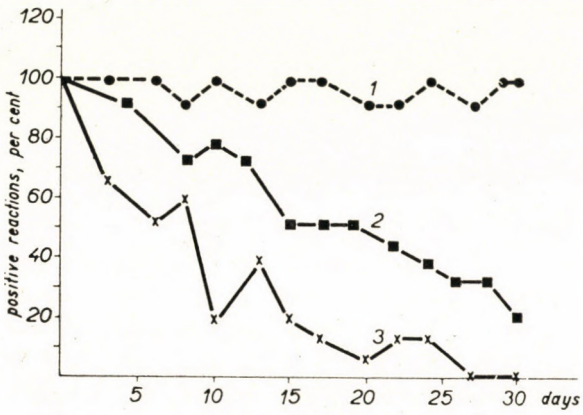


Fig. 1. Analgesic tolerance of morphine-HCl and R-875 in the rat
1: 1.2 mg/kg of R-875, subcutaneously, per day.
2: 24 mg/kg of morphine-HCl subcutaneously, per day.
3: 6 mg/kg of morphine-HCl subcutaneously, per day

Even the effect of the higher dose of morphine was considerably reduced by the end of the experimental period; 79 per cent of the animals developed tolerance to it. In contrast with this, the animals did not develop tolerance to R-875 during the experimental period, though some minor variations occurred. The animals exhibited also katatonia, sedation and exophthalmus. On the other hand, the animals treated with the smaller dose of morphine exhibited these symptoms only for 4 to 6 days, while those subjected to treatment with the larger dose, for 22 to 25 days.

Hyperglycaemic effect in the rabbit. The derivatives of opium and Methadone are known to give rise to hyperglycaemia [12, 13, 14, 15, 16]. According to D. T. WATTS, a correlation exists between the analgesic and the hyperglycaemic effects. Morphine and Methadone cause hyperglycaemia by acting on the supraspinal centres and mobilizing adrenaline. This effect can be inhibited by pentobarbital [15].

The hyperglycaemic potency of morphine and R-875 was investigated in rabbits, to determine whether the great difference existing between the analgesic effect of the two drugs would be reflected in their hyperglycaemic effects, and to estimate the ratio of the analgesic effect to the hyperglycaemic

one. We wished to find the dose of R-875 raising the blood sugar level in approximately the same measure as 10 mg/kg of morphine-HCl does. The results are presented in Table III.

Table III

Hyperglycaemic effect of morphine-HCl and R-875 in rabbits

Compound	mg/kg s. c.	Number of animals	Blood sugar mg per 100 ml mean ± S. E., hours after treatment			Maximum change
			0	1	2 hours	
Morphine-HCl	10.0	15	112.5 ± 6.35	165. ± 10.2	195.5 ±11.37	83.0 ± 9.7
R-875	0.25	6	116.0 ± 11.1	207.0 ± 19.8	167.0 ± 12.1	91.0 ± 14.3
R-875	0.5	9	105.0 ± 9.32	270.0 ± 18.9	160.0 ±12.18	165.0 ± 11.1

On subcutaneous administration, 0.25 mg/kg of R-875 was found to have the same hyperglycaemic effect as 10 mg/kg of morphine. 0.5 mg/kg of R-875 was about twice as potent as 10 mg/kg of morphine. Thus, the hyperglycaemic effect of R-875 was found to be about 40 times stronger than that of morphine.

The hyperglycaemic effect of morphine reached the maximum in 2 hours, whereas the effect of R-875 developed faster; the maximum was attained in the first hour. During the second hour the effect of both the 0.25 and the 0.5 mg/kg doses of R-875 showed a definite decline.

Tolerance to the hyperglycaemic effect. It is known that in chronic experiments tolerance develops to the hyperglycaemic effect of the opium alkaloids and of the sympathetic analgesics [17, 18, 19, 20, 21]. PHATAK *et al.* [21] have recommended this method for testing the tolerance to analgesics. The results are shown in Fig. 2 and Fig. 3.

It is clear from Fig. 2 that after one week of subcutaneous treatment there was hardly any more of a hyperglycaemic response to 10 mg/kg of morphine. Fig. 3 shows that the tolerance to R-875 in daily subcutaneous doses of 0.25 mg/kg developed less rapidly. After one week of treatment the hyperglycaemic response was still increasing, to decline after two weeks. Subsequently, the blood sugar level was raised much less, than initially.

Thus, the animals developed tolerance to the hyperglycaemic effect of morphine sooner than to that of R-875. In contrast with what happened in the case of the analgesic effect, in the late phase of the test period there did develop a tolerance to the hyperglycaemic effect of R-875.

Hypermotility in mice. It is well-known that analgesics cause excitation in the mouse [22, 23, 24]. This effect can be well measured quantitatively by the method of DEWS [11, 15].

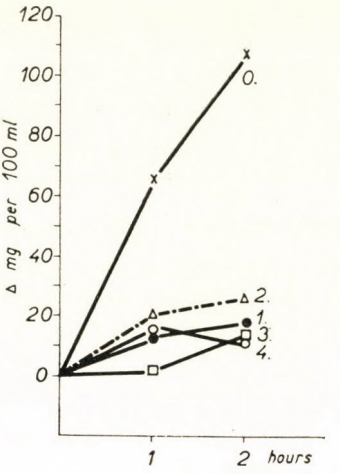


Fig. 2. Development of tolerance to the hyperglycaemic effect of 10 mg/kg of morphine-HCl subcutaneously. Rabbits. The figures indicate the weeks

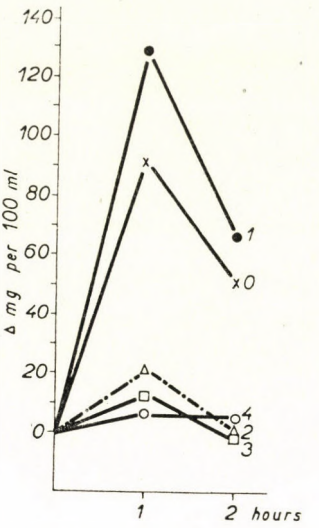


Fig. 3. Development of tolerance to the hyperglycaemic effect of 0.25 mg/kg of R-875, subcutaneously. Rabbits. The figures indicate the weeks

We have investigated the relation of the hypermotility caused by R-875 to the central nervous excitatory effect of morphine, as well as the quantitative correlation between the excitatory and analgesic potencies.

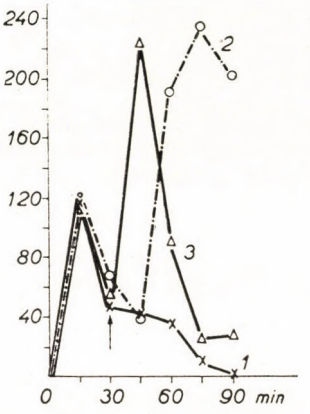


Fig. 4. Hypermotility caused in the mouse by morphine-HCl and R-875, respectively. Ordinata: number of interruptions of light.
1: physiological NaCl, intraperiteoneally.
2: Morphine-HCl, 20 mg/kg, intraperitoneally.
3: R-875, 1 mg/kg, intraperitoneally.
Arrows indicate injections of the drugs

The results are presented in Fig. 4.

The activity of the animals treated with physiological saline decreased gradually and ceased almost completely by the end of the experiment. Following the intraperitoneal administration of 20 mg/kg of morphine, after 30 minutes a significant increase of activity occurred, which reached its maximum 45 minutes after the injection, but was still significant after 60 minutes. In a

dose of 1 mg/kg, intraperitoneally, R-875 caused excitation faster than morphine and the maximum occurred 15 minutes after administration. Subsequently, excitation rapidly subsided. 1 mg/kg of R-875 injected intraperitoneally produced about the same degree of excitation as that caused by 20 mg/kg of morphine. Thus, as regards its hypermotility-inducing action, R-875 is 20 times more potent than morphine.

Tolerance to hypermotility. It is known that the forced motor activity caused by morphine in mice is the result of cortical stimulation [26, 27]. No tolerance develops either to this effect of morphine or to the tail reaction [26]. No comparable data are available for the synthetic analgesics.

In Fig. 5 is shown the excitation caused by the intraperitoneal injection of 20 mg/kg of morphine. After one week of treatment the excitatory response became stronger, then its intensity remained the same. No tolerance developed to this effect.

In Fig. 6 are shown the motor excitation responses to the intraperitoneal injection of 1 mg/kg of R-875. The same mild increase is observable which occurred with morphine. Like in the case of morphine, no tolerance to this effect developed during 28 days of treatment. The tail reaction of HERMANN—STRAUB was not abolished by either of the drugs.

Thus, mice developed no tolerance to the central nervous excitatory effect of either morphine or R-875.

Discussion

In both the mouse and the rat, D-2,2-diphenyl-3-methyl-4-morpholino-butyril-pyrrolidine has a more potent analgesic effect than any of the derivatives of morphine or Methadone thus far known. The relative potency data for mice are closely similar to those obtained by JANSSEN *et al.* [2, 3], who found R-875 to be 18.5 times more active than morphine. On the other hand, in the rat these authors found R-875 to be 40.5 times more potent than morphine. We observed no such difference between the two animal species. If the ED_{50} values were compared, R-875 proved to be about 20 times more potent than morphine in both species, but when testing the ED_{98}, the difference in potency was 26.6-fold in mice and only 5-fold in rats.

Tests in rabbits showed R-875 to raise the blood sugar level. Both the opiates and the synthetic analgesics are known to raise the blood sugar level [12, 13, 15, 16]. The similarity of its structure to that of Methadone suggests that R-875 might have raised the blood sugar level by acting on the supraspinal centres, mainly on the hypothalamus. Like in the case of morphine and Methadone derivatives, the excitation of sympathetic centres causes adrenaline mobilization, which, in turn, lowers the glycogen content of the liver [16]. Accordingly, hyperglycaemia induced by R-875 probably also develops as a result of sympathico-adrenal stimulation. In the rabbit, R-875 was found 40

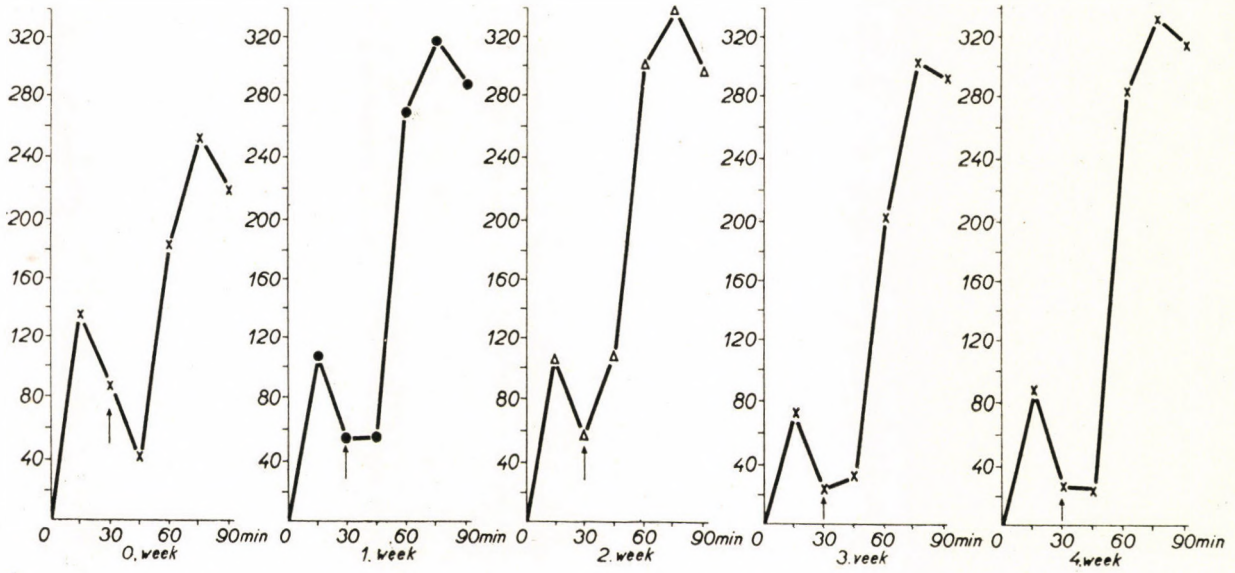


Fig. 5. Tolerance to the hypermotility caused by morphine-HCl in the mouse.
Ordinata : number of interruptions of light
Arrows indicate the injection of 20 mg/kg of morphine intraperitoneally

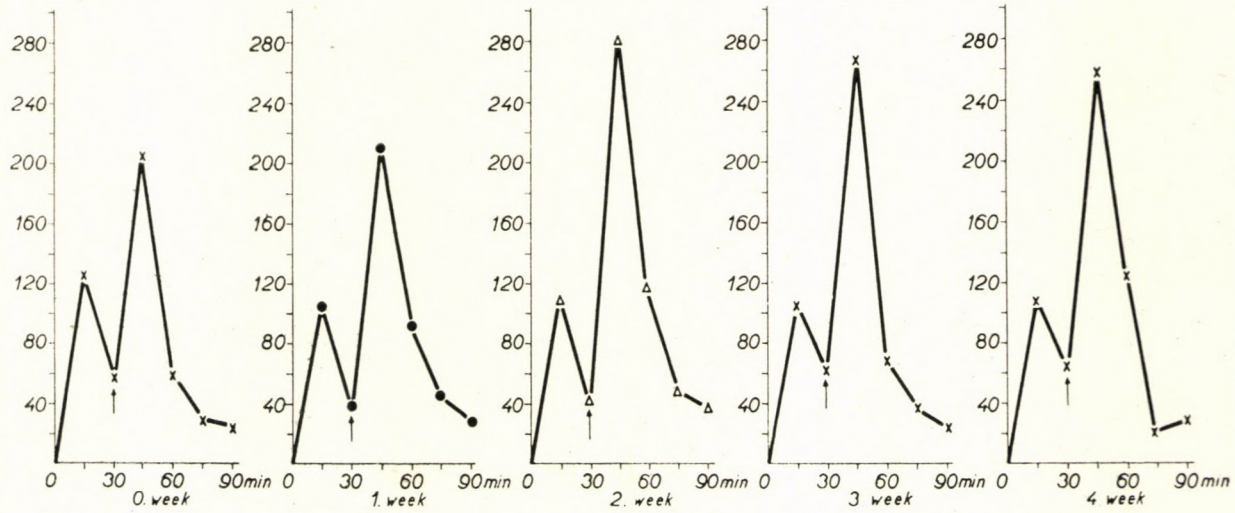


Fig. 6. Tolerance to the hypermotility caused by R-875 in the mouse.
Ordinata : number of interruptions of light.
Arrows indicate the injection of 1 mg/kg of R-875 intraperitoneally

times more potent than morphine as far as their hyperglycaemic effect is concerned.

In the mouse, R-875 caused hypermotility, just like morphine or Methadone. In this respect it was 20 times more active than morphine. This central excitatory effect showed the same quantitative values as the analgesic effect in mice. The central nervous stimulating effect being characteristic of analgesics, this method may prove useful in the determination of the potency ratio.

As to the duration of analgesic, hyperglycaemic and motor excitatory effects of R-875 it has been observed that these effects developed faster and disappeared faster than those of morphine. The maximum of the analgesic effect of morphine was reached in 30 minutes and then the activity declined slowly, whereas the same effect of R-875 attained the peak in 15 minutes, to disappear thereafter rapidly. The data obtained for the duration of the analgesic effect of R-875 agree well with those reported by JANSSEN *et al.* [3], but in our tests with morphine the maximum effect was observed to occur not after 60, but in 30 minutes. This finding is in agreement with the results of EDDY *et al.* concerning the duration of the morphine effect [28].

The maximum of the hyperglycaemic effect of R-875 was also reached earlier than that of morphine. The same applied to the hypermotility-inducing effect. Thus, as far as the duration and maximum of the effect are concerned, these phenomena changed in parallel. This proves that these effects of R-875 develop faster and disappear faster, obviously because of the rapid absorption and fast elimination of the drug.

In the tolerance of the rat to the analgesic action, differences were noted between R-875 and morphine. All the animals developed tolerance to the analgesic effect of 6 mg/kg morphine by the end of treatment (27 days), and even the 8-fold ED_{50} was tolerated by 79 per cent of the animals during the 30 days of treatment. In contrast with this, virtually no tolerance developed to the analgesic effect of R-875, except in one of 15 animals. These data agree well with those reported by JANSSEN *et al.* [2]. In addition to its analgesic actions, the drug retained its effects inducing sedation, katatonia and exophtalmus.

In the rabbit, the increase in the blood sugar content caused by equivalent doses of the two drugs decreased significantly in the course of treatment. With morphine, tolerance developed within 1 week, when the hyperglycaemic response to R-875 was still increasing. After two weeks of treatment, however, tolerance developed to the hyperglycaemic action of R-875, too. PHATAK *et al.* [21] made similar observations with Methadone. According to FINNEGAN *et al.* [15] and WATTS [16], the analgesics raise the blood sugar level by stimulating first the supraspinal autonomic centres (the hypothalamus, in the first place) and by a consecutive mobilization of adrenaline. Tolerance to this effect develops faster than to the analgesic effect, and may already be present when the analgesic effect is still demonstrable. However, species differences might

play a role and tests in some philogenetically higher animal would perhaps supply more information as to the response expectable in man. The data concerning both the analgesic and hyperglycaemic effects appear to indicate, however, that man will also develop tolerance to the new analgesic, although at a slower rate than to morphine or Methadone.

The motor excitation was increasing in intensity during treatment and no tolerance to that effect developed in the mice treated with either of the two drugs. Not only hypermotility, a cortical phenomenon, but also the HERMANN—STRAUB tail reaction, due to spinal excitation [30], was unaffected. Thus, according to our results, no tolerance develops during the test period to the cortical and spinal stimulating effects of morphine and R-875.

The results of the tolerance studies made in three different animal species indicate that of the effects tested it is the hyperglycaemic one (which is of hypothalamic origin), to which tolerance is developed at the fastest rate. The analgesic action of morphine on the postcentral gyrus comes next. No tolerance develops, however, to the cortical effect responsible for motor excitation and to the HERMANN—STRAUB phenomenon, which is spinal in origin.

Acknowledgement

We are indebted to Miss É. MÁRIÁSSY and Miss K. PÉCZER for technical assistance.

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Recensio

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»Die Muskeltätigkeit, Versuch einer Biophysik des quergestreiften Muskels«.

355 Seiten, 204 Abbildungen, Verlag der Ungarischen Akademie der Wissenschaften, Budapest, 1958.

Der Verfasser der sich seit vier Jahrzehnten mit dem Thema beschäftigt, behandelt nach einer kurzen historischen Einleitung das Problem in zwei Hauptteilen u. zw. »Zustandsformen der Aufbaubestandteile« und »Mechanik des Muskels«. Der erste Abschnitt, »Struktur des quergestreiften Muskels, Die Faser als die kleinste funktionelle Einheit« behandelt die strukturellen Eigenschaften des Muskels. Im zweiten Abschnitt, »Zustandsformen der einzelnen Muskelbestandteile« werden Rolle und Bedeutung des Myosins, Wassers und Kaliums besprochen. Der dritte Abschnitt, »Muskelerregung« befaßt sich hauptsächlich mit der Volumverminderung bei der Muskelkontraktion, ferner mit dem Aktionsstrom, Muskelschall und mit der Bedeutung weiterer Begleiterscheinungen, mit der Elektronenbetrachtung und der Halbleitertheorie im Erregungsproblem. Im vierten Abschnitt, »Spannung des Muskels« werden die Zustandsformen des Myosins bei Spannung und Druck besprochen. Der fünfte Abschnitt, »Die mechanische Seite der Muskeltätigkeit« beschäftigt sich mit der Latenzzeit, mit der spannungsfreien und spannungsliefernden Kontraktion, Arbeitsleistung und Wirkungsgrad. Der sechste Abschnitt, »Spezifische Funktion und Stoffwechsel des Muskels« gibt eine Übersicht über die Rolle des Muskels im Gesamtstoffwechsel, Arbeit und Stoffwechsel des isolierten Muskels und die mit der mechanischen Tätigkeit zusammenhängenden Stoffwechselprozesse. Zum Schluss werden im Abschnitt »Muskelmaschine« die Natur der Muskelerregung und die neueren Kontraktionstheorien ausführlich behandelt.

Nach jedem Abschnitt wird die Literatur angegeben, am Ende der Monographie ein ausführliches Namen- und Sachverzeichnis.

Die Monographie bedeutet in der Literatur unserer medizinisch-biologischen Wissenschaft eine in ihrer Art einzig dastehende, außerordentlich interessante Fachschrift mit ausdrücklich individueller Denkart und ist eine Wiedergabe einer beinahe 40jährigen, auf ein Problemkreis beschränkter konsequenter Forschungsarbeit. Der Leser erhält nicht nur eine gründliche Übersicht über die Physiologie der Muskeltätigkeit, sondern auch eine Orientierung über die prezise Methodik des Forschers, seine eigenartige Auffassung und originelle Denkart.

Die mit verschiedenen Methoden erhaltene Angaben werden stets vom Gesichtspunkt der strukturellen und funktionellen Einheit betrachtet. Die ständige Vervollkommung und Zeitgemäßheit der außerordentlich prezisen Methodik ermöglichte dem Verfasser, Mangelhaftigkeiten oder Fehler zu entdecken, die der Aufmerksamkeit gut bekannter Forscher dieses Gebietes entgingen. Die dialektische Denkart mit der inventionellen Bereitschaft für Methodik vereint führten zur Feststellung derartiger grundlegenden neuen Tatsachen, die dogmatisch herrschende Ansichten widerlegten und dieses Problem in vollkommen neue Beleuchtung setzten. Es soll nur als Beispiel die vom Verfasser ausgearbeitete Methode der Volummessung des Muskels erwähnt werden. Die große Empfindlichkeit dieser Methode ermöglicht — gleichzeitig mit dem Aktionsstrom — die Registrierung schneller und außerordentlich geringer Volumverminderungen, sowie bei der tetanischen Kontraktion auf die langsame größere Volumverminderung sich auflagernden geringeren und schnellen Volumveränderungen.

Charakteristisch für die konsequente Denkart des Verfassers ist, daß die Beantwortung dieses Problems ausschließlich auf Grund registrierbarer Tatsachen und Resultaten von Berechnungen, mit einer äußerst scharfen und unberücksichtigenden Logik auf ein bestimmtes »Ja« oder »Nein« eingeengt wird, ohne irgend welche Ausflüchte oder Kompromisse.

Es erscheint vielleicht ungewohnt, in einer derartigen Monographie die vor Jahrzehnten publizierten Versuche aufs Neue eingehend zu besprechen, oder über schein-

bar überwundene Probleme mit bereits verstorbenen Autoren zu disputieren. Nach der sorgfältigen Durchlesung der ganzen Monographie scheint das Bestreben zu dieser Vollkommenheit aber nicht überflüssig, da sich die Bedeutung und derzeitige Gültigkeit der vorher verschwiegenen oder unrichtig interpretierten Tatsachen in voller Stärke nur in diesem historischen Überblick erhebt. Dem konventionellen oberflächlichen Leser kann auch die Wiedererweckung früherer Prioritätsdebatten, sowie die dokumentierte Aufzählung der unrichtigen Zitate, das Verschweigen des Namens des Verfassers und seiner Ergebnisse, die Rücksendung seiner an verschiedene Redaktionen gesandten Manuskripte, usw. ebenfalls uninteressant und überflüssig erscheinen. Wenn man aber in Betracht nimmt, wie lehrreich eben das für ältere, hauptsächlich aber für jüngere Forscher ist, so ist es vielleicht besonders begründet, einmal auch über diese Frage, abweichend von der allgemeinen Sitte ein unverhüllt aufrichtiges Bild zu erhalten. Selbst durch das außerordentlich große Anwachsen der physiologischen Literatur in den letzten Jahrzehnten läßt sich die beinahe allgemeine Erscheinung der Polarisation auf sprachlichem und sonstigem Gebiet bei der Betrachtung und beim Zitieren der Literatur nicht berechtigen. Man muß allerdings annehmen, daß die offene und aufrichtige Bekanntgabe der Ereignisse der 35jährigen experimentellen Tätigkeit des Verfassers nicht allgemein mit Freude empfangen wird.

Die methodische Entwicklung und veränderte Betrachtung der letzten Jahre brachte es mit sich, daß die bisher als feststehend erscheinende Konzeptionen und Theorien auch auf engerem Gebiet der Muskeltätigkeit modifiziert, bzw. neue Wege gesucht werden müssen. Die völlige Liquidierung der bisherigen Theorien kann aber natürlich nur dann erwartet werden, wenn die neue Annahme restlos auf breiterem Gebiet mehr darbieten wird. Hier möchte ich erstens auf die Feststellungen des Verfassers über das »Alles-oder-Nichts-Gesetz« oder »Membrantheorie« der Erregung hinweisen.

Der Verfasser gibt eine breite und kühne Perspektive zur weiteren Forschung als er z. B. statt der Ionen-Theorie die Elektronen-Theorie in den Vordergrund setzt, oder auf die biologische Anwendung des Halbleiterprinzips hinweist.

Die Abbildungen der Monographie geben eine didaktische Ergänzung zu dem Text der prezisen Methoden des Verfassers. Die vorzüglich ausgestattete (Papier, Druck und Reproduktion der Abbildungen) Monographie wird ein hervorragendes, in Traditionen reiches Gebiet der ungarischen experimentellen Wissenschaft auch auf internationalem Niveau entsprechend representieren.

K. LISSÁK

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A kézirat nyomdába érkezett: 1958. XI. 7. — Terjedelem: 10,50 (A/5) ív, 48 ábra

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ACTA PHYSIOLOGICA

ACADEMIAE SCIENTIARUM HUNGARICAE

TOMUS XV. FASCICULUS 1

РЕЗЮМЕ

О ФУНКЦИИ ДЕНЕРВИРОВАННОЙ ПОЧКИ

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1. У наркотизированного животного выделение натрия и воды почкой, денервированной путем пересечения чревного нерва больше, чем иннервированной почкой противоположной стороны.

2. Гиперсалурия и полиурия в большинстве случаев сопровождаются большим инулиновым, или креатиновым клиренсами.

3. Между выделением натрия и разницей в инулиновом клиренсе не наблюдается корреляции. В отдельных периодах повышенное выделение натрия связано с меньшим инулиновым клиренсом. Повышенный диурез денервированной почки технически предрасполагает к более высоким клиренсам. На этой основе авторы считают одним фактором, вследствие денервации появляющихся гиперсалурии и полиурии, снижение канальцевой реабсорбции натрия.

4. Характерным для денервированной почки является то обстоятельство, что на натрий падает большая процентная ставка всех выделенных осмолов, чем в случае иннервированной почки.

5. У ненаркотизированного животного нет сигнификантной функциональной разницы между иннервированной и денервированной почками.

НАКОПЛЕНИЕ ИНУЛИНА И ПАГ В ПОЧЕЧНОЙ ТКАНИ

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1. При непрерывном вливании инулина и ПАГ и неизменной концентрации инулина и ПАГ в плазме, выделенное в одну минуту в моче количество меньшие, чем влитые дозы этих веществ. Следовательно, одна часть введенного количества инулина и ПАГ отлагается в организме или же употребляется иным путем. Меньшую часть исчисленной разницы авторы обнаружили в почках, где вещества накоплялись.

2. Кровопусканием вызывалась у собак анурия, а затем у них проводилось внутривенное вливание инулина и ПАГ. После двухчасовой анурии левая почка пересаживалась в шею собаки-реципиента, после чего авторы установили количества инулина и ПАГ в выделенной моче пересаженной почки, в моче собаки-реципиента, и количество, оставшееся в ткани пересаженной почки. Далее было установлено также содержание инулина и ПАГ в другой, непересаженной и «непромытой» почке. Оказалось, что «промытая» левая почка также содержит инулин и ПАГ.

3. Количество депонированного инулина и ПАГ не объясняет разницы, полученной обычными мочевыми или другими клиренсами. Полученных результатов нельзя рассматривать доказательством инулиновой секреции.

ДАННЫЕ К СПЕЦИФИЧЕСКОЙ ФУНКЦИОНАЛЬНОЙ АДАПТАЦИИ КОРЫ НАДПОЧЕЧНИКОВ

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Авторы дают общий обзор о своих исследованиях, проведенных в области производства гормонов корой надпочечников, и указывают на известные связи относительно

межжелезистой регуляции или же специфической гомеостатической функции коры надпочечников. Результаты их исследований можно резюмировать в нижеследующем:

1. В ходе биосинтеза наблюдались 19 таких кортикоидов, которые оказались $\triangle^4$-3-кетокортикоидами с α-кетол-боковой цепью на 17-ом С-атоме. Из этих кортикоидов 6 соединений выявляли более сильную полярность, чем гидрокортизон, 3 соединения обладали полярностью между гидрокортизоном и кортизоном; дальнейшие 3 между кортизоном и кортикостероном, в то время как полярность 4 соединений была меньше той кортикостерона.

2. Среди соединений, более полярных чем гидрокортизон, лимфолитическая активность двух соединений (III., IV.) была у крыс и мышей с удаленными надпочечниками выше активности гидрокортизона и кортизона, но их способность к накоплению гликогена была низкой.

3. Кортикоиды, наблюдящиеся в связи с биосинтезом, встречаются в венозной крови надпочечников различных видов животных, а также в ходе так наз. специфической адаптации.

Адаптационные изменения специфического характера при секреци надпочечников

а Половые стероиды вызывали качественные изменения в составе кортикоидов венозной крови надпочечников. У собак фолликулин (бензоат эстрона) в значительной мере уменьшает секрецию гидрокортизона, причем, в то же время появляется 17-гидроксипрогестерон. У кошек на действие фолликулина можно было выявить 2 новых соединения, а именно $\triangle^4$-3-кетокортикоида, полярность которых ниже той кортикостерона.

б При гипертиреозе в венозной крови надпочечников кошек появляются 5 новых кортикоидов; два из этих соединений задерживают секрецию тиреотропного гормона в большей степени, чем все известные нам кортикоиды. Проведенные наблюдения над человеческим материалом показали, что при гипертиреозе эти соединения выявляемы также и в моче. У собак с удаленной щитовидной железой, получившых метилтиоурацил удалось выявить повышение секреции кортикоидов и повышение соотношения гидрокортизон : кортикостерон.

в В венозной крови надпочечников различных видов животных, так например у диких и одомашенных крыс и у лисиц в секреции при покое наблюдаются такие кортикоиды, каких нельзя выявить в венозной крови надпочечников собак и кошек.

г Возбудители общего адаптационного синдрома, в том числе и успокаивающие средства, обусловливают специфическую реакцию, причем наблюдается лишь крайне повышенная секреция гидрокортизона. По мнению авторов, повышенная секреция кортикоидов объясняется процессами обмена веществ и потребностью организма в кортикоидах, ибо полярные кортикоиды сильно повышают центральную возбудимость и одновременно также вегетативный симпатический тонус. В случае применения успокаивающих средств, данное действие проявляется в качестве компенсаторного гомеостатического механизма. Подобное явление наблюдается в связи с изменением поведения после известных хирургических вмешательств в нервную систему (удаление ядра миндалины, фронтальная лейкотомия).

Резюмируя исследованные явления, авторы приходят к тому заключению, что кора надпочечников — сверх рамок общего адаптационного синдрома — участвует со специфической реакцией в поддержании гомеостаза организма. Наблюдаемые у различных видов животных явления указывают на то, что эта специфическая адаптация модифицируется также отклонениями отдельных видов, обусловленными разницами в тканевом обмене веществ коры надпочечников.

СЕКРЕЦИЯ КОРТИКОСТЕРОИДА В ПРИДАТОЧНЫХ НАДПОЧЕЧНИКАХ

П. ВЕЙС, Л. ХОРВАТ и Т. КАДАШ

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Авторы проводили в экспериментах над крысами с удаленными надпочечниками в различные сроки после операции определения кортикостерона в вытяжке венозной крови надпочечников. Взятием венозной крови надпочечников они хотели собирать кровь из ткани *придаточных надпочечников*, расположенных в жировой ткани, окружающей удаленные надпочечники. Согласно их результатам уже в первые дни после удаления

надпочечников можно выявить известное выделение кортикостерона, которое впоследствии дальше повышается. Следовательно, нельзя говорить об отсутствии гормона, а лишь о состоянии гипофункции.

ИЗМЕНЕНИЕ УГЛЕВОДНОГО ОБМЕНА ГОЛОВНОГО МОЗГА У КРЫС НА ДЕЙСТВИЕ РАБОТЫ

В. МАТЕ, ДЬ. КАШШАИ, Б. ХУНКАР, и Л. КИШ

Биохимическая лаборатория Государственного нервопсихиатрического института

Авторы исследовали у крыс обоих полов, не достигших половой зрелости, после их плавания в течение 3 часов, содержание гликогена, глюкозы, молочной кислоты и пировиноградной кислоты в мозге.

При сигнификантно повышенном содержании гликогена, в количестве остальных исследованных веществ сигнификантной разницы не наблюдалось.

Из результатов авторов следует, что общераспространенный взгляд о стабильности гликогена нуждается в проверке. По крайней мере в известных условиях между функциональной нагрузкой нервной системы и изменением содержания гликогена в мозге наблюдается параллельность.

ДЕЙСТВИЕ ГИПОТЕРМИИ НА ОСВОБОЖДЕНИЕ ГИСТАМИНА

Т. СИЛАДЬИ, Х. ЧЕРНЬЯНСКИ, О. ВЕРЕШ и Б. ЦЕНКАР

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Авторы, применяя методику Янчо, установили, что накопление красящих веществ уменьшается по мере снижения температуры тела ; данный эффект можно отнести к снижению освобождения гистамина. Большие дозы кортизона или ларгактила — главным образом последнего — уменьшают накопление красящих веществ, однако, их действие не обусловливается задержкой освобождения гистамина.

ДЕЙСТВИЕ НЕОРГАНИЧЕСКИХ ИОНОВ НА АДРЕНАЛИНОВЫЕ РЕАКЦИИ

Т. СИЛАДЬИ, И. САТАИ и Б. ЧАБА

Патофизиологический институт Медицинского университета, Дебрецен

Авторы исследовали задерживающее адреналиновые эффекты действие ионов кадмия, марганца, никкеля, олова и ванадия. Самыми эффективными из названных ионов оказались ионы кадмия — они задерживали адреналиновую реакцию на сердце лягушек, на Тренделенбургской лягушке, на кровяном давлении и селезенке собак. Авторы выявили, что задерживающий эффект не основывается на блокировании одновалентной группы (SH). По их имению в антиадреналитическом действии неорганических ионов важную роль играет изменение пермеабильности клеток.

ДЕЙСТВИЕ ТРИПТОФАНА НА ЧИСЛО ЛЕЙКОЦИТОВ

Я. РИГО, Ф. ТАКАЧ, и Й. ШОШ

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Авторы исследовали у крыс действие триптофана на число лейкоцитов и на качественную картину крови. Они установили :

1. Недостаток в триптофане вызывает лейкопению, которая после парентеральной подачи 10 мг/100 г. триптофана прекращается.

2. У нормальных животных введенные парентерально 10 мг/100 г триптофана вызывает в 24 час выраженный лейкоцитоз. Данного явления нельзя вызвать 6 другими аминокислотами, и поэтому авторы считают вероятным, что в данном случае речь может быть о специфическом действии триптофана.

3. На действие триптофана в первые часы возникают лимфопения и гранулоцитоз, проявляющиеся выраженне всего от 2—4 часов. Данное действие можно вызвать и другими аминокислотами (Стрессор эффект).

4. Путем экстирпации надпочечников удалось отражать возникающее на действие триптофана ранее изменение картины крови, однако, появляющийся в 24-ый час лейкоцитоз неизменно наблюдался и в этом случае.

ПОВЕДЕНИЕ ОБМЕНА У КРЫС ПОСЛЕ ПОВРЕЖДЕНИЯ ГИПОТАЛАМУСА В ТЕПЛОТЕ И ЕГО ОТНОШЕНИЯ К ТЕРМОРЕГУЛЯЦИИ В ХОЛОДНОЙ СРЕДЕ

ДЬ. МЕШТЬЯН, Б. МЕШ, ДЬ. СЕГВАРИ и С. ДОНХОФФЕР

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Двусторонние электролитические повреждения гипоталамуса препятствовали у приблизительно одной третьи подопытных крыс гипертермическому повышению теплообразования, которое при одинаковых экспериментальных условиях и одинаковой гипертермии у неповрежденных животных всегда состоится. Подобное явление наблюдается и после односторонних повреждений. Гипертермическое повышение обмена может не проявляться при сохранении химической регуляции, однако, оно может образоваться также в случае отсутствия последнего. С другой стороны, гипертермическое повышение обмена может полностью сохраняться также в случае отсутствия химической регуляции. Обсуждаются, далее, локализация, также как и результирующиеся заключения и выводы.

О ПОЛУЧЕНИИ ПРОТИВОСЫВОРОТКИ ФОСФОГЛЮКОМУТАЗЫ И ЕЕ СВОЙСТВАХ

ДЬ. БОТ и И. РЕДАИ

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Авторы изготовили из мышцы петуха в высшей степени очищенный препарат фосфоглюкомутазы. С этим препаратом они — при помощи подсобного средства — иммунизировали кроликов.

Сыворотка обработанных кроликов показала на 16 день незначительное, а на 28 день значительное тормозящее фосфоглюкомутазу действие. Далее они разработали методику для количественного измерения содержания антимутазы в сыворотках. Антимутаза тормозит мутазную активность домашних птиц (петухов, голубей, уток) не только в вытяжках мышц, но и сердца и печени, и следовательно фосфоглюкомутаза не обладает специфичностью по отношению к органам. Полученная авторами противосыворотка не тормозила мутазной активности у млекопитающих и лягушек, значит, фосфоглюкомутаза петухов обладает специфичностью по отношению к домашним птицам (= классовая специфичность).

БОЛЕУТОЛЯЮЩЕЕ, ГИПЕРГЛИКЕМИЧЕСКОЕ И ВЫЗЫВАЮЩЕЕ ДВИГАТЕЛЬНОЕ ВОЗБУЖДЕНИЕ ДЕЙСТВИЯ Д-2,2-ДИФЕНИЛ-3-МЕТИЛ-4-МОРФОЛИНОБУТИРИЛПИРРОЛИДИНА (П-875, ПАЛФИУМ) И ИССЛЕДОВАНИЯ ТЕРПИМОСТИ В ОТНОШЕНИИ ЭТИХ ДЕЙСТВИЙ

Й. БОРШИ, Э. ЧАНЬИ, Г. ЛАЗАР и Ж. ЧАК

Фармакологический научно-исследовательский институт, Будапешт

Авторы исследовали по сравнению с морфином болеутоляющее, гипергликемическое и вызывающее повышенную подвижность действия нового болеутоляющего сред-

ства д-2,2-дифенил-3-метил-4-морфолинобутирилпирролидин (Палфиум, П-875) и терпимость в отношении этих действий.

1. Как у мышей, так и у крыс П-875 оказался почти 20 раз более эффективным в своем болеутоляющем действии, чем морфин. Действие П-875 достиг своего максимума быстрее чем морфин, но оно снизилось быстрее.

2. У кроликов обусловленная 10 мг/кг морфина гипергликемия была вызвана 40 раз меньшим количеством П-875, а именно введенными подкожно дозами в 0,25 мг/кг. Его действие проявлялось быстрее чем в случае морфина.

3. У мышей П-875 вызывал 20 раз более интенсивную повышенную подвижность чем морфин. В данном случае соотношение эффективности действия то же самое, как в случае болеутоляющего эффекта. Действие П-875 быстро развивалось и также быстро прекращалось.

4. Все крысы, получившие ED_{98} (6 мг/кг) морфина, показали терпимость к болеутоляющему действию на 27 день обработки. На 30 день обработки 79 процентов животных показали терпимость к морфину в дозах 24 мг/кг (8 ED_{50}), в то время как интенсивность болеутоляющего действия ED_{98} (1,2 мг/кг) П-875 не уменьшалась в течение 30 дневной обработки животных.

5. У кроликов терпимость к гипергликемическому действию 10 мг/кг хлористоводородного морфина и 0,25 мг/кг П-875 проявлялась одинаковым образом. Терпимость к морфину развивалась в течение одной недели, тогда как гипергликемическое действие П-875 в это время еще повышалось ; терпимость к действию этого средства развивалась лишь после двунедельной обработки.

6. Обусловленная внутрибрюшинным впрыскиванием 20 мг/кг морфина или 1 мг/кг П-875 повышенная подвижность повышалось у мышей в течение хронической обработки. В течение 28 дневной обработки терпимости к этому действию не проявлялось.

Реакция Херрманна—Штрауба также осталась неизменной.

Авторы пришли к тому заключению, что из всех известных до сих пор болеутоляющих средств П-875 является в отношении болеутоляющего, гипергликемического и повышающего подвижность действий наиболее эффективным средством.

Терпимость развивается быстрее всего в отношении гипергликемического действия (которое исходит от гипоталамуса), равным образом, как в случае морфина, так и П-875. Затем следует болеутоляющее действие морфина. Ни при одном из исследованных средств не развивается терпимости по отношению к корковой повышенной подвижности и к спинной реакции Херманна—Штрауба.

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РОЛЬ КАЛЬЦИЯ В ПРОНИЦАЕМОСТИ ЧЕЛОВЕЧЕСКИХ ЭРИТРОЦИТОВ ДЛЯ КАЛИЯ

ДЬ. ГАРДОШ
Химический институт Медицинского университета, Будапешт

В присутствии NaF, $NaF+Na_2HAsO_4$, также как и моноиодуксусной кислоты (MJE) + аденозина, калий с большой скоростью выходит из эритроцитов в сыворотку. Этот выход калия состоится лишь в том случае, если в системе присутствуют ионы кальция. На этой основе можно предполагать, что кальций играет важную роль в регуляции ионного транспорта эритроцитов.

ИССЛЕДОВАНИЕ РАЗЛИЧИЙ МЕЖДУ МЕТАКОЛЛАГЕНОМ И КОЛЛАСТРОМИНОМ

И. БАНГА и Й. БАЛО
1. Институт Патологической анатомии и Экспериментальной-онкологии Медицинского университета, Будапешт

1. Авторы исследовали коллагенные фракции, полученные из Ахиллесова сухожилия рогатого скота. Было установлено, что метаколлаген и колластромин, несмотря на то, что оба вещества представляют собой нерастворимые компоненты коллагена, все же не идентичны.

2. Различия между метаколлагеном и колластромином можно выявить кислотным набуханием, способностью присоединить серебро, и сопротивлением против протеолитических групп. Результаты одновременно служат и доказательством того, что нерастворимые фракции не идентичны с исходным нативным коллагеном.

3. Авторы объясняют различия между нативным коллагеном, колластромином и метаколлагеном на основе содержания проколлагена, мукоида$_1$ и мукоида$_2$.

АКТИВИЗАЦИЯ ПРЕКОРТИКОТРОПИНА

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Изготовленная методом Юнга (Young) вытяжка передней доли гипофиза при 20-кратном разбавлении не уменьшает содержания аскорбиновой кислоты в надпочечниках у крыс с удаленным гипофизом. Однако, эта вытяжка активизируется, если ее pH с помощью соляной кислоты устанавливается на 3, или если к ней прибавляют раствор мочевины. Результаты опытов подтверждали предположение Дасгупта (Dasgupta) и Юнга о существовании прекортикотропина. Вытяжкой гипоталамуса при примененных авторами методах не удалось активизировать прекортикотропина.

ДЕЙСТВИЕ ПОВРЕЖДЕНИЯ ПАРАВЕНТРИКУЛЯРНОГО ЯДРА НА ФУНКЦИЮ СИСТЕМ ГИПОФИЗ—ЩИТОВИДНАЯ ЖЕЛЕЗА—КОРА НАДПОЧЕЧНИКОВ И ПОЛОВЫХ ЖЕЛЕЗ

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Авторы установили в своих опытах, что после повреждения паравентрикулярного ядра у крыс накопление J^{131} щитовидной железой и радиоактивность осаждаемого J^{131} в плазме уменьшаются, хотя потеря в весе наблюдалась лишь у самцов. Содержание кортикоидов в ткани надпочечников подопытных животных после операции выраженно уменьшается, что указывает на гиперактивность надпочечников. Метротрофная активность передней доли гипофиза повышается у обоих полов, однако, у самок повышение более выраженное чем у самцов.

О ДЕЙСТВИИ ПОВРЕЖДЕНИЙ НАДБУГОРЬЯ (EPITHALAMUS) НА ОБМЕН И ТЕМПЕРАТУРУ ТЕЛА У КРЫС, И СРАВНЕНИЕ С ПОВЕДЕНИЕМ ПОСЛЕ ПОВРЕЖДЕНИЙ ПОДБУГОРЬЯ (HYPOTHALAMUS)

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На электролитические повреждения надбугорья у крыс в большом процентном соотношении возникают нарушения регуляции обмена и температуры тела. Частота и комбинации отдельных нарушений показывают в своей совокупности характерную для повреждений надбугорья картину, которая отклоняется от той, наблюдаемой после повреждений подбугорья.Самые значительные отклонения по сравнению с повреждениями подбугорья суть следующие : *а)* гораздо чаще наблюдаемое отсутствие гипертермического повышения обмена, *б)* повышенный основной обмен реже сопровождается лихорадочно повышенной температурой тела, *в)* отсутствие снижения основного обмена до субнормальных величин, *г)* в окружающей среде 20—22° С в большинстве случаев наблюдается нормальная температура тела, даже при отсутствии химической регуляции, *д)* нарушение химической регуляции никогда не имеет места без одновременного отсутствия гипертермического повышения обмена. Сигнификантной разницы между повреждениями надбугорья и повреждениями подбугорья не наблюдалось : *а)* в частоте нарушения химической регуляции, *б)* в частоте отсутствия гипертермического повышения обмена при отсутствии и при сохранении химической регуляции, и *в)* в степени гипертермии в окружающей среде 35° С при сохраненном, отсутствующем и восстановленном гипертермическом повышении обмена. «Вторая химическая регуляция» температуры наблюдается также при отсутствии гипертермического повышения обмена. Авторы приписывают области надбугорья непренебрегаемое значение в регуляции обмена и температуры тела.

НЕМЕДЛЕННОЕ ДЕЙСТВИЕ Л-ТИРОКСИНА, Л-ТЕТРАИОДОТИРОУКСУСНОЙ КИСЛОТЫ, ДИИОДОТИРОНИНА, ДИИОДОТИРОУКСУСНОЙ КИСЛОТЫ И Л-ТИРОНИНА НА ПОТРЕБЛЕНИЕ КИСЛОРОДА И НА ТЕМПЕРАТУРУ ТЕЛА У КРЫС С УДАЛЕННЫМ ГИПОФИЗОМ. ДЕЙСТВИЕ КОРТИЗОНА

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Следующие препараты вызвали у крыс с удаленным гипофизом острую реакцию подобно ответу, вызванному трииодотиронином и его уксуснокислотным аналогом : л-тироксин (лаб. ГЛАКСО), л-тетраиодтироуксусная кислота (лаб. ГЛАКСО), л-дииодтиронин (лаб. ГЛАКСО), 3,5-дииодтиронин (ГОФФМАНН ЛА РОШ), л-дииодтироуксусная кислота (лаб. ГЛАКСО). Л-тиронин (ГОФФМАНН ЛА РОШ) оказался совершенно недействующим Кортизон отражал немедленный ответ на все исследованные иодтиронины. Авторы

излагают и оценивают различия, проявляющиеся в реакциях на различные препараты. Точную природу действующих веществ до тех пор нельзя выяснить, пока не будут получены чистые соединения, ибо дииодтиронин содержит больше, чем одно из иодированных соединений.

О ЗНАЧЕНИИ ГИПЕРТЕРМИЧЕСКОГО ПОВЫШЕНИЯ ОБМЕНА В ТЕРМОРЕГУЛЯЦИИ. ОПЫТЫ С ПОВРЕЖДЕНИЕМ ГИПОТАЛАМУСА И ЭПИТАЛАМУСА НА КРЫСАХ

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Авторы получили на основе своих опытов следующие результаты: у животных с наличием и с отсутствием гипертермического повышения обмена после повреждения гипоталамуса или эпиталамуса нельзя выявить разницы температуры тела в теплой среде. Из этого наблюдения следует: *а)* что гипертермическое повышение обмена не служит удовлетворению потребности в энергии теплоотдающих механизмов, ибо в этом случае отсутствие должно было бы сопровождаться более интенсивным повышением температуры тела, и *б)* что вызванная теплой средой гипертермия в известных пределах не может быть приписана пассивному удерживанию тепла, так как при повышенном теплообразовании следовало бы наблюдать и повышенную температуру тела. Следовательно, с одной стороны гипертермическое повышение обмена не является обусловленным повышением температуры тела (значит оно не находится в соответствии с правилом *ван'т Гоффа*), а с другой стороны, повышение температуры тела, в известных пределах, независимо от повышения обмена энергии. Оба явления представляют собой феномены, регулируемые центральной нервной системой, однако, они не находятся во взаимной тесной причинной связи.

ДЕЙСТВИЕ ПРЕПАРАТОВ ДЕКСТРАНА РАЗЛИЧНОГО МОЛЕКУЛЯРНОГО ВЕСА НА КРОВЯНОЕ ДАВЛЕНИЕ И ДЛИТЕЛЬНОСТЬ ВЫЖИВАНИЯ КОШЕК В ГИПОВОЛЕМИЧЕСКОМ ШОКЕ

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Авторы исследовали действие различных препаратов декстрана на кровяное давление кошек в гиповолемическом шоке, вызванном кровопотерей в 25 мл/кг в наркозе эвипан-натрия. Не было найдено разницы между измеренном в течение трех часов прессорным эффектом декстравена (препарата декстрана большого молекулярного веса) и измеренным таким же способом действием плазмодекса (препарата декстрана более низкого молекулярного веса). У декстравена, однако, летальное количество вторичного кровопускания было значительно больше. У кошек, содержанных после кровопускания такого же размера в течение одного часа в состоянии пониженного кровяного давления без подачи заменяющего плазму средства, жизнеспасающее действие обоих препаратов распространялось только на несколько часов, а не на дни. Если однако, после кровопотери такого же размера количество крови возмещалось в пределах приблизительно 15 минут с препаратами большого молекулярного веса (интрадекс) или более низкого молекулярного веса (плазмодекс) то жизнеспасающее действие обоих препаратов декстрана проявлялось у приблизительно двух третьей части животных еще и 48 часов спустя, тогда как в случае введения физиологического раствора поваренной соли только 17% кошек выживали тяжелую потерю крови.

В последних опытах при измерении кровяного давления кошек 3, 6, 12 и 22 часов после введения препаратов декстрана не наблюдалось значительной разницы между продолжительностью и интенсивностью повышающего кровяное давление действия двух видов препаратов. Ни препарат с большим молекулярным весом, ни тот с низким молекулярным весом не могли вызвать длительного восстановления кровяного давления у кошек, потерявших большое количество крови.

ФАРМАКОЛОГИЧЕСКОЕ ИССЛЕДОВАНИЕ НОВОГО ПРОТИВОСПАЗМАТИЧЕСКОГО СОЕДИНЕНИЯ

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1. Авторы исследовали в своих опытах новое соединение завода Хиноин, аналогичное соединение папаверину: хинопарин (1— (3′—4′-диметоксифенил-)6—7-диметоксиизохинолин).

2. Хинопарин проявляет одинаковое с папаверином спазмолитическое действие на коронарные сосуды у кошек, на изолированные легкие и двенадцатиперстную кишку in situ морских свинок, также как и на матку крыс. Его понижающее кровяное давление действие на 30% сильнее действия папаверина.

3. Хинопарин при примененных авторами дозах не повышает венозного давления, в то время как папаверин — при одинаковых дозах — значительно повышает его.

4. В исследованиях над сердцем кошек in situ (с помощью кардиометра Хендерсона) ослабляющее сердце действие папаверина значительно сильнее действия хинопарина.

5. У крыс, при внутрибрюшинном и внутривенозном введении, хинопарин оказался 2 раза или же 2,8 раз меньше токсическим чем папаверин.

ИССЛЕДОВАНИЕ НЕКОТОРЫХ ФАРМАКОДИНАМИЧЕСКИХ СВОЙСТВ ГИДРОКСИЗИНА (ATARAX)

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В первой серии опытов авторы исследовали действие гидроксизина (внутривенно 5—36 мг/кг) на вызванные раздражением гипоталамуса и прочих центральных частей вегетативной нервной системы изменения кровяного давления и электрокардиограммы. Подопытными животными были 8 кошек и 4 собаки. Электрическому раздражению однополюсными электродами подвергались 45 отдельных точек базальной области мозга. Раздражение 14 из этих точек вызвало изменения кровообращения. Вызванное раздражением гипоталамуса выделение адреналина удалось предотвратить рассечением спинного мозга (C_7—D_1, или D_2—D_3).

Полученные главные результаты следующие:

1. Гидроксизин снижает, и даже полностью прекращает, явления повышенного и пониженного кровяного давления, вызванные раздражением гипоталамуса и прочих центральных частей вегетативной нервной системы.

2. Гидроксизин оказывает меньшее действие на снижение кровяного давления, предшествующее вызванному раздражением гипоталамуса повышению кровяного давления, также как и на выделение адреналина, обусловленное раздражением гипоталамуса или обонятельного мозга.

Во второй серии опытов авторы исследовали над 9 кошками и 6 собаками (после подачи кураре) действие гидроксизина на электрокардиограмму в отношении стандартного и предсердечного верхушечного отведений. Эта серия опытов показала, что внутривенная подача гидроксизина в дозах 5—10 мг/кг способствует реполяризации сердечной мышцы: повышение амплитуды зубца Т или переход отрицательного зубца Т в положительный. Данное действие не находится в связи с вызванным гидроксизином изменением кровяного давления.

В третьей серии опытов авторы исследовали действие гидроксизина на изолированное сердце лягушки. Опыты показали, что большие дозы оказывают отрицательное инотропное влияние. Гидроксизин снижает или даже совершенно отражает отрицательное инотропное влияние ацетилхолина и калия. В противоположность этому гидроксизин не имеет действия на положительное инотропное влияние адреналина и кальция.

Biochemia

THE ROLE OF CALCIUM IN THE POTASSIUM PERMEABILITY OF HUMAN ERYTHROCYTES

By

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In the presence of NaF, NaF + Na_2HAsO_4, as well as iodoacetic acid + adenosine, K rapidly leaves the erythrocytes. This K outflow into the serum takes place exclusively in the presence of Ca ions. This allows to conclude to the important role of Ca in the regulation of the ion transport of erythrocytes.

*

In the human erythrocytes as in all anuclear red blood corpuscles, glycolysis is responsible for the maintenance of unequal ion distribution and of the apparent impermeability to cations. This unequal distribution of ions will cease and the exchange of ions between erythrocytes and serum will start if glycolysis is blocked by some inhibitor, for instance NaF or iodoacetic acid (IA). At higher concentrations of NaF, NaF + Na_2HAsO_4, as well as IA + adenosine, the rate of K outflow from the cells (and that of Na influx) is very high, 70 to 170 mg K/100 ml erythrocytes/hour [1, 2]. This rapid outflow of K can be prevented with ethylenediamine tetraacetate (EDTA), in the presence of which the rate of K outflow is reduced to 6 mg/100 ml erythrocytes/hour [3].

Investigations on the effect of EDTA showed that the presence of Ca is essential for the high rate of K outflow. Experiments carried out in this field will be described in the present report.

Methods

Fresh defibrinated human blood was used. The experiments were carried out *in vitro*, at 37° C, on whole blood or on erythrocytes suspended in isotonic NaCl solution. K was determined by flame photometer after deproteinization. EDTA-Ca was prepared according to PFEIFFER and OFFERMANN [4].

Experimental

It was investigated whether the inhibitory action of $2 \cdot 10^{-3}$ *M* of EDTA was suspended by $MgCl_2$. As it is clear from Fig. 1, 10^{-2} *M* of $MgCl_2$ had no effect on K transport under the experimental conditions employed.

In contrast with this, $CaCl_2$ completely blocked the action of EDTA. As it is shown in Fig. 2, the inhibitory effect of EDTA on K outflow was

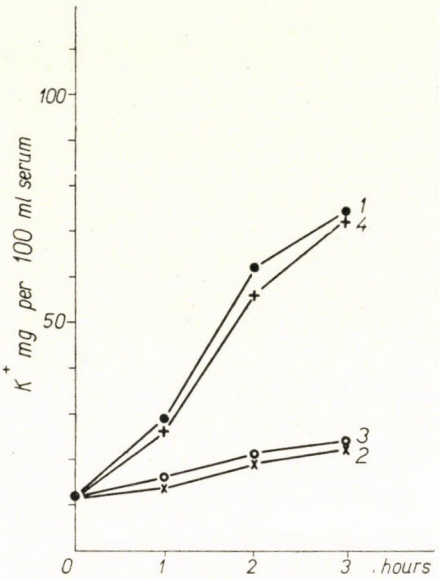

Fig. 1. Effect of Mg on the K permeability of blood containing IA + adenosine
1 : 10^{-3} *M* IA + 10^{-2} *M* adenosine. *2 :* 10^{-3} *M* IA + 10^{-2} *M* adenosine + $2 \cdot 10^{-3}$ *M* EDTA. *3 :* 10^{-3} *M* IA + 10^{-2} *M* adenosine + $2 \cdot 10^{-3}$ *M* EDTA + 10^{-2} *M* $MgCl_2$. *4 :* 10^{-3} *M* IA + 10^{-2} *M* adenosine + 10^{-2} *M* $MgCl_2$

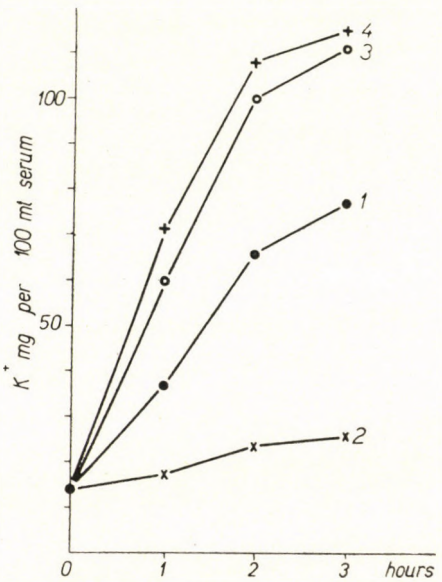

Fig. 2. Effect of Ca on the K permeability of blood containing IA + adenosine
1 : 10^{-3} *M* IA + 10^{-2} *M* adenosine. *2 :* 10^{-3} *M* IA + 10^{-2} *M* adenosine + $2 \cdot 10^{-3}$ *M* EDTA. *3 :* 10^{-3} *M* IA + 10^{-2} *M* adenosine + $2 \cdot 10^{-3}$ *M* EDTA + $5 \cdot 10^{-3}$ *M* $CaCl_2$. *4 :* 10^{-3} *M* IA + 10^{-2} *M* adenosine + $5 \cdot 10^{-3}$ *M* $CaCl_2$

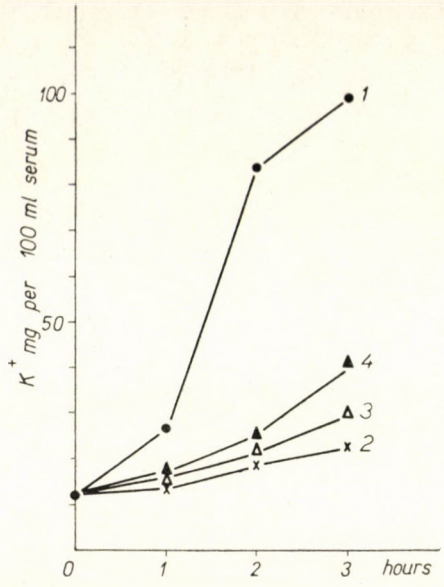

Fig. 3. Effect of Calgon and Na oxalate on the K permeability of blood treated with NaF
1 : $1.6 \cdot 10^{-2}$ *M* NaF. *2 :* $1.6 \cdot 10^{-2}$ *M* NaF + $2 \cdot 10^{-3}$ *M* EDTA. *3 :* $1.6 \cdot 10^{-2}$ *M* NaF + + 5.10^{-3} *M* Calgon. *4 :* $1.6 \cdot 10^{-2}$ *M* NaF + 10^{-2} *M* Na oxalate

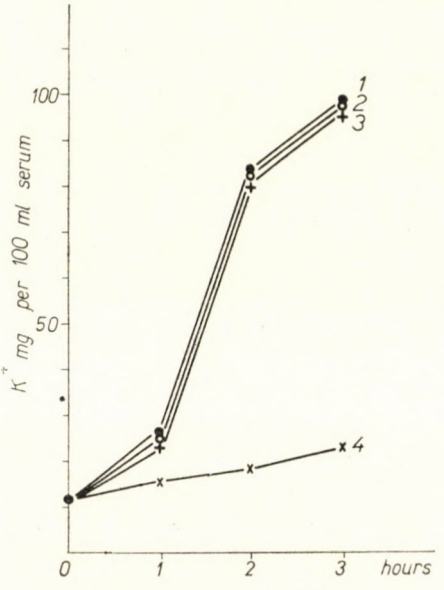

Fig. 4. Effect of different concentrations of EDTA on the K permeability of NaF-treated blood
1 : $1.6 \cdot 10^{-2}$ *M* NaF. *2 :* $1.6 \cdot 10^{-2}$ *M* NaF + $5 \cdot 10^{-4}$ *M* EDTA. *3 :* $1.6 \cdot 10^{-2}$ *M* NaF + + 10^{-3} *M* EDTA. *4 :* $1.6 \cdot 10^{-2}$ *M* NaF + $2 \cdot 10^{-3}$ *M* EDTA

totally absent in the presence of $5 \cdot 10^{-3}$ *M* of $CaCl_2$. On the other hand, $CaCl_2$ increased the rate of K outflow in blood containing IA + adenosine.

An effect similar to that of EDTA can be achieved also by other agents forming a complex with Ca. Calgon (Na-hexametaphosphate) and higher concentrations of sodium oxalate likewise inhibit K outflow (Fig. 3). At a concentration of 10^{-2} to 10^{-3} *M* sodium citrate has no effect on K permeability.

As to the relation of inhibition by EDTA to its concentration, 10^{-3} *M* was ineffective, whereas $2 \cdot 10^{-3}$ *M* was fully effective (Fig. 4). Inhibition

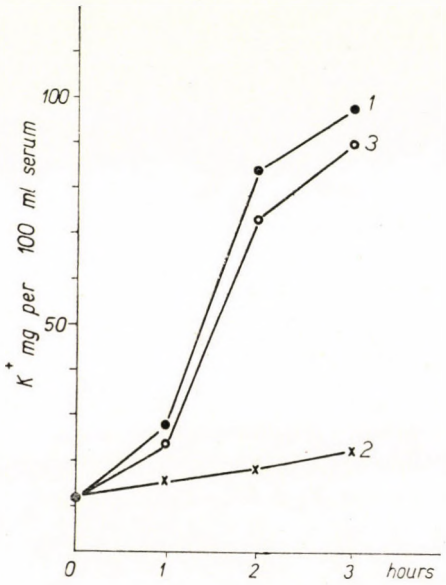

Fig. 5. Effect of EDTA-Ca on the K permeability of NaF-treated blood
1 : $1.6 \cdot 10^{-2}$ *M* NaF. *2 :* $1.6 \cdot 10^{-2}$ *M* NaF + $2 \cdot 10^{-3}$ *M* EDTA. *3 :* $1.6 \cdot 10^{-2}$ *M* NaF + + $2 \cdot 10^{-3}$ *M* EDTA-Ca

started at a much lower concentration in the case of washed erythrocytes, as washing removes a considerable part of serum Ca.

Fig. 5 shows the effect of the Ca complex of EDTA on K permeability. This compound was obviously ineffective.

Discussion

According to the experiments, the effect of EDTA is based on a binding of the ionized Ca of serum. This was proved first of all by the fact that the inhibition caused by EDTA was suspended by $CaCl_2$. Moreover, $CaCl_2$ gave rise to an effect opposite to that of EDTA, because it increased the rate of K outflow. A further evidence confirming the role of Ca is that other Ca-binding

agents, such as Calgon and oxalate, have a similar effect. The lowest inhibitory concentrations are, EDTA $2 \cdot 10^{-3}$ *M*; Calgon $5 \cdot 10^{-3}$ *M*; sodium oxalate 10^{-2} *M*, when the order of activity corresponds to the order of the dissociation constants of Ca complexes. No inhibition takes place in the presence of citrate, because even at high citrate concentrations there may be present such amounts of Ca ion as suffice for starting K outflow.

It is generally assumed that the serum contains about 10 mg per 100 ml ($2.5 \cdot 10^{-3}$ *M*) of Ca and 65 per cent ($1.6 \cdot 10^{-3}$ *M*) is present in the ionized form. Fig. 4 shows that the EDTA effect develops when the quantity of EDTA present in the system can bind all the ionized Ca present.

The ineffectiveness of EDTA-Ca is another proof of the role of Ca. This compound can no longer combine with Ca and thus has no effect in K permeability.

In our experiments such conditions were created which allowed a separation of the inhibitory action on glycolysis from that on K transport. EDTA, Calgon and sodium oxalate have namely no effect at all on the inhibition of glycolysis by NaF, whereas they completely inhibit K outflow [3]. By seperating the double effect of the glycolytic inhibitors we have come nearer to the elucidation of the mode of action of ion transport.

The experiments have shown that in response to glycolytic inhibitors K ions will start their rapid outflow from the erythrocytes only when the system contains Ca ions. This lets to conclude on the significant role of Ca in the maintenance of the physiological ion balance of blood.

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DIFFERENCES BETWEEN METACOLLAGEN AND COLLASTROMIN

By

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Collagen fractions prepared from the Achilles tendon of the cattle have been subjected to analysis. It has been found that metacollagen is different from collastromin, although both substances represent the insoluble component of collagen.

Differences between metacollagen and collastromin were demonstrated in regard to swelling in acid, silver-binding capacity, and in the resistance to proteolytic enzymes. At the same time, the insoluble fractions are not identical with the original native collagen.

The differences between native collagen, collastromin and metacollagen have been explained on the basis of the procollagen, mucoid$_1$, and mucoid$_2$, contents.

*

The submicroscopic structure of the collagen fibre suggested the hypothesis [6, 2] that collagen was composed not of a single homogeneous protein, but of at least two different protein components.

TUSTANOVSKI, ZAIDES, ORLOVSKAYA and MIKHAILOV [11] who arrived at a similar conclusion, have separated two components of collagen : procollagen, dissolved with a citrate buffer of pH 4, and the residue insoluble in citrate, collastromin. According to these authors, the complete isolation of collastromin from procollagen requires heating at 50° C for 1 hour 30 minutes. By this treatment, however, the properties of collastromin are altered.

The insoluble component of collagen, which remains after the thermal and chemical relaxation following the thermal and chemical contraction of the collagen fibre, is called metacollagen [7, 8]. It is questionable, however, whether this substance is identical with collastromin. In the course of chemical contraction, collastromin and metacollagen prepared from rat tail tendon behave in the same way. On this basis was concluded to the identity of these two kinds of insoluble collagen. However, to treatment with elastase they respond differently, indicating that they might still differ from each other.

To decide the problem, we prepared collastromin and metacollagen from the Achilles tendon of the cattle, a tissue which contains so-called "mature" collagen fibres, and which is available in quantities sufficient for the isolation of the above kinds of collagen.

To study the properties of the two substances in question, we examined them for swelling in water, resp. acid, silver-binding capacity and lysis by elastase.

According to our theory, in addition to the soluble procollagen, two soluble mucoproteins also take part in the building up of native collagen. These have been called mucoprotein$_1$ (mucoid$_1$) and mucoprotein$_2$ (mucoid$_2$) [3]. Mucoid$_1$ is dissolved during hydrothermal heat contraction [1], whereas mucoid$_2$ is dissolved by a specific enzyme, collagen mucoproteinase, isolated from the pancreas. We investigated the ratio of the single soluble components in the different collagen preparations ; the composition of the latter will be given on this basis.

Methods

Native collagen. The Achilles tendon of young (1 to 2-year old) cattle was freed from the adjacent cartilage, minced into pieces 2 to 3 mm thick and placed without delay into a large amount of acetone. After several days, the acetone was repeatedly changed, until the sample had become bone-hard after drying. The residue dried at 60° C was ground. A fine cotton-like material was thus obtained.

Collastromin. Wet Achilles tendon was finely ground in a Tourmix apparatus and extracted six times in succession with a five-fold volume of a 0.1 *M* citrate buffer of pH 4. Each extraction lasted 24 hours. The single citrate extracts were removed by filtration through cloth under pressure. From the 6th residue the citrate buffer was washed out with water. This was followed by dehydration with acetone and drying.

Metacollagen. The cotton-like acetone-dried and minced tendon was allowed to stand for 10 minutes at 75° C in the presence of 25 volumes of distilled water. After cooling and filtration the residue was washed in water and dried in acetone, obtaining in this way the metacollagen fraction.

Determination of aqueous and acetic acid swelling. To 250 mg of the collastromin, metacollagen and control preparations in weighed (13 ml) centrifuge tubes were added 10 ml volumes each of water or 0.1 *M* acetic acid resp. After repeated shaking, the ability to absorb water was tested 24 hours later. The tubes were centrifuged, the supernatant was decanted, the tubes were turned upside down for 10 minutes to allow the fluid to flow out. After wiping the tubes dry they were weighed by analytical scales. As collagen binds 8 to 24 times its own weight of water, the error of measurement is slight and the results are reproducible.

Determination of bound silver. 250 mg amounts of the dried collagen preparations were shaken for 1 hour in dark flasks in the presence of 0.001 *N* $AgNO_3$ dissolved in 50 ml of a pH 6.2 acetate buffer (equivalent to 5.39 mg of Ag), as described by GRASSMANN and KUSCH [9]. After filtration in the dark, 20 ml aliquots of the filtrate were titrated with 0.01 *N* NH_4SCN, in the presence of Fe ammonium alum, as indicator (Silver determination according to VOLHARD). As the control, 250 mg finely minced filter paper (*Schleicher* and *Schüll* 2043/B) was treated in the same way. This served to determine the surface-adsorbed silver, which was then substracted from the values obtained. The amount of silver bound by 1 g of collagen was computed from the difference.

Lysis by elastase. 50 mg quantities of the collagen preparations were incubated in weighed tubes at 37° C with 5 ml of a pH 8.8 0.025 *M* veronal acetate solution (control) and 1 mg elastase (designated 213/F, with an activity of 140 elastase units) for various lengths of time. The amount of dissolved protein in the supernatant was estimated by FOLIN's phenol method [10]. As the control, the non-soluble part was weighed. In this way, the lysis of collagen was controlled by two methods.

Lysis of mucoid$_2$. The different collagen preparations were treated for 15 minutes at 37° C with pancreatic collagen mucoproteinase [4], adding to 100 mg of collagen 5 ml of a pH 7.4 veronal acetate buffer and 0.1 mg of enzyme. The supernatant was tested for polysaccharide by the anthrone method.

The quantities of the single components are given in terms of mg of protein N per 100 mg of collagen.

Results

The data for the swelling in water or acetic acid, for the silver binding capacity, and lysis by elastase of the native collagen, collastromin and metacollagen prepared from Achilles tendon are summarized in Table I and Table II. According to the results, collastromin differs from metacollagen in the properties examined. However, it also differs from the control collagen. Collastromin swells markedly in water and this swelling is unaffected by acetic acid. The control Achilles tendon swells only slightly in water, but markedly in acetic acid. Metacollagen shows only slight swelling in water as well as in acetic acid. As far as the ability to bind silver is concerned (Table II), collastromin binds the most, then follows the control Achilles tendon. As compared to the control, metacollagen binds about half the amount bound by the control tendon. On the other hand, metacollagen alone is lysed without residue by elastase. We succeeded in demonstrating the presence of a substance showing green fluorescence in the soluble fraction of Achilles tendon, in the procollagen. Sulphuric acid and the water-soluble Molisch reagent (1 naphthol 2 sulphate) increase the intensity of the fluorescence, so that it is clearly visible by the naked eye.

The experiments with the enzyme collagen-mucoproteinase indicated that metacollagen contains the same amount of the $mucoid_2$ fraction as native collagen does, whereas collastromin much less. Although our collagen-muco-

Table I

Swelling of collagen fractions in water and acetic acid

	Swelling bound g H_2O/g collagen	
	in water	in acetic acid
Control Achilles tendon	8.7±1	25±2
Collastromin	25.0±2	25±2
Metacollagen	6.8±1	10±1

Table II

Binding of silver and lysis by elastase of collagen fractions

	Bound mg Ag per g collagen	Lysis by elastase in 24 hours
Control Achilles tendon	8.6±1	15 per cent
Collastromin	20.0±4	4 per cent
Metacollagen	4.0±1	100 per cent

Table III

Composition of Achilles tendon, collastromin and metacollagen

	Procollagen per cent	$Mucoid_1$ per cent	$Mucoid_2$ per cent
Control Achilles tendon ...	20	5	15
Collastromin	∅	5	4
Metacollagen	∅	∅	15

proteinase enzyme was not pure, in Table III we venture to present the quantitative correlation of the single components, as established in the above experiments. According to the data in Table III, collastromin differs from metacollagen in that the latter contains no $mucoid_1$, whereas collastromin does. Neither of them contains procollagen. Metacollagen contains the full amount of $mucoid_2$, whereas collastromin contains only a minimum quantity.

Discussion

Our earlier data [8, 5] already indicated that collastromin was not identical with metacollagen, although both contain the so-called insoluble fraction of collagen. The experiments described in the present report, which involved the use of mature Achilles tendon fibres, revealed significant differences between collastromin and metacollagen in their responses to treatment with water and acetic acid, silver and elastase. These differences can be explained only by the presence or absence of the various components building up collagen. At the same time, it has been proved that both collastromin and metacollagen differ from the original native collagen.

As compared with the control Achilles tendon, collastromin shows excessive swelling in water. According to our unpublished experiments with isolated rat tail tendon, the fibres exhibit marked swelling in water, while in 40 per cent KI they are converted to metacollagen. After drying, however, metacollagen loses this property, as it is indicated also by the data in Table I. The excessive swelling in water shown by collastromin is a phenomenon which at present we are unable to explain.

As regards swelling in acetic acid, collastromin does not differ from the original Achilles tendon collagen. We ascribe this property to its $mucoid_1$ content. On the other hand, metacollagen does not swell in acetic acid, because hydrothermal treatment dissolves the fraction $mucoid_1$ from the collagen tissue. The presence of $mucoid_1$ is essential for the binding of water molecules at acid reaction by the electrovalent bonds in the collagen fibre. Our experiments

on rat tail tendon yielded similar results [5]. The same mucoid$_1$ component is largely responsible for the binding of silver. This explains the fact that metacollagen binds only 50 per cent as much silver as is bound by the native Achilles tendon. It is not quite clear why the silver binding capacity of collastromin increases more than 100 per cent as compared with the native Achilles tendon and at the same time, also the extracted procollagen binds a high amount of silver. (Unpublished experiments.) We assume that procollagen is dissolved as a result of splitting, through which Ag-binding groups become free in the residual collastromin similarly as in procollagen. In collastromin the Ag-binding groups thus liberated are contained by a molecule group dissolved in water at 50° C. As a result, after heating in water at 50° C collastromin binds only a small amount of silver, whereas the substance dissolved in water has a marked silver-binding capacity.

We have observed years ago [1, 3] that metacollagen was lysed by elastase, and collastromin was not. This also indicates that, as a result of the dissolution of mucoid$_1$, the molecule is so altered that collagen becomes digestible by proteolytic enzymes (trypsin, too, lyses metacollagen).

As regards their amino acid composition, there is no difference between native collagen and metacollagen (personal communication by J. H. BOWES). Neither is there a difference in the main amino acid components (glycine, oxyproline, alanine, leucine). Thises proves that the properties peculiar to collagen, such as swelling in acetic acid, binding of silver, and digestibility by protease, depend on the minute amounts of polysaccharide-containing components that take part in the build-up of the molecule not as contaminants, but as integral parts of collagen.

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THE ACTIVATION OF PRECORTICOTROPIN

By

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Dasgupta & Young [1] found that certain preparations of ox pituitary glands had little or no activity in the ascorbic-acid depletion test for corticotropin [2] but acquired such activity when the solution was acidified (pH 3). They found also that treatment with 6 *M* urea solution for ten minutes similarly activated such preparations. The material, that was present in such preparations in a form inactive in the ascorbic acid depletion test but which became active on mild treatment, was called "precorticotropin" by Dasgupta & Young [1].

Precorticotropin could be a physiological precursor of corticotropin or it might be produced during extraction, for example by the binding of corticotropin to some other substance in the extract to form a complex that is inactive until the corticotropin is liberated by treatment with acid or urea. If precorticotropin were a physiological precursor, the animal should have a system for its activation. There is much evidence [3—8] that the hypothalamus contains a substance that causes the release of corticotropin from the pituitary gland but some of this evidence might also be interpreted as the activation of a precursor. We therefore decided to search for an activating system for precorticotropin in hypothalamic tissue. We also investigated the possibility that precorticotropin could be activated by adrenal extracts.

Ox pituitary glands were homogenized and centrifuged. The supernatant, equivalent to 10 mg dry pituitary tissue per ml, was inactive in the Sayers test when diluted 1 : 20 with saline, but active if treated for 10 min. with 6 *M* urea or acidified to pH 3 before dilution. Specimens of the supernatant were mixed with hypothalamic homogenate from ox or rat for varying times. In some experiments the pituitary and hypothalamic extracts were separately prepared and mixed one hour before dilution ; in one experiment they were incubated for four hours at 37° C. In other experiments the pituitary and hypothalamic tissues were homogenized together. The hypothalamic tissue of one ox or of four to eight rats was used in each experiment. Since Slusher & Roberts [8] found that the pituitary-stimulating activity of the hypo-

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thalamus is completely lost 45 min. after the death of the animal, in our experiments with rat hypothalamus the preparation was made immediately after decapitation of the rat.

In all experiments control tests were made with urea or acidification and in every instance the expected activation was seen, which indicated the presence of precorticotropin in the pituitary supernatant fluid (Table I).

Table I

Response in SAYERS *test of pituitary extract treated in various ways*

The supernatant of a homogenate of 10 mg/ml ox pituitary gland was diluted 1:20 for each test

Treatment	None	pH 3	Urea (6*M*)	Ox hypothalamus	Rat hypothalamus	Rat adrenal
Ascorbic-acid change mg/100 g adrenal ± S. E. M.	—2±4	—37±10	—64±8	—6±7	—11±5	+4±10
(no. of rats)	(25)	(8)	(19)	(9)	(13)	(9)

Urea solutions of 6 *M* and 3 *M* concentrations, but not that of 1 *M* concentration, activated precorticotropin (Table II). The activation of precorticotropin by urea and acidification [1] was thus confirmed, and urea was found to act at 3 *M* as well as 6 *M* concentration, but not at 1 *M* concentration.

Table II

Effect of urea concentration on the activation of precorticotropin

The supernatant of a homogenate of 10 mg/ml ox pituitary gland was diluted 1:20 after 10 min. treatment with urea solution

Urea concentration	0	1 *M*	3 *M*	6 *M*
Sayers test responses Ascorbic-acid change mg/100 g adrenal	—61, —7, —9, —8	—26, +3	—48, —35, —104, —13	—69, —38, —49
Mean response	—21	—12	—50	—52

The results failed to show significant activation of precorticotropin by hypothalamic or adrenal extracts (Table I). Thus we found no evidence for the presence in hypothalamic or adrenal tissues of an enzyme capable of converting precorticotropin into a substance capable of reducing the adrenal-ascorbic acid in hypophysectomized rats, although the activating effect of treatment with urea or acid was confirmed.

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Physiologia

EFFECT OF THE LESION OF PARAVENTRICULAR NUCLEUS ON THE FUNCTION OF THE PITUITARY, THYROID, ADRENAL CORTEX AND GONADAL SYSTEMS

By

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The effect on endocrine function of the lesion of the paraventricular nucleus has been investigated in the rat. I^{131} uptake by the thyroid was decreased after the operation. The activity of precipitable I^{131} in the plasma similarly diminished. Thyroid weight changed only in male animals. The corticoid content of the adrenals underwent a marked decrease, as a sign of adrenal hyperactivity. The metrotrophic activity of the anterior pituitary was elevated in both sexes, but the increase was more marked in female than in male rats.

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A great number of observations agree that the hypothalamus plays a fundamental role in the regulation of pituitary hormone secretion. Uotila [24], Bogdanove, Spirtos and Halmi [1], Westman and Jacobsohn [25], Dey [5, 7], DeGroot, Colfer and Harris [4], Hume and Wittenstein [20], Endrőczi, Kovács and Szalay [8], as well as many other authors have pointed out that both transsection of the pituitary stalk and electrocoagulation or stimulation of certain hypothalamic nuclei altered the regulatory influences exerted by the hypothalamus on the pituitary secretion of adrenocorticotrophic, thyreotrophic, gonadotrophic and somatotrophic hormones.

In spite of the fact that a vast literature has accumulated concerning the role played by certain hypothalamic nuclei in the regulation of endocrine functions, the demonstration of a direct hypothalamic representation of any endocrine function is still lacking. This lack is to be accounted for primarily by the fact that the very same subcortical nuclei are capable of initiating complex changes in both the endocrine functions and the vegetative processes simultaneously. The present report will deal with the simultaneous changes induced by electrocoagulation of the paraventricular nucleus in the function of the pituitary, thyroid, adrenal cortex and gonads, respectively.

Methods

The experiments were performed on 78 albino rats of the same breed each weighing 200 to 250 g. The rats were separated according to sex and divided into groups of ten to fourteen animals. The experiments began fourteen to fifteen days following the operation. Hypothalamic lesion was induced under intraperitoneal Sodium Evipan anaesthesia (10 mg/100 g

body weight), with the aid of a HORSLEY—CLARK stereotaxic instrument adapted to the rat. Electrocoagulation was made with a current intensity of 4 mA, for ten seconds. After experimentation the brain of the animals was fixed in 10 per cent formol, embedded in paraffine and stained with cresyl violet. The lesions were localized in the fronto-occipital plane.

Study of thyroid function

24 hours before experimentation the animals were subjected to intraperitoneal injection of potassium iodide containing 5.0 μC I^{131}. The thyroids were removed under barbiturate anaesthesia and weighed on a torsion balance with an accuracy of 0.5 mg. Precipitable (protein-bound) and non-precipitable I^{131} in the plasma were determined as follows. After administering heparine in a dose of 2 mg/100 g body weight, blood was withdrawn by puncture of the aorta. After centrifugation with 3000 r. p. m. for ten minutes the plasma was treated with two volumes of twenty per cent trichloroacetic acid and then centrifugated again, the latter procedure allowing the separation of bound and of precipitable I^{131}, respectively. Radioactivity in the plasma and thyroid was measured by the method of WOLLMAN and SCOW [26] using a 3.0 mg/cm^2 end window GM tube and a scintillation counter with a scale to 1024.

Study of adrenocortical function

The adrenals were removed, weighed with accuracy of 0.5 mg, and the pooled glands from ten to fourteen animals were homogenized in acetone. Extraction and determination of corticoids were performed by the methods already described (ENDRŐCZI, BATA and MARTIN [11]; ENDRŐCZI, BATA and MARTIN [12]; ENDRŐCZI and LISSÁK [13]; LISSÁK, ENDRŐCZI and MEDGYESI [22]. For separation of the components by paper chromatography, the method of BURTON, ZAFFARONI and KEUTMAN [2] was used. Evaluation was made according to the alkaline fluorescence technique of BUSH [3].

Study of the gonadotropic hormone

The pituitaries were removed and weighed. The anterior lobes were isolated and homogenized in two ml physiological saline. The extracts were stored at —15° C until experimentation. Metrotrophic activity was measured on infantile mice, according to the method of KLINEFELTER, ALBRIGHT and GRISWOLD [21].

The gonads from both control rats and those with hypothalamic lesion were removed and weighed on torsion balance.

Results

Electrocoagulation led to bilateral destruction of the paraventricular nucleus, and reached occasionally also the basomedial border of the thalamus. In most cases the lesion extended in the fronto-occipital plane from the optic

chiasm to the medial niveau of the tuber. A tipically localized lesion is shown in Fig. 1.

Changes in the weight of the endocrine organs after hypothalamic lesion were found to depend on the sex of the animals. Female rats showed namely no change in thyroid weight after the operation, while male animals reacted

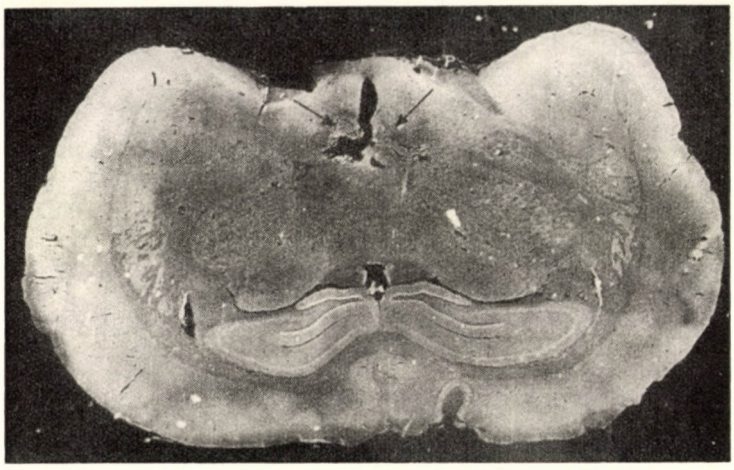

Fig. 1. Section in the fronto-occipital plane. The arrow shows the site of the electrocoagulation, which involved the nucleus paraventricularis on both sides, extending from the anterior niveau of the tuber to the anterior border of the posterior hypothalamus

with a considerable altered thyroid weight ($P > 0.001$). On the contrary, adrenal weight did not change either in the male or in the female rats.

Table I

Thyroid gland, mg/100 g body weight		
Normal (10) (males)	11.2 ±1.1	t : 6.53 $P>0.001$ DF : 22
Operated (14) (males)	8.65±1.33	
Normal (10) (females)	10.22±1.29	t : 1.68 $P<0.1$ DF : 18
Operated (10) (females)	10.29±1.22	

After destruction of the paraventricular nucleus, significant changes occurred in the I^{131} activity of both precipitable and non-precipitable fractions of the plasma. Activity of I^{131} precipitable with trichloroacetic acid was namely

Table II

Adrenal gland, mg/100 g body weight		
Normal (14) (males)	20.0±3.05	t : 0.72 $P<0.1$ DF : 22
Operated (10) (males)	21.5±3.63	
Normal (10) (females)	26.0±4.1	t : 0.98 $P<0.05$ DF : 18
Operated (10) (females)	27.6±3.45	

considerably decreased, a finding indicating that by the thyroid less anorganic iodine was converted into the organic form. The radioactivity of the total I^{131} was similarly significantly higher in the controls than in the operated animals.

Table III

Precipitable I^{131} 24 hours after radioiodine injection		
Normal (10) (males)	193±138	t : 2.92 $P>0.01$ DF : 22
Operated (14) (males)	82± 39	
Normal (10) (females)	218± 95	t : 6.33 $P>0.001$ DF : 18
Operated (10) (females)	61± 13	

Total I^{131} 24 hours after radioiodine injection		
Normal (10) (males)	354±140	t : 3.63 $P>0.001$ DF : 18
Operated (10) (males)	216± 68	
Normal (10) (females)	327±121	t : 4.89 $P>0.001$ DF : 18
Operated (10) (females)	146± 31	

Lesion of the paraventricular nucleus evoked a considerable decrease in the I^{131} uptake of the thyroid in both male and female rats. These findings confirm the results obtained by measuring the precipitable I^{131} content of the plasma. The difference was present also in female rats, even though these animals failed to show any change in thyroid weight.

Table IV

Total I^{131} in the thyroid gland, 24 hours* after radioiodine injection

Normal (10) (males)	26 200±2 320	t : 3.93 $P>0.001$ DF : 22
Operated (14) (males)	19 000±4 400	
Normal (10) (females)	30 320±7 200	t : 2.62 $P>0.05$ DF : 18
Operated (10) (females)	22 750±5 700	

* Calculated per weight of thyroid gland/100 g body weight

To study the function of the adrenal cortex, the tissue corticoid content was analized by paper chromatography. This method yielded information not merely as regards the quantitative aspects, but also to the qualitative corticoid pattern. The qualitative corticoid pattern of the adrenals was not substantially altered by the operation. The main component was always corticosterone. In addition, trace amounts of two new derivates appeared. One of those components was less polar than corticosterone, while the other was more polar, as already described by BUSH [3] as well as by ENDRŐCZI and LISSÁK [13]. The changes in the tissue corticoid content induced by paraventricular lesion showed a special relation to the activity of the adrenal cortex. As it can be seen from Table V, the first administration of ACTH resulted in a marked increase of the corticoid content, whereas which, however, diminished on the maintenance of the hyperfunctional state. This finding agrees with the recent observations of HOLZBAUER and VOGT [19]. In our operated animals, the corticoid content of the adrenals underwent a marked decrease, as an indirect sign of continuous adrenal hyperactivity. This latter was demonstrated further by the finding that, as far as adrenal weight is concerned, there was no indication of a hypofunctional decrease ; in fact, slight signs of hypertrophy could be observed.

Determination of the metrotrophic activity of the anterior pituitary revealed primarily the presence of gonadotrophic hormone, even though the

Table V

The amount of corticoids in the adrenal gland, expressed in μg/100 g adrenal tissue

Normal (males, 10)	820	
(males, 10)	745	
F (females, 10)	980	
(females, 8)	864	
Operated (males, 14)	282	
(females, 10)	320	
Treatment with ACTH 1.0 IU/100 g body weight		Percentual changes in the weight of adrenal gland
3 days (8)	1860	∅
6 days (6)	720	+11.4
11 days (10)	345	+14.0

method of KLINEFELTER, ALBRIGHT and GRISWOLD [21] is not absolutely specific for the determination of that hormone. Table VI shows that the metrotrophic activity of the operated male and female rats was more intensive than that of normal animals. Table VI demonstrates, further, that female rats exhibited a significantly greater metrotrophic activity than did male animals.

Table VI

Metrotrophic activity of the anterior pituitary from normal and operated rats

Uterine weight, mg/100 g body weight	
Untreated control mice .	127 ±18
Normal (10) (males)	156 ±16
Normal (10) (females) ..	169 ±18
Operated (14) (males) ...	178 ±22
Operated (10) (females)	194 ±23

Discussion

Numerous authors have investigated the role played by the paraventricular nucleus in the regulation of vegetative processes. As far as endocrine systems are concerned, no specific regulatory function was, however, demonstrated to be connected to that nucleus. GREER [14, 15], as well as GREER and ERWIN [16], placed lesions in the paraventricular nucleus and, ventral to it, in the anterior hypothalamus. They found that the storage of iodine by the thyroid was unaltered, only the proliferation of thyroid tissue was de-

creased. The function of the adrenals was essentially normal after stimulation or electrocoagulation of the above areas (HARRIS [18]; ENDRŐCZI and MESS [10]; ENDRŐCZI, KOVÁCS and LISSÁK [9]). These observations, however, referred to the reactivity of the pituitary-adrenocortical system and to the sensibility of anterior pituitary to stimuli initiating ACTH secretion, but gave no information concerning an eventual chronic adrenal hyperfunction.

HILLARP [17] was the first to point out that the lesion of the paraventricular nucleus or of the areas situated ventral of it, induces constant oestrus and decreases the secretion of luteinizing hormone (DEY *et al.* [5, 6, 7]). Our present investigations demonstrated an increased metrotrophic activity in the pituitary after operation. Although no change was found in the weight of the uterus, the ovaries or the male gonads after the surgical intervention, the possibility cannot be excluded that the functional changes observed in the experiments of DEY and HILLARP occurred also in our cases, and manifested themselves with an metrophic activity.

According to morphological studies the lesion of the paraventricular nucleus does not lead to any substantial alteration in the function of the endocrine organs (OLIVECRONA, [23]). Our functional investigations, however, revealed that the above lesion induced a hypofunction of the thyroid, adrenal hyperfunction and an increase of the metrotrophic activity of the anterior pituitary. On the other hand, it cannot be stated for certain whether the changes observed in the endocrine organs after destruction of the paraventricular nucleus were consequences of a disturbed regulation of the pituitary secretion of trophic hormones or whether the altered function of one single organ had initiated a functional change in the other organs.

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ÜBER DIE THERMOREGULATORISCHE BEDEUTUNG DER HYPERTHERMISCHEN UMSATZSTEIGERUNG. VERSUCHE AN RATTEN MIT LÄSIONEN DES HYPOTHALAMUS UND DES EPITHALAMUS.

Von

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Durch Versuche wurde festgestellt, daß die Körpertemperaturen von Tieren mit erhaltener und mit fehlender hyperthermischer Umsatzsteigerung lassen in warmer Umgebung sowohl nach Hypothalamus- als auch nach Epithalamusläsionen keinen Unterschied erkennen. Daraus folgt, daß *a)* die hyperthermische Umsatzsteigerung nicht dem Energiebedarf der wärmeabgebenden Mechanismen dient, da in diesem Falle ein Fehlen derselben mit einem stärkeren Anstieg der Körpertemperatur verbunden sein müßte; *b)* daß die durch eine warme Umgebung ausgelöste Hyperthermie innerhalb gewisser Grenzen keiner passiven Wärmestauung zugeschrieben werden kann, da man bei höherer Wärmeproduktion auch eine höhere Körpertemperatur beobachten müßte.

*

Die Erhöhung der Wärmeproduktion bei Umgebungstemperaturen über der thermoneutralen Zone [16] wird allgemein mit der VAN'T HOFFschen Regel erklärt und so als direkte Folge der Hyperthermie angesehen [4, 10, 17, 18]. Diese Auffassung läßt sich jedoch nicht aufrechterhalten, da bei unter Urethan oder Chloralhydratwirkung stehenden intakten Ratten [8], sowie nach Entfernung der Schilddrüse, der Hypophyse, oder während Thiouracilbehandlung [2, 3, 6, 9], bei gleich hohen oder gar noch höheren Umgebungstemperaturen bei gleicher Hyperthermie keine Erhöhung der Wärmeproduktion beobachtet wurde, und auch Läsionen des Hypothalamus und des Epithalamus die hyperthermische Umsatzsteigerung ausschalten können [6, 8, 13, 15]. Eine andere, weit weniger verbreitete Ansicht führt die Steigerung der Wärmeproduktion in Hyperthermie auf den erhöhten Energiebedarf der wärmeabgebenden Mechanismen (erhöhtes Minutenvolumen, etc.) zurück, und spricht damit der Umsatzerhöhung in der Wärme eine — wenn auch indirekte — Rolle in der Thermoregulation zu [18]. Besonders ausführlich befaßten sich THAUER und WEZLER [19] mit dieser Frage. Nach ihrer Schätzung entfielen auf diese spezifische, im Dienste der Thermoregulation stehende Organarbeit, von der in ihren Versuchen an vier Studenten beobachteten hyperthermischen Umsatzsteigerung von 110 ml O_2/min, mindestens 50 ml O_2/min. Da sich für die restlichen 60 ml O_2/ml ein Temperaturkoeffizient von bloß 1,9 errechnen ließ, kamen sie zum Schluß, daß sich hinter der hyperthermischen Umsatzsteigerung eine mindestens 5 Prozent betragende regulative Senkung verberge. Dieser Auffassung gemäß wäre also die hyperthermische Umsatzsteigerung die Resultante der durch diese regulative

Senkung geringen Umfanges verminderten Summe des durch spezifische Organarbeit bedingten Mehrbedarfes und der durch die VAN'T HOFFsche Regel bedingten Erhöhung des Umsatzes.

Unser ansehnliches Versuchsmaterial an Ratten mit Hypothalamus- und Epithalamusläsionen eignet sich gut zu einer erneuten Überprüfung des Problems der hyperthermischen Umsatzsteigerung, da falls die erhöhte Wärmeproduktion — wenn auch indirekt — im Dienste der Regulation steht, ein Fehlen derselben ein Ausbleiben der Funktionssteigerung der Mechanismen der Wärmeabgabe andeuten würde, und dies zu einer stärkeren Hyperthermie führen müßte. Steht jedoch die Steigerung der Wärmeproduktion nicht im Dienste der Thermoregulation, so wäre zu erwarten, daß ein Ausbleiben dieser mit einer geringeren Hyperthermie einherginge.

Eine diesbezügliche Analyse der Versuche an schilddrüsenlosen, sowie an hypophysektomierten Ratten, und der an unter Urethan- oder Chloralhydratwirkung stehenden Tieren gewonnenen Ergebnisse zeigten jedoch, entgegen dem Erwarten, eine Hyperthermie gleichen Grades bei fehlender und erhaltener Steigerung der Wärmeproduktion [6, 7]. Es schien daher angezeigt, diese Frage auch an Ratten mit Hypothalamus- und Epithalamusläsionen zu prüfen, umso mehr, da in den Urethan- und Chloralhydratversuchen die Körpertemperaturen in der thermoneutralen Umgebung signifikant unterhalb jener der Kontrollversuche lagen, und die thyreoidektomierten und hypophysektomierten Ratten naturgemäß eine niedrige Körpertemperatur und einen stark erniedrigten Grundumsatz hatten.

Versuchsanordnung

Der O_2-Verbrauch wurde in einer thermoneutralen (29° C), sowie in einer zur Hyperthermie führenden warmen (35° C) Umgebung in 3—4 Perioden von je 15 min in einem modifizierten [1] Apparat von BELÁK und ILLÉNYI [5] bestimmt; die Körpertemperatur wurde nach der letzten Bestimmung des O_2-Verbrauches bei der gegebenen Umgebungstemperatur rektal mit einem Quecksilberthermometer gemessen. Die Tiere wurden während des ganzen Versuches sorgfältig beobachtet und Perioden, in welchen sich die Ratten bewegten, wurden außer Betracht gelassen. Da zum vollständigen Temperaturausgleich zwischen Wasserbad und Kammer etwa 20 min benötigt werden, befanden sich die Tiere meistens etwa 90 min in der gegebenen Umgebungstemperatur, als die Körpertemperatur gemessen wurde. Die Luft der Kammer war praktisch mit Wasserdampf gesättigt, und dies erklärt, daß schon bei 35° C eine beträchtliche Hyperthermie bestand. Es sei bemerkt, daß unter den gleichen Versuchsbedingungen bei intakten Ratten eine Erhöhung der Wärmeproduktion niemals vermißt wurde. Die Versuche wurden frühestens 24 Stunden nach der Läsion ausgeführt, und in vielen Fällen nach einigen Tagen, manchmal auch nach mehreren Wochen — je nach Dauer der Störung — wiederholt. Die elektrolytischen Läsionen (4—6 mA für 5—6 sec) wurden mit einem auf dem HORSLEY und CLARKEschen [11] Prinzip beruhenden Zielgerät, mit wenigen Ausnahmen, bilateral gesetzt. Die Hypothalamusläsionen betrafen verschiedene Gebiete des Hypothalamus, die epithalamischen Läsionen beschränkten sich in der überwiegenden Mehrzahl der Fälle auf das Gebiet der Ggl. habenulae. Näheres über Lokalisation, sowie über andere sich aus dieser Versuchen ergebende Fragen wird an anderer Stelle berichtet [12, 13, 14, 15].

Versuchsergebnisse

a) *Läsionen des Hypothalamus*

Insgesamt wurden 55 Ratten mit Hypothalamusläsionen in 73 Versuchen einer Umgebung von 35° C ausgesetzt. Von diesen reagierten 33 Tiere normal: die Wärmeproduktion erhöhte sich mit der Hyperthermie wie in der intakten Ratte. Bei 18 Ratten blieb dagegen in 30 Versuchen trotz ausgesprochener Hyperthermie die Erhöhung der Wärmeproduktion vollkommen aus. Dies bietet die Möglichkeit, das Verhalten der Körpertemperatur letzterer Gruppe *a)* mit jenem der lädierten Ratten mit erhaltener Umsatzsteigerung, und *b)* mit dem Verhalten intakter Ratten zu vergleichen. Letztere Möglichkeit scheidet als Grundlage eines direkten Vergleiches aus, da die Körpertemperatur in warmer Umgebung auch bei lädierten Tieren mit erhaltener Umsatzsteigerung hochsignifikant ($P < 0{,}001$) über jener intakter Ratten ($39{,}2 \pm 0{,}10$) liegt [13].

Tabelle I

O_2-Verbrauch und Körpertemperatur nach Läsionen des Hypothalamus bei Umgebungstemperaturen von 29° und 35° C, bei erhaltener und bei fehlender hyperthermischer Umsatzsteigerung

$$\left[M \pm \sqrt{\frac{\Sigma \Delta x^2}{n\,(n-1)}}\right]$$

	O_2-Verbrauch ml/dm³/Stunde		Körpertemperatur °C	
	bei 29° C	bei 35° C	bei 29° C	bei 35° C
Hyperthermische Umsatzsteigerung erhalten (n = 36)	$71 \pm 2{,}3$	$94 \pm 2{,}8$	$37{,}3 \pm 0{,}11$	$39{,}9 \pm 0{,}13$
Hyperthermische Umsatzsteigerung fehlt (n = 30)	$77 \pm 3{,}3$	$77 \pm 3{,}2$	$37{,}6 \pm 0{,}17$	$39{,}9 \pm 0{,}14$

Die Ergebnisse in Tabelle I sind ganz eindeutig: obwohl die Wärmeproduktion in der Gruppe mit erhaltener Umsatzsteigerung mit mehr als 30 Prozent ansteigt und hochsignifikant ($P < 0{,}001$) höher liegt als der Umsatz bei fehlender Steigerung der Wärmeproduktion, kommt es zu einer Hyperthermie gleichen Grades.

b) *Läsionen des Epithalamus*

Insgesamt wurden 56 Ratten mit epithalamischen Läsionen einer Umgebung von 35° C ausgesetzt. 20 Tiere verhielten sich normal: der Energieumsatz erhöhte sich in der Wärme wie bei intakten Ratten. In 104 Versuchen an 36 Tieren blieb jedoch die Umsatzsteigerung unter den gleichen Bedin-

Tabelle II

O_2-Verbrauch und Körpertemperatur nach Läsionen des Epithalamus bei Umgebungstemperaturen von 29° und 35° C, bei erhaltener und bei fehlender hyperthermischer Umsatzsteigerung

$$\left[M \pm \sqrt{\frac{\Sigma \Delta x^2}{n(n-1)}}\right]$$

	O_2-Verbrauch ml /dm²/ Stunde		Körpertemperatur °C	
	bei 29° C	bei 35° C	bei 29° C	bei 35° C
Hyperthermische Umsatzsteigerung erhalten (n = 20)	72±1,8	93±1,8	37,4±0,10	40,1±0,14
Hyperthermische Umsatzsteigerung fehlt (n = 104)	80±1,0	79±1,5	37,4±0,06	39,9±0,05

gungen aus. Da bei einer Umgebungstemperatur von 35° C auch die Körpertemperaturen der Tiere mit erhaltener Umsatzsteigerung signifikant höher liegen als in der intakten Ratte ($P < 0{,}001$), können auch bei Läsionen des Epithalamus nur die beiden Gruppen der lädierten Tiere direkt verglichen werden.

Ratten mit Läsionen des Epithalamus verhalten sich also betreffs des gestellten Problems ganz wie die Tiere mit Hypothalamusläsionen. In einer Umgebung von 35° C besteht in der Körpertemperatur kein Unterschied zwischen den Gruppen mit erhaltener und fehlender Steigerung der Wärmeproduktion, obwohl der Umsatz in der ersteren mehr als 30 Prozent anstieg, und die Wärmeproduktion hochsignifikant ($P < 0{,}001$) höher lag als in der Gruppe mit fehlender Umsatzsteigerung.

Die höhere Wärmeproduktion bei fehlender hyperthermischer Umsatzsteigerung in *thermoneutraler* Umgebung ist im Falle der epithalamischen Läsionen (Tab. II) statistisch gesichert ($P > 0{,}001$), die nach Hypothalamusläsionen (Tab. I) beobachtete Differenz, sowie der in thermoneutraler Umgebung beobachtete Unterschied in der Körpertemperatur der beiden Gruppen mit Läsionen des Hypothalamus (Tab. I), ist nicht signifikant.

c) *Verhalten nach Wiederherstellung der hyperthermischen Umsatzsteigerung*

Sind die beschriebenen Ergebnisse auch noch so eindeutig, so schien es doch nicht überflüssig die Wirkung der hyperthermischen Umsatzsteigerung und deren Fehlen am selben Tiere zu vergleichen. Die Möglichkeit dazu wird dadurch geboten, daß früher oder später die hyperthermische Umsatzsteigerung auch bei den Ratten wieder beobachtet werden kann, bei welchen diese nach der Hypo- oder Epithalamusläsion nicht ausgelöst werden konnte. Insgesamt standen zu einem solchen Vergleich 10 Ratten mit Hypothalamusläsionen, und 22 Tiere mit Epithalamusläsionen zu Verfügung. Tabelle III

enthält die Werte bei an die Läsion sich anschließendem totalem Ausfall, und nach vollkommener Wiederherstellung der hyperthermischen Umsatzsteigerung.

Tabelle III zeigt, daß der Grad der Hyperthermie von der Restitution der hyperthermischen Umsatzsteigerung nicht berührt wird ; bei der gleichen Umgebungstemperatur erreicht die Körpertemperatur die gleiche Höhe, unabhängig davon, ob sich hiebei die Wärmeproduktion erhöht oder nicht, und auch die Ausgangstemperatur bei 29° C hat keinen Einfluß.

Tabelle III

O_2-Verbrauch und Körpertemperatur nach Läsionen des Hypo- und Epithalamus bei totalem Ausfall, und nach vollkommener Wiederherstellung der hyperthermischen Umsatzsteigerung

$$\left[M \pm \sqrt{\frac{\Sigma \Delta x^2}{n(n-1)}}\right]$$

	O_2-Verbrauch ml/dm²/Stunde		Körpertemperatur °C	
	bei 29° C	bei 35° C	bei 29° C	bei 35° C
Hypothalamusläsion, Umsatzsteigerung fehlt (n = 10)	90±4,0	90±4,2	38,2±0,30	40,1±0,33
Dieselben Tiere nach Wiederherstellung der Umsatzsteigerung	65±3,5	89±3,6	37,0±0,18	40,0±0,24
Epithalamusläsion, Umsatzsteigerung fehlt (n = 22)	87±2,2	85±1,7	37,8±0,11	40,0±0,12
Dieselben Tiere nach Wiederherstellung der Umsatzsteigerung	75±1,4	90±2,3	37,3±0,10	39,9±0,15

Die Wärmeproduktion ist bei voller Manifestation der Störung in thermoneutraler Umgebung erhöht, und gleicht jener, welche nach Restitution der hyperthermischen Umsatzsteigerung bei 35° C beobachtet wurde. Man könnte daher geneigt sein, die eigentliche Störung in dem schon in thermoneutraler Umgebung erhöhten Umsatz zu erblicken. Doch ist dies nicht der Fall : die hyperthermische Umsatzsteigerung kann so bei erhöhtem, wie bei normalem, oder — nach Hypothalamusläsionen — auch bei erniedrigtem Grundumsatz vollkommen fehlen, oder voll erhalten sein [13, 15]. Der Unterschied zwischen Tabelle I und II einerseits, und Tabelle III andererseits, findet seine Erklärung darin, daß in den beiden ersteren zahlreiche Tiere mit mehreren Versuchen repräsentiert sind, dagegen ist in Tabelle III von jedem Tier nur je ein Versuch vor, und ein Versuch nach der Restitution aufgenommen. Da Erhöhungen des Umsatzes und der Körpertemperatur in thermoneutraler Umgebung mehr vorübergehender Natur sind als der Ausfall der hyperthermischen Umsatzsteigerung, umfaßt Tabelle I und II einen bedeutend höheren Prozentsatz von Versuchen mit in thermoneutraler Umgebung normaler Körpertemperatur und normalem Grundumsatz wie Tabelle III.

In thermoneutraler Umgebung liegen Körpertemperaturen so nach Hypo- wie nach Epithalamusläsionen signifikant höher als nach der Restitution ($P < 0{,}01$), dabei unterscheiden sich die in Tabelle I und III zusammengefaßten Versuche (Hypothalamusläsionen) nicht signifikant ($P > 0{,}05$). Im Falle der Epithalamusläsionen (Tab. II und III) ist die Differenz wohl signifikant ($P < 0{,}01$), findet aber die gleiche Erklärung wie der höhere Grundumsatz.

Besprechung und Zusammenfassung

Die Versuchsergebnisse sind eindeutig: die Körpertemperaturen von Tieren mit erhaltener und mit fehlender hyperthermischer Umsatzsteigerung lassen in warmer Umgebung sowohl nach Hypothalamus- wie nach Epithalamusläsionen keinen Unterschied erkennen. Aus dieser Beobachtung folgt: *a)* daß die hyperthermische Umsatzsteigerung nicht dem Energiebedarf der wärmeabgebenden Mechanismen dient, da in diesem Falle ein Fehlen derselben mit einem stärkeren Anstieg der Körpertemperatur verbunden sein müßte, und *b)* daß die durch eine warme Umgebung ausgelöste Hyperthermie innerhalb gewisser Grenzen keiner passiven Wärmestauung zugeschrieben werden kann, da man dann bei höherer Wärmeproduktion auch eine höhere Körpertemperatur beobachten müßte. Es ist also einerseits die hyperthermische Umsatzerhöhung nicht durch den Anstieg der Körpertemperatur (also nicht durch die VAN'T HOFFsche Regel) bedingt, andererseits ist auch der Anstieg der Körpertemperatur, innerhalb gewisser Grenzen, unabhängig von der Erhöhung des Energieumsatzes. Beide sind zentralnervös regulierte, jedoch ursächlich nicht streng miteinander verbundene Phänomene.

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THE IMMEDIATE ACTION OF L-THYROXINE, L-TETRAIODOTHYROACETIC ACID, DIIODOTHYRONINE, DIIODOTHYROACETIC ACID AND L-THYRONINE ON OXYGEN CONSUMPTION AND BODY TEMPERATURE IN THE HYPOPHYSECTOMIZED RAT. THE ACTION OF CORTISONE

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The following preparations were found to be followed in the hypophysectomized rat by an acute response similar to that elicited by triiodothyronine and its acetic acid analogue : L-thyroxine (GLAXO), L-tetraiodothyroacetic acid (GLAXO), L-diiodothyronine (GLAXO), 3,5-diiodothyronine (HOFFMANN—LA ROCHE), L-diiodothyroacetic acid (GLAXO). L-thyronine (HOFFMANN—LA ROCHE) was completely inactive. Cortisone abolished the immediate response to all the iodothyronines investigated. Differences in the response to the various preparations have been discussed and evaluated. The exact nature of the active substances remains in doubt until pure compounds are available, since all contain more than one iodinated compound.

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DONHOFFER *et al.* have demonstrated that the intravenous administration of a few micrograms of triiodothyronine, its acetic acid derivative, and with lesser regularity also the administration of some thyroxine preparations are followed in the case of the triiodo-derivatives within 2 hours, in the case of thyroxine usually in the third or fourth hour, by a marked increase in oxygen consumption and a rise in body temperature in the hypophysectomized rat [4, 5, 6, 7, 12]. The immediate response to triiodothyronine and triiodothyroacetic acid could be abolished by appropriate pretreatment with adrenocorticotrophic hormone or cortisone [4, 5, 7]. It seemed therefore desirable to test in similar experiments some other iodothyronine derivatives and, as a control, not iodinated thyronine.

Methods

1 mg of the substances studied [L-thyroxine (GLAXO), L-tetraiodothyroacetic acid (GLAXO), 3,5-diiodothyronine (GLAXO and HOFFMANN—LA ROCHE), L-diiodothyroacetic acid (GLAXO), and L-thyronine (HOFFMANN—LA ROCHE)] was dissolved in 2 drops of 0.1 *N* NaOH and immediately made up to 5 ml with bidistilled water. Before use an aliquot part of this solution was further diluted with physiological saline to contain the required amount in 0.1 ml. The solutions were kept in a refrigerator and not used for more than a few days. The solvents were tested for pyrogens. The thyronine derivatives were injected intravenously; cortisone (CIBA) was administered subcutaneously in doses of 1 mg the previous evening, and another

mg on the morning of the experiment, approximately 3 hours prior to the injection of the iodothyronine.

Oxygen consumption was measured in a closed system, evolved from the apparatus of BELÁK and ILLÉNYI [2] in this laboratory [1, 10], for at least three 15 min periods before injecting the material under investigation, and for six hours thereafter in two or three 15 min periods every hour, with the exception of the first hour following the injection, in which either one single estimation was carried out, or estimations were started after one hour had elapsed. The data concerning the first hour have therefore been omitted from some of the Tables. Enviromental temperature was kept between 29—30° C throughout the experiment, and body temperature was measured with a mercury thermometer 6 cm from the anus. All rats were accustomed to the laboratory and to handling. Hypophysectomy had been performed at least two or three weeks before the experiments were carried out.

All figures for oxygen consumption are based on two or three closely agreeing estimations of 15 min, only in the hour of the main rise was the agreement less close and in this the mean may represent constantly rising figures. Standard error of the mean: $\pm \sqrt{\frac{\Sigma \Delta x^2}{n(n-1)}}$; significance: STUDENT's "t"-test. Table VIII is based on χ^2, calculated with YATES' modification for small series [11].

Results

a) L-thyroxine

Table I

Effect of 10 μg L-thyroxine (GLAXO) on oxygen consumption and body temperature in the hypophysectomized rat, without and after pretreatment with cortisone

Rat	Treatment		Oxygen consumption ml/dm²/h, and body temperature						Maximum rise
			Before thyroxine	Hour after thyroxine					
				2nd	3rd	4th	5th	6th	
63	without cortisone	O_2	47	46	48	48	58	58	23%
		°C	36.9	36.6	—	37.1	—	37.5	0.6 °C
	with cortisone	O_2	51	51	51	52	52	50	2%
		°C	37.0	36.5	—	36.0	—	36.0	—
65	without cortisone	O_2	47	47	50	55	60	70	49%
		°C	35.8	35.8	—	36.8	37.4	38.0	2.2 °C
	with cortisone	O_2	50	50	49	49	49	48	0%
		°C	36.0	36.5	—	36.3	—	36.8	0.8 °C
67	without cortisone	O_2	38	37	53	58	56	—	53%
		°C	36.0	36.0	—	36.8	37.3	—	1.3 °C
	with cortisone	O_2	50	50	48	50	51	50	2%
		°C	36.0	—	36.4	36.8	36.7	36.5	0.4 °C

Table I shows that all three hypophysectomized rats responded to the intravenous injection of 10 μg L-thyroxine with an increase in oxygen consumption and two of them with a rise in body temperature exceeding 1.0° C.

Cortisone abolished both effects: oxygen consumption was practically unchanged in all three rats, and the rise in body temperature was reduced to 0.4 and 0.8 °C in two, and replaced by a fall in the third rat. The augmented metabolic rate in the cortisone experiment in rat No 67 was due to the animal having received thyroxine 4 days earlier. Pretreatment with thyroxine is known not to interfere with the acute action of the iodothyronines [4, 5].

b) L-tetraiodothyroacetic acid (Tetrac)

Table II

The effect of 5 μg tetraiodothyroacetic acid (GLAXO) *on oxygen consumption and body temperature in the hypophysectomized rat*

(n = 16)

	Oxygen consumption and body temperature						
	Before injection	Hour after injection of Tetrac					
		1st	2nd	3rd	4th	5th	6th
Oxygen consumption ml/dm²/h	37±1.5	38±1.3	42±2.1	48±2.3	50±2.4	51±2.0	51±2.4
Compared with initial level	—	$P>0.6$	$P>0.05$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$
Body temperature °C...	36.2 ±0.12	— —	36.2 ±0.13	36.6 ±0.25	36.6 ±0.30	36.7 ±0.20	36.4 ±0.30
Compared with initial level	—	—	$P>0.9$	$P>0.1$	$P>0.2$	$P>0.05$	$P>0.3$

Table II demonstrates that the administration of tetraiodothyroacetic acid (Tetrac) was followed by a highly significant increase in oxygen consumption in the third hour; the level reached being maintained until the end of the experiments. In contrast with the effect of triiodothyronine (TIT) and triiodothyroacetic acid (Triac), the average increase in body temperature was small, and approached, but did not reach, significance in the fifth hour. Detailed analysis revealed that oxygen consumption responded in 15 out of 16 experiments with a rise greater than 20 per cent, the smallest rise observed amounting to 27 per cent, the greatest to 97 per cent. Body temperature rose by more than 1.0 °C in one, and by 1.0 °C in three experiments; in 9 the change was less than 0.5 °C. Fig. 1 shows a fairly typical experiment in detail.

c) Diiodothyronine (DIT)

8 of the experiments in Table III, and 3 in Table IV were performed with 3,5-diiodothyronine HOFFMANN—LA ROCHE and the rest with L-diiodo thyronine GLAXO. The results being identical there is no need to treat them separately. The rise in oxygen consumption was significant by the third hour,

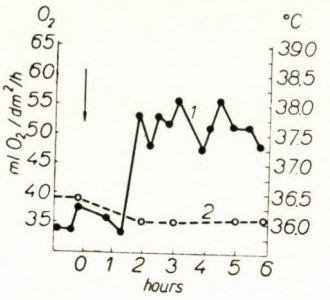

Fig. 1. The response of a hypophysectomized rat to the intravenous injection of 5 μg tetrathyroacetic acid
1 : oxygen consumption ; 2 : body temperature

Table III

The effect of 10 μg diiodothyronine (GLAXO *or* HOFFMANN — LA ROCHE) *on oxygen consumption and body temperature in the hypophysectomized rat*

(n = 15)

	Oxygen consumption and body temperature						
	Before injection	Hour after injection of diiodothyronine					
		1st	2nd	3rd	4th	5th	6th
Oxygen consumption ml/dm²/h	45±1.7	49±4.9	49±2.5	54±2.2	59±2.1	59±1.5	59±1.4
Compared with initial level	—	$P>0.4$	$P>0.2$	$P<0.01$	$P<0.001$	$P<0.001$	$P<0.001$
Body temperature °C ..	36.2 ±0.14	—	36.8 ±0.19	37.0 ±0.17	37.3 ±0.15	37.2 ±0.27	37.4 ±0.18
Compared with initial level	—	—	$P<0.05$	$P<0.01$	$P<0.001$	$P<0.001$	$P<0.001$

Table IV

The effect of diiodothyronine (DIT) on oxygen consumption and body temperature in hypophysectomized rats pretreated with cortisone

(n = 6)

	Oxygen consumption and body temperature						
	Before injection	Hour after injection of DIT					
		1st	2nd	3rd	4th	5th	6th
Oxygen comsumption ml/dm²/h	50±3.9	—	49±3.4	50±3.9	50±5.0	50±3.7	49±3.8
Compared with initial level	unchanged						
Body temperature °C...	36.4 ±0.18	—	36.5 ±0.10	36.1 ±0.11	36.5 ±0.21	36.4 ±0.13	36.3 ±0.18
Compared with initial level	unchanged						

the rise in body temperature already by the second. Both effects were completely abolished by previous treatment with cortisone. Analysis of the individual experiments revealed a failure to respond with an increase in oxygen consumption and body temperature in one case ; in another four experiments the rise in body temperature did not exceed 1.0 °C, but increased nevertheless

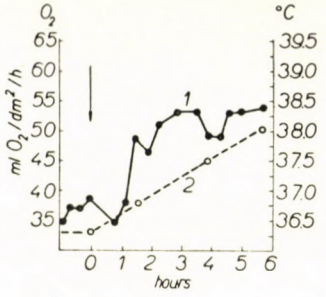

Fig. 2. The response of a hypophysectomized rat to the intravenous injection of 10 μg diiodothyronine
1 : oxygen consumption ; 2 : body temperature

in two of these by 0.9 °C and in the other two by 0.6 and 0.7°, respectively. In each of the six cortisone experiments oxygen consumption varied only within 5 per cent of the initial level, and body temperature within 0.4 °C. Fig. 2 demonstrates one of the diiodothyronine experiments in detail.

d) Diiodothyroacetic acid (Diac)

The statistical analysis of the response to Diac (Table V) revealed a highly significant and sustained increase in oxygen consumption from the

Table V

The effect of 5 μg L-*diiodothyroacetic acid* (GLAXO) *on oxygen consumption and body temperature in the hypophysectomized rat*

(n = 12)

	Oxygen consumption and body temperature						
	Before injection	Hour after injection of diiodothyroacetic acid					
		1st	2nd	3rd	4th	5th	6th
Oxygen consumption ml/dm²/h	48±1.2	46±1.5	57±2.0	62±1.5	62±1.2	64±1.4	61±1.6
Compared with initial level	—	$P > 0.3$	$P < 0.01$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Body temperature °C...	36.0 ±0.13	—	36.6 ±0.26	37.1 ±0.31	36.7 ±0.22	36.8 ±0.37	37.0 ±0.20
Compared with initial level	—	—	$P > 0.05$	$P < 0.01$	$P < 0.02$	$P > 0.05$	$P < 0.001$

second hour. The rise in body temperature seems to have been less regular, but this is due partly to the fact that it was not measured every hour in every animal. Oxygen consumption failed to increase in one experiment only; in the other 11 the maximum increase ranged between 28 and 57 per cent. Body temperature rose by more than 1.0 °C in 7 experiments, in one there was an increase of 0.9; there was no response ($\leq$ 0.5 °C) in 4. Fig. 3 demonstrates an experiment in detail.

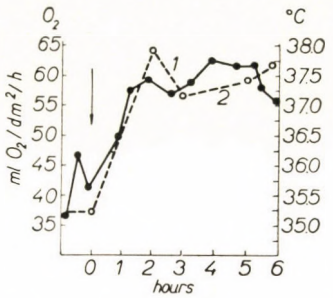

Fig. 3. The response of a hypophysectomized rat to the intravenous injection of 5 μg diiodothyroacetic acid
1 : oxygen consumption ; 2 : body temperature

Three rats pretreated with cortisone failed to respond to diiodothyroacetic acid with an increase in oxygen consumption and a rise in body temperature ; the greatest deviations from the initial level were in oxygen consumption —8 and +8 per cent ; in body temperature, —0.3 and +0.5 °C.

e) L-thyronine

The variety of iodothyronines capable of eliciting a response in the hypophysectomized rat made it desirable to observe whether this is confined to iodinated compounds, or shared also by the non-iodinated moiety.

Table VI demonstrates that the intravenous injection of L-thyronine failed to elicit a response in the hypophysectomized rat.

f) Comparison of the action of the various iodothyronines

The acute responses in the hypophysectomized rat to the various brands of thyroxines on the one hand, and to triiodothyronine and its acetic acid derivative on the other [6], already suggested that the time course of the response depends on the nature of the iodothyronine used, the rise being more rapid and sustained after the triiodo-compounds than after the thyroxines. Therefore, a somewhat more detailed analysis seemed desirable.

Responses in a single experiment may set in early (1st or 2nd hour) or late (3rd or 4th, rarely in the 5th hour) ; the maximum reached may be

Table VI

The action of 10 μg L-*thyronine* (HOFFMANN—LA ROCHE) *on oxygen consumption and body temperature in the hypophysectomized rat*

Rat No.		Oxygen consumption and body temperature							Maximum change
		Before injection	Hour after the injection of thyronine						
			1st	2nd	3rd	4th	5th	6th	
1	Oxygen consumption	60	55	60	59	59	60	61	8%
	Body temperature	36.3	—	36.9	—	36.9	—	36.5	0.6 °C
2	Oxygen consumption	61	61	59	62	—	62	62	3%
	Body temperature	37.0	36.5	—	36.5	—	36.5	36.5	0.5 °C
3	Oxygen consumption	48	48	48	49	48	47	48	2%
	Body temperature	36.4	—	36.9	—	36.6	—	36.6	0.5 °C
4	Oxygen consumption	49	49	51	53	52	51	49	8%
	Body temperature	36.5	—	36.5	36.5	36.8	—	36.8	0.3 °C

maintained throughout the experiment, or oxygen consumption may start to decline soon after reaching a peak level, whichever of the iodothyronines or their acetic acid analogues have been injected. The same applies to changes in body temperature. Comparison had therefore to be based on the frequencies of certain characteristics, and the time of onset of the rise in oxygen consumption or the failure to do so, and a rise in body temperature of 1.0 °C or more, were chosen for this purpose (Table VII).

Table VIII contains the statistical evaluation of the data of Table VII. The experiments with the various iodothyronines and their acetic acid derivatives having been performed with intervals ranging from a week or two to several months, extra caution seems to be warranted in drawing conclusions. Apart from the difference in the dosage of the iodothyronines (10 μg) and their acetic acid analogues (5 μg), a clear difference emerges between the tetra- and the triiodo-compounds, and is rather probable between the tri- and the diiodo-analogues, the rise in oxygen consumption setting in earlier after the administration of the triiodo-analogues. The frequency of failure to respond within 6 hours with a rise in oxygen consumption was significantly greater after the injection of thyroxine, than after the administration of the triiodo-analogues ; compared with the incidence of failure to respond to any of the other compounds, the difference was fairly evident, though statistically not significant.

Table VII

Comparison of the acute action of various iodothyronines and their acetic acid derivatives on oxygen consumption and body temperature in the hypophysectomized rat

	No. of experiments	Rise of oxygen consumption by more than 20 per cent		Failure to respond within six hours	Body temperature	
		within first two hours	in the 3rd to 5th hour		rise $\geqq$1.0 °C	change $<$1.0 °C
Diiodothyronine (DIT) ...	15	4	10	1	9	6
Triiodothyronine (TIT)	29	19	9	1	21	8
Thyroxine	70	17	32	21	45	25
Diiodothyroacetic acid (Diac)	12	6	5	1	7	5
Triiodothyroacetic acid (Triac)	37	27	10	0	32	5
Tetraiodothyroacetic acid (Tetrac)	16	5	10	1	4	12

Table VIII

Statistical evaluation of the differences between the acute responses to various iodothyronine analogues recorded in Table VII

	Probability of null hypothesis (P)	
Between	Onset of rise in oxygen consumption	Change in body temperature
TIT and DIT	$P<0.02$	$P>0.7$
Triac and DIT	$P<0.02$	$P>0.05$
TIT and Thyroxine	$P<0.001$	$P>0.5$
Triac and Thyroxine	$P<0.001$	$P<0.05$
TIT and Diac	$P>0.7$	$P>0.7$
Triac and Diac	$P<0.05$	$P>0.05$
TIT and Triac	$P>0.7$	$P>0.3$
TIT and Tetrac	$P>0.05$	$P<0.01$
Triac and Tetrac	$P<0.05$	$P<0.001$
TIT + Triac and DIT + Diac	$P<0.01$	$P>0.05$
TIT + Triac and Thyroxine + + Tetrac	$P<0.001$	$P<0.01$
Thyroxine + Tetrac and DIT + Diac	$P>0.2$	$P>0.9$

Discussion

The experimental data leave no doubt that the acute response of oxygen consumption in the hypophysectomized rat is not confined to the triiodo-analogues (TIT and Triac), but can be observed with a similar regularity after the intravenous injection of the diiodo-analogues and the acetic acid derivative of thyroxine (Tetrac). The administration of thyroxine itself, as already reported [6], is less regularly followed within 6 hours by a rise in oxygen consumption. Although in the single experiment the time of onset was not strictly characteristic, statistical analysis revealed clearly the greater rapidity of action of the triiodo-analogues. The remarkable activity of the diiodo-compounds was rather unexpected, because in more chronic types of experiments for instance 3,5-diiodothyronine is considered to be only 1/25th as active as thyroxine.

The increase in body temperature was — accepting the arbitrary dividing line of a rise of 1.0 °C or more — less regular in all types of experiments. The significance of the difference between Triac and thyroxine is due to the fact that both oxygen consumption and body temperature failed to respond to thyroxine with a rise in about one third of the experiments, and so this difference cannot be considered independently. A most convincing dissociation of the effects on oxygen consumption and body temperature was observed after the injection of Tetrac: oxygen consumption failed to increase only in 1 of 16 experiments, while a rise in body temperature was missed in 12. This remarkable dissociation of the effects on oxygen consumption and body temperature raises a number of questions regarding the nature of their association ; these must for the present remain unanswered.

Like the action of the triiodo-analogues [4, 5, 7], the acute effect of all the other iodothyronines and their acetic acid analogues was completely abolished by previous administration of cortisone. (In some instances 2 × 1 mg may not suffice, as in more recent experiments somewhat more cortisone was needed to suppress the immediate action of Triac completely.)

The observation that in the hypophysectomized rat cortisone abolishes the acute effects of the investigated diiodo- and tetraiodo-analogues, furnishes further supporting evidence of the interaction of iodothyronines and the adrenal cortex, postulated already in the course of an analysis of the action of the triiodo-analogues of thyroxine [4, 5, 7].

The fact that all investigated preparations were found by paper chromatography [3, 8, 9] to contain more than one iodinated component (DIT : 3 ; Diac 3 ; L-thyroxine : 3 ; Tetrac : 3), raises the question which of these, or how many of these, are active, and whether there are more than simple quantitative differences in their activity. The problem cannot be answered definitely before absolutely pure compounds have been studied, and with

due reserve only so much can be said that at present it seems probable that the immediate response is not confined to a single compound contained in all preparations, and that more than a simple quantitative difference is needed to explain the differences in the time of onset of the rise in oxygen consumption, and in the behaviour of body temperature. The unequivocally negative results with L-thyronine show conclusively that the described effect is at least confined to iodinated compounds.

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Acknowledgements. Authors are indebted for the supplies of L-thyroxine, L-tetraiodothyroacetic acid, L-diiodothyronine, and L-diiodothyroacetic acid to GLAXO LABORATORIES, for 3,5-diiodothyronine and L-thyronine to HOFFMANN—LA ROCHE LTD., and for the cortisone to CIBA LTD.

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ÜBER DIE WIRKUNGEN VON EPITHALAMUSLÄSIONEN AUF UMSATZ UND KÖRPERTEMPERATUR DER RATTE UND DEREN VERGLEICH MIT DEM VERHALTEN NACH LÄSIONEN DES HYPOTHALAMUS

Von

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Elektrolytische Läsionen des Epithalamus der Ratte werden in einem hohen Prozentsatz von Störungen der Regulation des Umsatzes und der Körpertemperatur gefolgt. Die Häufigkeit und die Kombination der einzelnen Störungen ergeben in ihrer Gesamtheit ein für die epithalamischen Läsionen charakteristisches, von dem nach Hypothalamusläsionen beobachteten, abweichendes Bild. Die wichtigsten Unterschiede gegenüber Hypothalamusläsionen sind: *a)* bedeutend häufigeres Fehlen der hyperthermischen Umsatzsteigerung, *b)* seltenere Vergesellschaftung eines erhöhten Grundumsatzes mit einer fieberhaft erhöhten Körpertemperatur, *c)* Fehlen eines Absinkens des Grundumsatzes auf subnormale Werte, *d)* meistens normale Körpertemperatur in einer Umgebung von 20—22° C selbst bei fehlender chemischer Regulation, *e)* keine Störung der chemischen Regulation, ohne gleichzeitiges Ausfallens der hypothermischen Umsatzsteigerung. Kein signifikanter Unterschied konnte zwischen Epithalamus und Hypothalamusläsionen festgestellt werden *a)* in der Häufigkeit der Störung der chemischen Regulation, *b)* in der Häufigkeit des Fehlens der hyperthermischen Umsatzsteigerung bei fehlender und bei erhaltener chemischer Regulation, und *c)* in dem Grad der Hyperthermie in einer Umgebung von 35° C bei erhaltener, fehlender, und wiederhergestellter hyperthermischen Umsatzsteigerung. Die »zweite chemische Wärmeregulation« wird auch bei fehlender hyperthermischer Umsatzsteigerung beobachtet. Dem epithalamischen Gebiet wird eine nicht zu vernachlässigende Bedeutung in der Regulation von Umsatz und Körpertemperatur zu gesprochen.

Anmerkung. In einem Teil der Versuche erfreuten wir uns der Mitarbeit von DR. I. TÓTH-NAGY, DR. E. OBRINCSÁK-PAP und DR. L. NAGY.

*

Die Vielfalt der bei Ratten nach Läsionen des Hypothalamus beobachteten thermoregulatorischen Störungen (12—15) ließ es wünschenswert erscheinen diese Versuche auch auf benachbarte Gebiete auszudehnen, wenn auch diesen gewöhnlich keine besondere Rolle in den thermoregulatorischen Vorgängen zugesprochen wird. Von besonderem Interesse schien das epithalamische Gebiet, da nach Läsionen des Areales der Trig. habenulae öfters ein Ausfall der hyperthermischen Umsatzsteigerung und der hypoxischen Umsatzsenkung beobachtet wurde [3, 4, 17].

Versuchsanordnung

Läsionen, Bestimmungen des O_2-Verbrauches und histologische Untersuchungen wurden auf dieselbe Weise ausgeführt, wie dies in früheren Mitteilungen bereits beschrieben wurde [13, 14]. Da die Luft der Stoffwechselkammer praktisch mit Wasserdampf gesättigt ist, entwickelt sich bereits bei einer Temperatur von 35° C ausnahmslos eine beträchtliche Hyperthermie, wie sie z. B. in einem gut durchlüfteten Thermostaten nur bei höheren Umgebungstemperaturen beobachtet wird.

Die einzelnen Säulen der Abbildungen wiedergeben auch in dieser Arbeit — wenn nicht anders angegeben — den Mittelwert dreier gut übereinstimmender Perioden von je 15 min. Da etwa 20 min zum Temperaturausgleich zwischen Wasserbad und Kammer benötigt werden, wurde die erste Bestimmung des O_2-Verbrauches erst nach diesem Zeitpunkt vorgenommen. Die Ratten befanden sich daher meistens etwa 70—90 min in der angegebenen Umgebungstemperatur als die Körpertemperatur gemessen wurde. Die Schemata wurden nach dem Atlas von SZENTÁGOTHAI [20] gezeichnet. Statistische Auswertung: in Tabelle VII. STUDENTS »t«-Test, ansonsten χ^2 mit der YATESschen Modifikation für kleine Versuchsreihen [24].

Die Versuche wurden insgesamt an 56 Ratten ausgeführt, deren jede bei thermoneutraler (29—30° C), und bei zur Hyperthermie führenden warmen Umgebungstemperatur (35° C), in einigen Versuchen auch noch bei 36 und 37° C beobachtet wurde; 23 Tiere wurden zur Prüfung der chemischen Wärmeregulation auch kühlen Umgebungstemperaturen (20—22° C) ausgesetzt. Der erste Versuch wurde gewöhnlich 24, seltener 48 Stunden nach der Läsion ausgeführt, und in den folgenden Tagen, bei einigen Tieren auch durch mehrere Wochen, öfters wiederholt. Bloß von 4 Ratten steht nur ein einziger Versuch zur Verfügung; in 3 wurde keine Abweichung von dem Verhalten intakter Tiere beobachtet, in einer blieben hyperthermische Umsatzsteigerung und chemische Regulation aus, doch ging das Tier 72 Stunden nach der Läsion, ehe der Versuch wiederholt wurde, ein. Insgesamt wurden die 56 Ratten in 124 Versuchen einer warmen, und 23 Tiere in 63 Versuchen einer kühlen Umgebung ausgesetzt.

Versuchsergebnisse

a) *Umsatz und Körpertemperatur in thermoneutraler Umgebung*

Ähnlich dem nach Läsionen des Hypothalamus beobachteten Verhalten, sieht man auch nach epithalamischen Läsionen recht häufig einen erhöhten Grundumsatz. Von den 56 Ratten lag der Grundumsatz in 37 mindestens 15, in den meisten 20—30 Prozent über der Norm; die Körpertemperatur verblieb dabei in 33 innerhalb des Normalbereiches, und bloß in 4 Tieren wurden in thermoneutraler Umgebung Körpertemperaturen von über 38,5° C beobachtet. In vielen Fällen kehrt der erhöhte Umsatz bereits am 2. oder 3. Tag nach der Läsion zur Norm zurück. Dies könnte als ein Hinweis auf eine unspezifische Natur gewertet werden; dagegen spricht, daß — wenn auch ausnahmsweise — auch viel länger währende Umsatzerhöhungen beobachtet wurden, und zwar ohne daß diese mit einer Erhöhung der Körpertemperatur verbunden gewesen wären (Tab. I).

Tabelle I

Ratte No. 547. Umsatz und Körpertemperatur in thermoneutraler Umgebung (29°) *nach der Läsion. (Lokalisation der Läsion: In der Mitte zusammenfließende Herde mit einem antero-posteriorem Durchmesser von 1,1 mm vom hinteren Niveau der Regio supraoptica bis zum vorderen Niveau der Regio infundibuli. Die Läsion umfaßt die beiden N. habenulae, die dorsalen Kerne des Thalamus und berührt durch das Dach der III. Kammer deren Plexus chorioideus.)*

	Vor der Läsion	Nach der Läsion									
		1. Tag	2. Tag	9. Tag	15. Tag	18. Tag	21. Tag	22. Tag	42. Tag	43. Tag	53. Tag
O_2-Verbrauch ml/dm²/Stunde	68	82	79	84	90	91	93	90	72	65	69
Körpertemperatur	37,5	37,7	37,2	36,9	36,8	37,1	37,0	37,0	37,1	37,3	37,0

In der Häufigkeit der Grundumsatzerhöhung besteht kein signifikanter Unterschied zwischen Hypothalamus- und Epithalamusläsionen (Tab. II); bei etwas eingehenderem Vergleich fällt aber auf, daß nach Epithalamusläsionen die Erhöhung des Grundumsatzes viel seltener mit einer fieberhaften Erhöhung der Körpertemperatur vergesellschaftet ist (Tab. II), und daß — im Gegensatz zu Hypothalamusläsionen — niemals eine Erniedrigung des Grundumsatzes beobachtet wurde (Tab. III).

Ein Unterschied von ähnlicher Signifikanz geht auch aus Tabelle III hervor. Nach Hypothalamusläsionen sieht man oft ein mehr oder weniger rasches Absinken des Grundumsatzes auf ausgesprochen subnormale Werte, wogegen nach Epithalamusläsionen dies niemals beobachtet wurde. Da auch ein in den ersten 1—2 Tagen erhöhter Umsatz in den folgenden Tagen auf

Tabelle II

Zahl der Ratten mit erhöhtem Grundumsatz nach Läsionen des Hypothalamus und des Epithalamus und das Verhalten der Körpertemperatur in thermoneutraler Umgebung

Läsion	Zahl der Ratten	Grundumsatz erhöht				P
		Insgesamt	P	Körpertemperatur		
				≤38,5	>38,5	
Hypothalamus	91	73		27	46	
			$P>0{,}2$			$P<0{,}001$
Epithalamus	56	40		35	5	

subnormale Werte sinken kann, sind in Tabelle III nur solche Tiere berücksichtigt, in denen auch am vierten Tage nach der Läsion, oder noch später, Bestimmungen des Grundumsatzes vorlagen.

Tabelle III

Zahl der Ratten mit erniedrigtem Grundumsatz (unter 60 ml O_2 pro dm^2 und Stunde) nach Hypothalamus-, und nach Epithalamusläsionen nach einer Beobachtungsdauer von mindestens 4 Tagen

Läsion	Zahl der Ratten	Grundumsatz		P
		erniedrigt	erhöht oder normal	
Hypothalamus	24	12	12	$P<0{,}01$
Epithalamus	18	0	18	

Es sei noch hinzugefügt, daß nach Epithalamusläsionen in thermoneutraler Umgebung niemals Körpertemperaturen über 38,5° C ohne einer gleichzeitigen Erhöhung des Grundumsatzes beobachtet wurden, auch beobachteten wir in thermoneutraler Umgebung bloß einmal eine subnormale Körpertemperatur (35,6° C), dagegen kam nach Hypothalamusläsionen beides öfters vor.

b) *Umsatz und Körpertemperatur nach Versetzen in eine kühle Umgebung*

Von 23 Ratten, die einer Umgebung von 20—22° C ausgesetzt wurden, zeigten 6 keine Erhöhung des O_2-Verbrauches, und in 4 weiteren wurde ein stark verzögertes Einsetzen der chemischen Regulation beobachtet.

Das Fehlen eines signifikanten Unterschiedes in der Häufigkeit des Ausbleibens der Umsatzsteigerung in kühler Umgebung ist auffallend (Tabelle IV), besteht doch zwischen den Zentren der epi- und der hypothalamischen Läsionen ein Tiefenunterschied von 3,5—4,5 mm. Da sich die Störung der

Tabelle IV

Verhalten der chemischen Wärmeregulation in einer Umgebung von 20—22° C nach Hypothalamus- und nach Epithalamusläsionen

Läsion	Zahl der Tiere	Chemische Regulation		P
		fehlt	erhalten	
Hypothalamus	91	32	59	$P > 0{,}5$
Epithalamus	23	6	17	

chemischen Wärmeregulation nach Epithalamusläsionen auf die ersten Tage nach der Läsion beschränkt, könnte wohl angenommen werden, daß diese auf einer akuten Fernwirkung beruhe. Das Fehlen eines Unterschiedes in der Häufigkeit scheint gegen diese Annahme zu sprechen, da Ödem, usw. bei Hypothalamusläsionen viel häufiger zu einem Ausfall der chemischen Regulation führen sollte als bei den entfernteren epithalamischen Läsionen. Eine sichere Entscheidung wäre wohl schwer zu fällen.

Ein etwas anderes Bild ergibt sich, wenn man das Verhalten der Körpertemperaturen vergleicht: nach Hypothalamusläsionen sieht man in einer Umgebung von 20—22° C selbst bei erhaltener Umsatzsteigerung öfters ein Absinken der Körpertemperatur auf ausgesprochen subnormale Werte. Ein ähnliches Verhalten wurde nach epithalamischen Läsionen niemals beobachtet, und selbst bei fehlender chemischer Regulation blieb die Körpertemperatur in 2 Ratten unverändert, in 2 anderen sank sie zwar einige Zehntel °C, blieb aber innerhalb normaler Grenzen, und bloß in 2 kam es zu Hypothermie (Absinken der Rektaltemperatur von 36,6 auf 35,8° C, bzw. von 35,5 auf 33,2° C.)

Von den beiden Ratten, in welchen es in einer kühlen Umgebung zur Hypothermie kam, sank in der einen die Körpertemperatur bloß mit 0,8° C auf 35,8° C, und der Temperaturabfall blieb von der selben Größe auch nach

Tabelle V

Hypothermie, bzw. Sinken der Körpertemperatur um mehr als 1,0° C nach Versetzen in eine Umgebung von 20—22°C

Läsion	Zahl der Tiere	Körpertemperatur		P
		fällt	unverändert	
Hypothalamus	91	30	61	$P<0{,}05$
Epithalamus	23	2	21	

Wiederherstellung der chemischen Regulation (Abb. 1). Die Versuche an der zweiten Ratte werden später eingehender dargestellt (Abb. 3).

Abb. 2 dient als Beispiel eines verzögerten Einsetzens der chemischen Wärmeregulation. In keinem der 4 Tiere war dieses Verhalten mit einem Abfall der Körpertemperatur verbunden, wogegen dies nach Läsionen des Hypothalamus mehrmals beobachtet wurde.

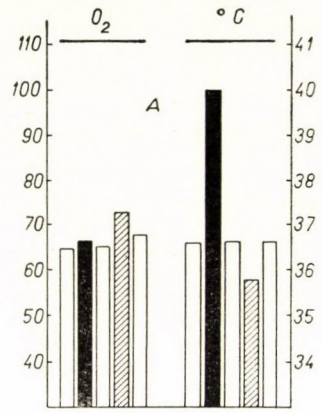
c) *Umsatz und Körpertemperatur bei Versetzen in eine warme, zu Hyperthermie führende Umgebungstemperatur*

Von den 56 Ratten die nach Läsionen des Epithalamus einer Umgebungstemperatur von 35° C ausgesetzt wurden, reagierten 20 normal: der Umsatz erhöhte sich wie in der intakten Ratte; bei 36 Tieren blieb jedoch trotz ausgesprochener Hyperthermie eine Umsatzerhöhung vollkommen aus. In einigen Versuchen wurden mehrere dieser Ratten auch höheren Umgebungstemperaturen (36,0 bis 37,5° C) ausgesetzt, ohne daß es zu einer Erhöhung des Umsatzes gekommen wäre. Ein Ausbleiben der hyperthermischen Umsatzsteigerung wurde auch nach Hypothalamusläsionen beobachtet, doch besteht in der Häufigkeit dieser Störung — im Gegensatz zur Störung der chemischen Regulation — zwischen den beiden Gruppen ein prägnanter Unterschied (Tab. VI).

Tabelle VI

Verhalten des Umsatzes bei Versetzung in eine warme, zu Hyperthermie führende Umgebungstemperatur

Läsion	Zahl der Tiere	Hyperthermische Umsatzerhöhung		P
		fehlt	erhalten	
Hypothalamus	51	18	33	$P<0{,}01$
Epithalamus	56	36	20	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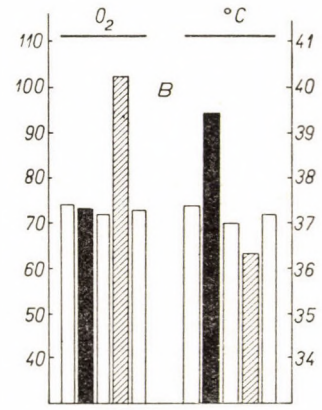
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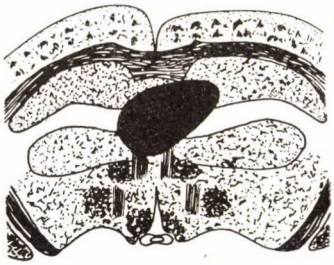
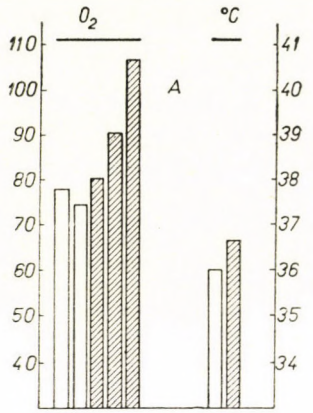


Abb. 1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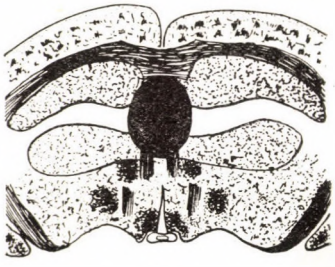

Abb. 2

Es mag auffallen, daß die Zahl der in thermoneutraler Umgebung einen erhöhten Umsatz aufweisenden Ratten (Tab. II) mit der Zahl der Tiere übereinstimmt, in welchen die hyperthermische Umsatzsteigerung ausblieb. Die beiden Phänomene sind jedoch keineswegs gekoppelt; ein erhöhter Grundumsatz kann auch bei erhaltener hyperthermischer Umsatzsteigerung beobachtet werden, und die hyperthermische Umsatzerhöhung kann ausbleiben, ohne daß der Grundumsatz überhaupt erhöht gewesen wäre. Der Grundumsatz kehrt auch in der großen Mehrzahl der Fälle viel rascher zur Norm zurück, ehe sich die hyperthermische Umsatzsteigerung wieder einstellen würde. Es sei noch hinzugefügt, daß von den 104 Versuchen in welchen eine hyperthermische Umsatzsteigerung vermißt wurde, der Grundumsatz in 36 zwischen 65 und 75 ml O_2 pro dm^2 und Stunde betrug.

Die Dauer des Ausfallens der hyperthermischen Umsatzsteigerung war bei den einzelnen Tieren verschieden; meistens kam es binnen 8—10 Tagen, öfters auch schon nach 2—3 Tagen zur Restitution, doch wurde in 2 Ratten noch 32 Tage nach der Läsion, und in einer Ratte sogar 43 Tage nach der Läsion eine hyperthermische Umsatzerhöhung vermißt. Schließlich kam es auch in dieser Ratte zur Restitution, 47 Tage nach der Läsion wurde eine

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Abb. 1. O_2-Verbrauch [ml/dm^2/Stunde] und Körpertemperatur bei 29° C (weiß), bei 35° C (schwarz), und bei 21° C (gestrichelt).

Ratte No. 574. Läsion: 17. III. 1953; Versuch *A*: 19. III. 1953; Versuch *B*: 23. III. 1953, eingegangen: 8. IV. 1953. Lokalisation: In der Mittellinie zu einem einzigen Herde zusammenfließende asymmetrische Läsionen im mittleren Niveau der Regio tuberalis. Der Herd liegt vorwiegend auf der einen Seite, und erfaßt beiderseits die N. habenulae, einseitig den Hippocampus, und erreicht die Wand der III. Kammer.

Diagramm: Niveau des Infundibulum.

Versuch *A*: 2 Tage nach der Läsion fehlen so die hyperthermische Umsatzsteigerung, wie die chemische Regulation. Die Körpertemperatur fällt in der kühlen Umgebung 0,8° C, und sinkt damit auf 35,8° C.

Versuch *B*: 6 Tage nach der Läsion ist, bei weiterhin fehlender hyperthermischer Umsatzsteigerung, die chemische Wärmeregulation wieder intakt. Die Wärmeproduktion liegt in kühler Umgebung mehr als 40 Prozent über der in Versuch *A* beobachteten, dennoch fällt die Körpertemperatur gegenüber den in thermoneutraler Umgebung gemessenen 37,4, 36,8 und 37,1° C auf 36,3° C, und unterscheidet sich daher kaum von dem Absinken der Körpertemperatur in Versuch *A*.

Abb. 2. O_2-Verbrauch [ml/dm^2/Stunde] und Körpertemperatur bei 29° C (weiß), und be 20,5° C (gestrichelt). Die Säulen wiedergeben Einzelbestimmungen von je 15 min.

Ratte No. 554. Läsion: 31. I. 1953; Versuch: 2. II. 1953; getötet: 13. II. 1953. Lokalisation: Bilaterale, etwas asymmetrische, zu einem einzigen Herde zusammenfließende Läsionen. Der Herd reicht vom vordersten Niveau der Regio supraoptica bis zum kaudalen Niveau der Regio tuberalis. In der Regio supraoptica erfaßt die Läsion unilateral den einen Seitenventrikel und dessen Plexus chorioideus, das Septum pellucidum, das Dach der III. Kammer und deren Plexus chorioideus, sowie die dorsalen Kerne des Thalamus. In der Regio tuberalis trifft die Läsion den Hippocampus, die III. Kammer und die beiden N. habenulae, sowie die dorsalen Kerne des Thalamus. Im kaudalen Niveau der Regio tuberalis ist der N. habenulae auf der einen Seite, im Niveau der Regio mamillaris auf beiden Seiten unversehrt.

Diagramm: Niveau des Chiasma opticum (*a*) und des Infundibulum (*b*).

Verzögertes Einsetzen der chemischen Wärmeregulation, ohne nachweisbare Störung im Verhalten der Körpertemperatur. Gleichzeitig wurde auch die hyperthermische Umsatzsteigerung vermißt. Trotz der sehr ausgedehnten Läsion wurde bereits am nächsten Tage ein promptes Einsetzen der chemischen Regulation, und 7 Tage später eine normale hyperthermische Umsatzerhöhung beobachtet.

Tabelle VII

Körpertemperaturen bei 35° C Umgebungstemperatur bei erhaltener, fehlender, und bei wiederhergestellter hyperthermischen Umsatzsteigerung, im Vergleich mit der intakten Ratte $\left(M \pm \sqrt{\frac{\Sigma \Delta x^2}{n(n-1)}}\right)$

Läsion	Hyperthermische Umsatzsteigerung	Körpertemperatur °C	Vergleich mit intakten Ratten
Epithalamus	erhalten (n = 20)	40,1±0,14	$P<0{,}001$
	fehlt (n = 104)	39,9±0,05	$P<0{,}001$
	wiederhergestellt (n = 22) ...	39,9±0,15	$P<0{,}001$
Hypothalamus	erhalten (n = 36)	39,9±0,13	$P<0{,}001$
	fehlt (n = 30)	39,9±0,14	$P<0{,}001$
	wiederhergestellt (n = 10) ...	40,0±0,24	$P<0{,}01$
Intakt	(n = 26)	39,2±0,10	—

angedeutete, am 54. Tage eine ausgesprochene hyperthermische Umsatzsteigerung beobachtet. Von den beiden anderen Tieren stellte sich bei einem die hyperthermische Umsatzerhöhung auch wieder ein, das andere wurde nach dem 32. Tag nicht mehr untersucht.

Vergleicht man das Verhalten der Tiere mit Epithalamusläsionen mit dem intakter Tiere, so sieht man, daß das Fehlen der hyperthermischen Umsatzsteigerung nicht die einzige Veränderung in der Wärme darstellt, dasselbe gilt für Ratten mit Hypothalamusläsionen.

Die Körpertemperaturen der lädierten Tiere, und zwar gleichgültig ob die Läsion im Hypothalamus oder im Epithalamus sitzt, sind in einer gleich warmen Umgebung signifikant höher wie die intakter Tiere. Dieses Verhalten ist keineswegs an die hyperthermische Umsatzsteigerung oder deren Fehlen gebunden, sondern bleibt nach der Restitution der Umsatzsteigerung noch weiter bestehen und wird auch nach Läsionen beobachtet, bei denen die hyperthermische Umsatzsteigerung voll erhalten blieb (Abb. 4—6).

Die sogenannte »zweite chemische Wärmeregulation« [18] wurde nicht systematisch untersucht, da in einem Teile der Versuche nach Rückversetzung in die thermoneutrale Umgebung sogar eine Stunde verging bis mit der Bestimmung des O_2-Verbrauches begonnen wurde. Immerhin fand sich bei 12 Ratten in 17 Versuchen eine deutliche »zweite chemische Regulation«, ohne daß es vorher in der Wärme zu einer hyperthermischen Umsatzsteigerung gekommen wäre. Diese Beobachtung stimmt mit den an thyreoidektomierten und an hypophysektomierten Ratten, sowie mit nach Hypothalamusläsionen gewonnenen Erfahrungen überein [1, 2, 15].

Tabelle VIII

Verhalten der hyperthermischen Umsatzsteigerung bei fehlender chemischer Wärmeregulation

Läsion	Chemische Wärmeregulation fehlt			P
	Insgesamt	Hyperthermische Umsatzsteigerung		
		erhalten	fehlt	
Hypothalamus	24	18	6	$P<0{,}01$
Epithalamus	6	0	6	

d) *Zusammenhang zwischen den Störungen der chemischen Regulation und der hyperthermischen Umsatzsteigerung*

In der Häufigkeit des Ausfallens der chemischen Regulation konnte zwischen Hypothalamus- und Epithalamusläsionen kein signifikanter Unterschied festgestellt werden (Tab. IV); untersucht man jedoch das gegenseitige Verhältnis der chemischen Regulation und der hyperthermischen Umsatzsteigerung, so zeigt sich ein bemerkenswerter Unterschied (Tabelle VIII).

Vergleicht man das Verhalten der chemischen Wärmeregulation bei fehlender hyperthermischer Umsatzsteigerung, so ergibt sich bloß ein statistisch nicht signifikanter Unterschied (Tab. IX).

Tabelle IX

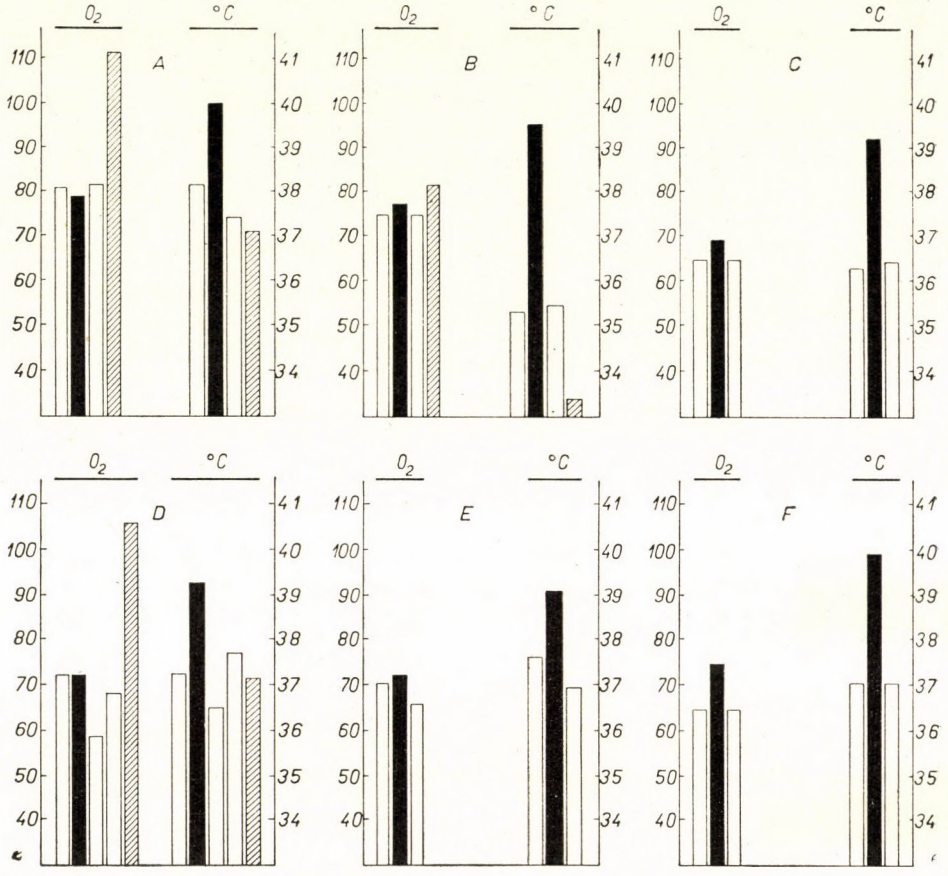
Verhalten der chemischen Wärmeregulation bei fehlender hyperthermischer Umsatzsteigerung

Läsion	Hyperthermische Umsatzsteigerung fehlt			P
	Insgesamt	Chemische Wärmeregulation		
		erhalten	fehlt	
Hypothalamus	18	12	6	$P>0{,}2$
Epithalamus	36	30	6	

e) *Einige Einzelversuche*

Lassen sich auch aus einem ziemlich großen Versuchsmaterial, wie das vorliegende, die grundlegenden Veränderungen und Beziehungen mit einfachen statistischen Mitteln scharf hervorheben und sicherstellen, so ist damit notgedrungen ein Verlust vieler Einzelheiten verbunden, die auch nicht jedes Interesses entbehren. Es schien daher angebracht den bereits wiedergegebenen Einzelversuchen (Abb. 1 und 2) noch einige weitere folgen zu lassen.

Abb. 3 wiedergibt 6 Versuche aus den der Läsion folgenden 15 Tagen. Dies war die einzige Ratte, in welcher sich nach anfänglich erhaltener chemischer Regulation ein Verlust derselben einstellte, und auch die einzige, in welcher es in kühler Umgebung zu einer ausgesprochenen Hypothermie kam.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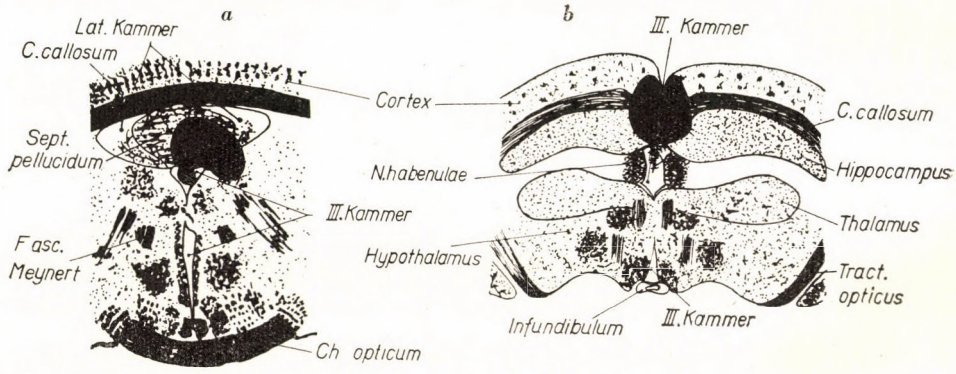

a
Lat. Kammer
C.callosum
Cortex
Sept. pellucidum
N.habenulae
III.Kammer
F asc. Meynert
Hypothalamus
Ch opticum
b
III. Kammer
C.callosum
Hippocampus
Thalamus
Tract. opticus
Infundibulum
III.Kammer

Abb. 3

All dies mag auf eine vorübergehende, sekundäre Wirkung auf den Hypothalamus deuten, doch war die Läsion weder ausgedehnter, noch mehr ventral gelegen als viele andere.

Abb. 4 und 5 wiedergeben Versuche vor und nach Restitution der hyperthermischen Umsatzsteigerung, und zeigen auch im Einzelversuch die relative Unabhängigkeit der Hyperthermie und des Umsatzes, wie dies schon aus Tabelle VII statistisch überzeugend hervorging.

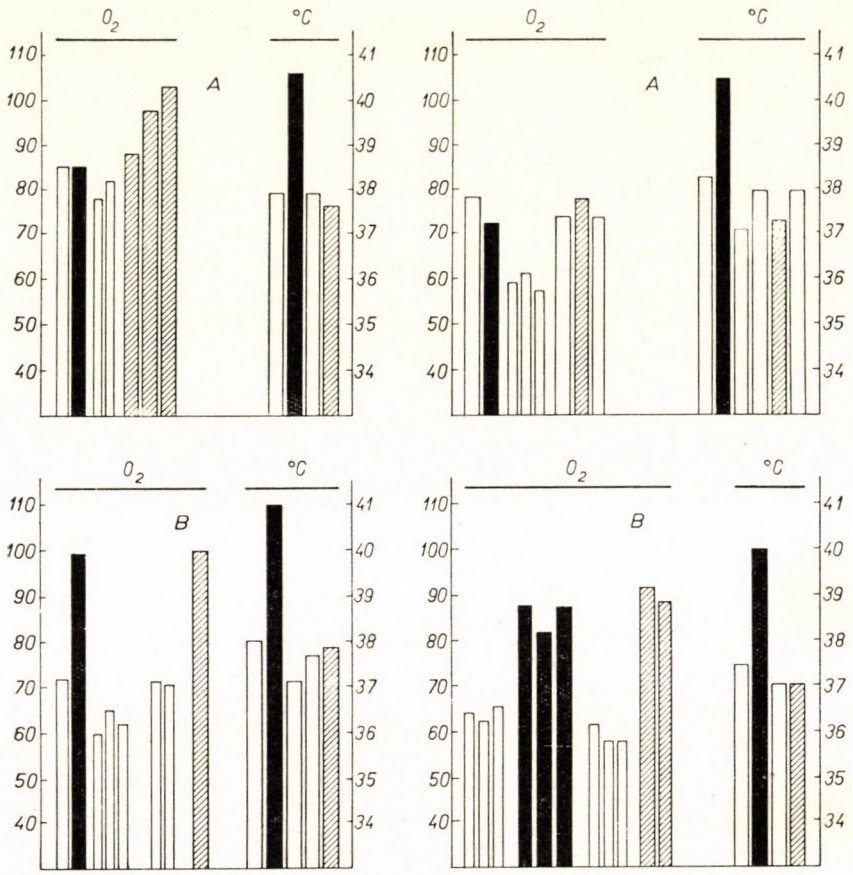
Abb. 6 demonstriert einen der Versuche, in welchen nach der Läsion die hyperthermische Umsatzsteigerung voll erhalten blieb, und bloß der erhöhte Grundumsatz und der Grad der Hyperthermie auf eine Störung hinweisen. Die Geringfügigkeit dieser Störung ist auch im Vergleich mit der recht ausgedehnten Läsion beachtenswert.

f) *Lokalisation der Läsionen*

Im Vergleich zu den Läsionen des Hypothalamus (12—15), die recht verschiedene Teile dieses Hirnabschnittes trafen, weist die Lokalisation der Epithalamusläsionen eine geringere Variabilität auf. Wie es aus den bereits angeführten Einzelbeispielen hervorging, flossen die bilateralen Läsionen — von wenigen Ausnahmen abgesehen — in der Mittellinie zu einem einzigen Herd zusammen. Dieser umfaßte, oder wenigstens berührte in allen Tieren das Areal der N. habenulae, erreichte meistens das Dach des III. Ventrikels, den dorsalen Thalamus und die Hippocampi. Da die Läsionen in den meisten Fällen auch die dorsalen Kerne des Thalamus mehr oder weniger berührten, ist es wohl nicht ganz gerechtfertigt von epithalamischen Läsionen zu sprechen, doch der Kürze halber sei es erlaubt diesen, das Zentrum der Läsion bezeich-

Abb. 3. O_2-Verbrauch [ml/dm²/Stunde] und Körpertemperatur bei 29° C (weiß), bei 35° C (schwarz), und bei 20° C (gestrichelt).

Ratte No. 573. Läsion: 10. III. 1953; Versuch *A*: 11. III. 1953; Versuch *B*: 12. III. 1953; Versuch *C*: 13. III. 1953; Versuch *D*: 14. III. 1953; Versuch *E*: 17. III. 1953; Versuch *F*: 25. III. 1953; getötet: Anfang April, 1953. Lokalisation: in der Mittellinie zu einem einzigen Herd zusammenfließende bilaterale Läsionen. Der Herd reicht von der Grenze der Regio supraoptica und der Regio tuberalis bis zur Regio mamillaris, trifft die Hirnrinde, die Hippocampi und berührt an einer kleinen Stelle das Dach des III. Ventrikels. In der Regio mamillaris erfaßt die Läsion eben noch das kaudale Ende der N. habenulae. Diagramm: Niveau des Chiasma opticum (*a*) und des Infundibulum (*b*). Am ersten Tag nach der Läsion ist bei fehlender hyperthermischer Umsatzsteigerung und erhöhtem Grundumsatz die chemische Regulation erhalten, und auch das Verhalten der Körpertemperatur zeigt keine gröbere Störung (Versuch *A*). Einen Tag später fehlt die chemische Wärmeregulation und auch die Regulation der Körpertemperatur ist schwer gestört (Versuch *B*). Am dritten Tag ist der Grundumsatz bei weiterhin fehlender hyperthermischer Umsatzsteigerung wieder normal (Versuch *C*). Am folgenden Tag ist die chemische Regulation wieder vollkommen normal und auch das Verhalten der Körpertemperatur zeigt keine Störung. Bei Rückversetzung aus der Wärme in eine thermoneutrale Umgebung kommt es trotz Fehlen der hyperthermischen Umsatzsteigerung zu einer deutlichen, länger als 90 min dauernden »zweiten chemischen Wärmeregulation« (Versuch *D*). 7 Tage nach der Läsion kommt es in der Wärme noch immer zu keiner hyperthermischen Umsatzsteigerung (Versuch *E*); nach weiteren 8 Tagen wurde endlich erstmalig wieder eine deutliche Umsatzsteigerung bei 35° C beobachtet (Versuch *F*).



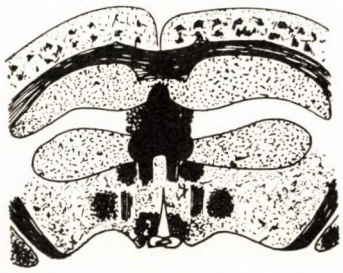


Abb. 4

Abb. 5

nenden Ausdruck zu gebrauchen. Die Beobachtung, daß sich nach unseren »epithalamischen« Läsionen nur selten eine Hyperthermie entwickelte (Tabelle II), spricht gegen eine bedeutendere Beteiligung des Thalamus an den Folgen der Läsion, und gleichzeitig auch gegen eine Wirkung dieser auf entferntere Gebiete, da ja gerade Läsionen des Thalamus besonders häufig zu fieberhaften Temperatursteigerungen führen [8, 9, 16, 19, 21—23].

Der Durchmesser der zusammengeflossenen Herde schwankte meistens zwischen 1,0 und 1,6 mm ; in einigen war er, namentlich in sagittaler Richtung, auch bedeutend größer, und erreichte in einem Fall 2,2 mm, in einem anderen nahezu 3 mm. Die den Abbildungen beigefügten Schemata geben einen guten Überblick über die große Mehrzahl der Läsionen.

Ein Zusammenhang zwischen Lokalisation und Ausdehnung der Läsion einerseits, und dem Verhalten der Körpertemperatur und des Umsatzes andererseits, konnte unter den angewandten Versuchsbedingungen nicht festgestellt werden. Diesbezüglich gleichen die nach epithalamischen Läsionen gemachten Beobachtungen, den mit Hypothalamusläsionen gewonnenen Erfahrungen.

Abb. 4. O_2-Verbrauch [ml/dm²/Stunde] und Körpertemperatur bei 29° C (weiß), bei 35° C (schwarz), und bei 20° C (gestrichelt). Die Säulen 3—7 in A und B wiedergeben Einzelbestimmungen von je 15 min.

Ratte No. 544. Läsion : 7. I. 1953 ; Versuch *A* : 9. I. 1953 ; Versuch *B*: 13. I. 1953; getötet : 10. III. 1953. Lokalisation : Bilaterale, in der Mittellinie zu einem einzigen Herd zusammenfließende Läsionen. Der Herd erstreckt sich vom mittleren Niveau der Regio tuberalis bis in das dorsale Mesencephalon, und erfaßt das Dach der III. Kammer, deren Plexus chorioideus, die dorsale Schicht des Thalamus, sowie im Niveau der Regio mamillaris den kaudalen Teil der N. habenulae. Diagramm : Niveau des Infundibulum.

Versuch *A* : 2 Tage nach der Läsion ist der Grundumsatz beträchtlich erhöht, die hyperthermische Umsatzsteigerung fehlt, die chemische Regulation setzt verzögert ein. Das Verhalten der Körpertemperatur zeigt, von der höheren Hyperthermie abgesehen, keine Abweichung von der Norm. Nach Rückversetzung aus der Wärme in eine thermoneutrale Umgebung eine eben angedeutete »zweite chemische Wärmeregulation«.

Versuch *B* : 6 Tage nach der Läsion ist der Grundumsatz normal, die hyperthermische Umsatzsteigerung ist wieder hergestellt, und auch die chemische Regulation setzt prompt ein. Die Körpertemperatur erreicht in der Wärme in Versuch *A* und *B* praktisch die gleiche Höhe, obwohl im letzteren die Wärmeproduktion ansteigt. Die »zweite chemische Wärmeregulation« ist recht ausgesprochen.

Abb. 5. O_2-Verbrauch [ml/dm²/Stunde] und Körpertemperatur bei 29° C (weiß), bei 35° C (schwarz), und bei 22° C (gestrichelt). Die schmalen Säulen in A und alle in B wiedergeben Einzelbestimmungen von je 15 min.

Ratte No. 540. Läsion : 5. I. 1953 ; Versuch *A*: 6. I. 1953 ; Versuch *B* : 23. I. 1953 ; getötet : 10. III. 1953. Lokalisation : Bilaterale, in der Mittellinie zu einem Herde zusammenfließende Läsionen. Der Herd erstreckt sich vom kaudalen Niveau der Regio supraoptica bis zum mittleren Niveau der Regio tuberalis, und erfaßt in der ersteren das Septum pellucidum und den dorsalen Thalamus, in der letzteren das Dach der III. Kammer, deren Plexus chorioideus, sowie die vorderen Teile der N. habenulae. Kaudal vom mittleren Niveau der Regio tuberalis sind die N. habenulae intakt. Diagramm : Niveau des Chiasma opticum.

Am Tage nach der Läsion fehlen so die hyperthermische Umsatzsteigerung, wie die chemische Wärmeregulation ; die »zweite chemische Regulation« ist dabei erhalten (Versuch *A*). 17 Tage später verhält sich der Umsatz vollkommen normal, bloß der Grad der Hyperthermie in der Wärme kann noch als Zeichen einer Störung gedeutet werden (Versuch *B*).

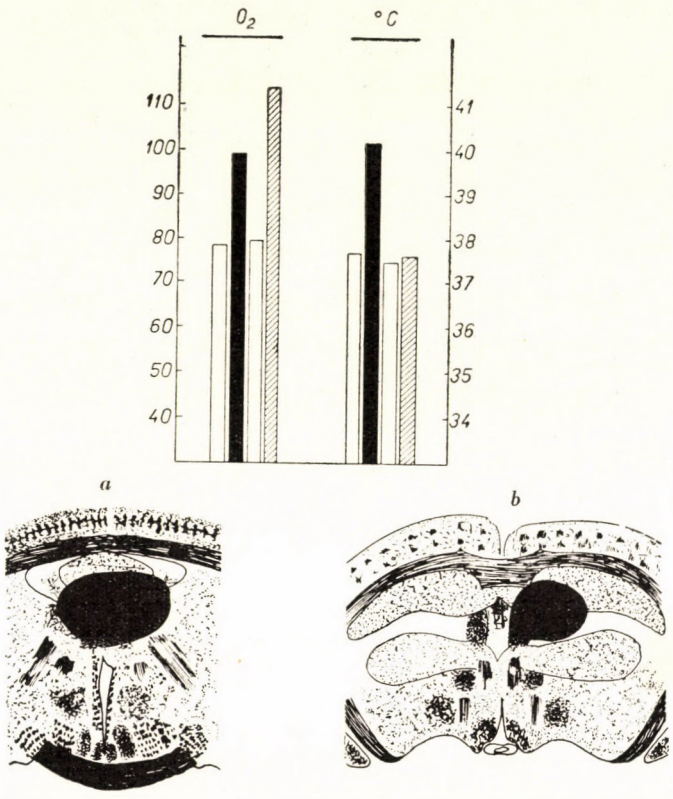


Abb. 6. O_2-Verbrauch [ml/dm²/Stunde] und Körpertemperatur bei 29° C (weiß), bei 35° C (schwarz), und bei 21.5° C (gestrichelt).

Ratte No. 556. Läsion: 10. II. 1953; Versuch: 11. II. 1953; getötet: 10. III. 1953· Lokalisation: Bilaterale Läsion, die in der Mittellinie zusammenfließend einen großen, von dem vordersten Niveau der Regio supraoptica bis zur Regio mamillaris reichenden Herd bilden. Dieser erfaßt in der Regio supraoptica das Septum pellucidum, die Seitenventrikel und berührt ventral die Commissura anterior. In der Regio tuberalis erstreckt sich die Läsion auf das Dach des III. Ventrikels und dessen Plexus chorioideus, erfaßt unilateral den Hippocampus und den dorsalen Thalamus, sowie beiderseits den vordersten Teil der N. habenulae. Kaudal von der Grenze des vorderen und des mittleren Niveaus der Regio tuberalis sind die N. habenulae intakt. Diagramm: Niveau des Chiasma opticum (*a*) und des Infundibulum (*b*).

Ausgedehnter Herd bei erhaltener hyperthermischer Umsatzsteigerung und intakter chemischer Regulation. Bloß der leicht erhöhte Umsatz und die hohe Hyperthermie in der warmen Umgebung deuten auf eine Störung des Umsatzes und der Körpertemperatur. Den nächsten Tag verlief der Versuch bei etwas höherem Grundumsatz (83 ml pro dm² und Stunde) auf ganz ähnliche Weise.

Besprechung

Eigentlich ist die Vielfalt der Störungen des Wärmehaushaltes die nach Läsionen des Epithalamus beobachtet wurden, ein recht bemerkenswerter Befund, handelt es sich doch um ein Gebiet, dem gewöhnlich keine besondere Rolle in der Regulation des Umsatzes und der Körpertemperatur zugeschrieben wird, und welches vom Hypothalamus, also dem zentralen Regulationsapparat

des Wärmehaushaltes, ziemlich ferne liegt. Letzteres spricht auch gegen eine unspezifische Wirkung auf den Hypothalamus. In demselben Sinne muß der Befund gewertet werden, daß fieberhafte Störungen der Körpertemperatur viel seltener beobachtet werden als nach Hypothalamusläsionen, obwohl der Thalamus, dessen Läsionen besonders häufig zu Hyperthermie führen, viel näher liegt und dessen dorsale Kerne in vielen Fällen in die Läsion einbezogen sind. Auch die mehrwöchige Dauer einzelner Störungen ließe sich schwer mit der Annahme einer unspezifischen Fernwirkung in Einklang bringen. Sieht man aber eine solche unspezifische Wirkung via Hypothalamus als

Tabelle X

Zusammenfassender Vergleich des Verhaltens nach Läsionen des Epithalamus und des Hypothalamus

Unterschied signifikant		Kein signifikanter Unterschied	
Vergesellschaftung eines erhöhten Grundumsatzes mit Hyperthermie (Tabelle II)	$P<0{,}001$	Häufigkeit der Grundumsatzerhöhung (Tabelle II)	$P>0{,}2$
Absinken des Grundumsatzes auf subnormale Werte (Tabelle III)	$P<0{,}01$	Häufigkeit der Störung der chemischen Regulation (Tabelle IV)	$P>0{,}5$
Absinken der Körpertemperatur in einer Umgebung von 20—22° C (Tabelle V)	$P<0{,}01$	Grad der Hyperthermie in warmer Umgebung (Tabelle VII)	$P>0{,}9$
Häufigkeit des Ausfallens der hyperthermischen Umsatzsteigerung (Tabelle VI)	$P<0{,}01$	Verhalten der chemischen Regulation bei fehlender hyperthermischer Umsatzsteigerung (Tabelle IX)	$P>0{,}2$
Verhalten der hyperthermischen Umsatzsteigerung bei fehlender chemischer Regulation (Tabelle VIII)	$P<0{,}01$		

unwahrscheinlich an, so ist man gezwungen anzunehmen, daß dem lädierten Gebiet eine bedeutsame Rolle in thermoregulatorischen Reaktionen zukommt.

Eine solche Annahme würde eine starke Stütze finden, wenn sich die nach Epithalamusläsionen beobachteten Störungen von den nach Hypothalamusläsionen registrierten klar unterscheiden würden. Im ersten Augenblick scheint dies nicht der Fall zu sein, denn alle nach Epithalamusläsionen beobachteten Störungen (Umsatzsteigerung, Hyperthermie in thermoneutraler Umgebung, Ausfallen der chemischen Regulation, Fehlen der hyperthermischen Umsatzsteigerung) kommen auch nach Läsionen des Hypothalamus vor [12—15]. Ein, durch statistische Verarbeitung der Beobachtungen ermöglichter, eingehender Vergleich fördert jedoch bemerkenswerte

Unterschiede zu Tage. Zur besseren Übersicht sind die in den vorangehenden Tabellen ausführlich dargestellten Ergebnisse in Tabelle X kurz zusammengefaßt.

Die viel seltenere Vergesellschaftung eines erhöhten Grundumsatzes mit Hyperthermie, das Fehlen einer Erniedrigung des Grundumsatzes, das viel seltenere Absinken der Körpertemperatur in einer kühlen Umgebung nach epithalamischen Läsionen, wären in sich allein schwer zu bewerten, da sich in diesem Vergleich immer die Störungen nach Hypothalamusläsionen als die ausgeprägteren erweisen. Dagegen fällt die hyperthermische Umsatzsteigerung nach Epithalamusläsionen bedeutend häufiger aus, als nach Hypothalamusläsionen. Diese Beobachtung spricht schon in sich allein entschieden dafür, daß im Zustandekommen der hyperthermischen Umsatzsteigerung den lädierten epithalamischen Gebieten eine besondere Rolle zukommt. Ein weiterer auffallender, und auch statistisch signifikanter Unterschied ist, daß nach den Epithalamusläsionen niemals ein Fehlen der chemischen Regulation ohne gleichzeitigem Ausfallen der hyperthermischen Umsatzsteigerung beobachtet wurde, während nach Hypothalamusläsionen die hyperthermische Umsatzsteigerung bei fehlender chemischer Regulation in 18 von 24 Ratten erhalten blieb (Tabelle VIII).

Überblickt man die Gegenüberstellung der Folgen von Hypothalamus- bzw. von Epithalamusläsionen in ihrer Gesamtheit, so enthüllen sich bezeichnende Unterschiede. Sind auch die einzelnen Störungen keine ausschließlichen Eigenschaften der einen, oder der anderen Lokalisation, so kommt es doch durch die Häufigkeit und die Kombination der verschiedenen Störungen zu einem charakterischem Bild (»pattern«) der Folgen von epithalamischen Läsionen. Jedenfalls muß man diesem Gebiet eine weit größere Bedeutung, als allgemein üblich, in der Regulation des Umsatzes und der Körpertemperatur zusprechen.

Die Bedeutung dieses Gebietes in der Regulation wird noch erhöht, wenn man in Betracht zieht, daß die im vorangehenden beschriebenen Veränderungen im Verhalten des Umsatzes und der Körpertemperatur nicht die einzigen sind, die nach Läsionen des Epithalamus beobachtet wurden. Aus einem größeren Versuchsmaterial wurde in 7 Ratten mit Läsionen des Epithalamus in insgesamt 20 Versuchen in einer Umgebung von 20—22° C und einem Luftdruck von 450 mm Hg eine, bei intakten Ratten nie fehlende, hypoxische Senkung des Umsatzes und der Körpertemperatur vollkommen vermißt [3, 17].

In einer beträchtlichen Anzahl von Ratten mit Epithalamusläsionen wurden auch die endokrinen Drüsen histologisch untersucht. Dabei wurden Befunde erhoben, die für eine Beeinflußung der Schilddrüsenfunktion zu sprechen schienen. MESS [10, 11] untersuchte dann diese Frage ausführlicher und kam zu dem Schluß, daß bilaterale Läsionen der N. habenulae 1. die strumigene Wirkung kleiner Methylthiouracilgaben verhindern, die Wirkung

großer Dosen aber kaum hemmen, 2. das histologische Bild von Jodmangelschilddrüsen normalisieren, 3. nach Entfernung der Schilddrüse das Absinken des Gehalts an thyreotropem Hormon in der Hypophyse hemmen. Dieser Befund könnte dahin gedeutet werden, daß dem Ausfallen der hyperthermischen Umsatzsteigerung eine verminderte Schilddrüsensekretion zugrunde liegt, da nach Entfernung der Schilddrüse, oder der Hypophyse, sowie nach Methylthiouracilbehandlung die hyperthermische Umsatzsteigerung ausblieb [1, 2, 5]. Der oft erhöhte, und niemals subnormale Grundumsatz, das sofortige Ausfallen der hyperthermischen Umsatzerhöhung nach der Läsion, das Fehlen einer Senkung des Grundumsatzes selbst bei wochenlang vermißter hyperthermischer Umsatzsteigerung, lassen jedoch eine solche Erklärung nicht zu.

Die auffallendste Folge der Läsionen des Epithalamus, das häufige Fehlen der hyperthermischen Umsatzsteigerung, bietet auch Gelegenheit zu einer eingehenderen Analyse des Mechanismus und der thermoregulatorischen Bedeutung dieser Erscheinung. Da diese, und die damit verbundenen Probleme bereits ausführlich behandelt wurden [6, 7], wird an dieser Stelle von einer Besprechung dieser Frage abgesehen.

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Pharmacologia

EFFECT OF DEXTRANS OF DIFFERENT MOLECULAR WEIGHT ON THE BLOOD PRESSURE AND SURVIVAL OF CATS IN HYPOVOLAEMIC SHOCK

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The effect of different dextran preparations has been investigated on the blood pressure of cats in hypovolaemic shock, induced by the withdrawal of 25 ml/kg blood. No difference was found between the pressor effect of Dextraven, a preparation of high molecular weight, and of Plasmodex, a blood substitute of low molecular weight. The lethal rebleeding volume was considerably greater with Dextraven. In cats made hypotensive for one hour, the life-saving effect of these preparations was restricted to a few hours. When the hypotension was maintained for 15 minutes only, both large and small molecular weight dextrans (Intradex and Plasmodex) produced a 48-hour survival in about two thirds of the animals, while physiological saline was effective in 17 per cent only. Three, 6, 12 and 22 hours following the infusion, both dextrans exhibited a pressor effect of identical duration, though neither of them was able completely and permanently to restore blood pressure.

*

The blood substitute dextran is known to be a mixture of numerous fractions differing in molecular weight. Two types of dextran preparation are commercially available. The Swedish, American and Hungarian dextrans consist of particles with a molecular weight from 50 000 to 80 000 and with an intrinsic viscosity of 0.18 to 0.28. In 24 hours 30 to 50 per cent of the amount administered is excreted in the urine. On the other hand, the British preparations have a higher molecular weight, generally between 170 000 and 250 000. Their intrinsic viscosity is 0.32 to 0.37. Correspondingly, the urinary excretion during 24 hours of these preparations does not exceed 25 per cent of the amount introduced.

It is still questionable whether or not the dextrans of high molecular weight offer some advantage as to their therapeutic effect [1]. High molecular weight dextrans are generally supposed to have a prolonged action. At the same time they increase the sedimentation rate of the erythrocytes to a greater degree than do dextrans of low molecular weight, an effect probably not indifferent for the organism. The doubtless therapeutical value of low molecular weight dextrans is shown by the observations that *a)* the Swedish dextran protected dogs from an otherwise lethal haemorrhagic shock [2]; *b)* when administered to man after a blood loss of 1000 ml, an American preparation was capable of maintaining for 24 hours 70 to 86 per cent of the elevated plasma volume [3].

The present experiments were devoted to a further study of the above question. Two high molecular weight dextran preparations were used, the English makes Dextraven and Intradex. The Hungarian Plasmodex, having a much lower molecular weight, served as comparison. The intrinsic viscosity at 25° C of this latter preparation lay between 0.19 and 0.22, varying with the different production lots of the past years. 35 per cent of the amount administered to rabbits appears in the urine during the first 24 hours.

Experiments with Dextran

Dextraven, Batch No. 3755 (1955), with an intrinsic viscosity of 0.38 at 25° C was used, a preparation fractionated within narrow limits. Accordingly, only a small part of it has a molecular weight lower than 40 000 and is, therefore, excreted with urine. The bulk of the fractions lies between 100 000 and 250 000 [4].

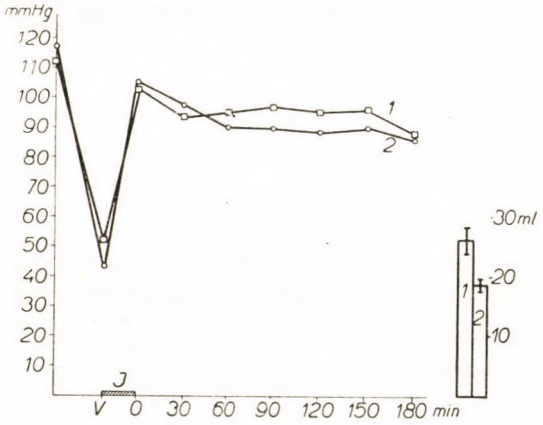

Fig. 1

In the first experiments, performed in 8 cats, the method described previously was used [5]. Under Sodium-Evipan anaesthesia, blood amounting to 25 ml/kg was successively withdrawn from the animals. This amount generally sufficed to induce a continuous drop in blood pressure to 60 mm Hg or below. If it did not, further 5—5 ml blood was withdrawn. Some minutes after the desired hypotension had been reached the plasma substitute was introduced intravenously. Blood pressure was subsequently recorded for three hours and then the additional blood volume to be withdrawn to induce death was determined. The results obtained with Plasmodex [5] on 31 cats in the same year served as comparison. Mean values of the results with Dextraven are illustrated in Fig. 1.

Fig. 1 demonstrates that, in the first three hours following haemorrhage, there was no difference between the blood pressure effect of Dextraven and of

Plasmodex. The lethal rebleeding volume was, however, significantly more with Dextraven than with Plasmodex, a fact indicating that the circulation of the cats treated with Dextraven remained in a better condition than that of the animals treated with the low molecular weight dextran.

Another question to be decided was whether the more advantageous circulatory influence of Dextraven had been accompanied by a higher survival rate. The effect of Dextraven on survival was, therefore, compared to that of Plasmodex and physiological saline, respectively. In these experiments protracted hypotension was produced by the following technique. Under Sodium-Evipan anaesthesia, 25 ml/kg blood was withdrawn from the animals. A 10 ml portion of this blood was saved after heparinization. If the blood-pressure had not sunken to 50 mm Hg or below, after half an hour further 5 ml/kg was let at ten minute intervals until the desired level (50 mm Hg) was reached. In the case of a fall below 30 mm Hg, 5 to 10 ml/kg of the heparinized blood was reinfused. It was our experience that cats with blood pressure below 30 mm Hg could live but 15 minutes. Hypotension was then maintained for one hour, without plasma substitute. Thereafter, the preparation under investigation was introduced and the blood pressure recorded through 30 minutes. Some minutes after administering the plasma substitute, the animals received a last dose of Evipan (20 mg), 30 minutes after the infusion the cannulas were removed, the wound was treated with antibiotic and closed. The animals were then carefully wrapped up and kept in the laboratory at a temperature between 17 and 24° C. 19 control cats were treated similarly, but without any plasma substitute and the survival of the two groups was compared. The results of these experiments are summarized in Table I.

Table I

Effect of dextrans of different molecular weight on blood pressure and survival of hypovolaemic cats. Hypotension maintained for one hour before giving dextran

Preparation	Number of animals	Blood withdrawn ml	Mean blood pressure, mm Hg after dextran				Survival		
			initial	before dextran	0′	30′	4	24	48 h
Dextraven	16	26.4	133	41	100	119	1/16	8/16	12/16
Plasmodex	15	26.2	121	34	97	116	2/15	9/15	10/15
Phys. saline	15	25.3	127	37	82	62	4/15	8/15	10/15
Control	19	25.3	121	—	—	—	14/19	16/19	16/19

Table I shows that 14 of the 19 animals not treated with plasma substitute died within four hours. After 24 hours further two cats died so that only 3 remained alive. The effect of the plasma substitute manifested itself mainly in the first four hours following haemorrhage. At this time even physiological saline produced a significantly longer ($P = 0.01$) survival than that of the untreated controls. When evaluated 24 hours after the treatment, Dextraven led to 50 per cent survival, saline to 53.5 per cent and Plasmodex to 60.6 per cent. The increase in the percentage of survivors evoked by Dextraven was statistically significant ($P = 0.05$), while that produced by the other two materials was not significant. After 48 hours the number of survivors was less and, if judged at that time, neither of the preparations ensured a survival rate significantly greater than that of the controls, in spite of some numerical improvement.

Hypotension of one hour duration without added plasma substitute seems, therefore, sufficient to render the shock irreversible. Thus, in most cases the effect of plasma substitutes was restricted to a few hours, and no life-saving effect was evident 48 hours after bleeding. This irreversibility of the shock is probably a process developing slowly. It is different from that type of irreversibility, in which not even a transient effect is exerted by plasma substitutes and the animals die of respiratory failure and hypotension within a few minutes. This latter phenomenon has been repeatedly observed during the three years when the biological effect of various production lots of Plasmodex was controlled.

Experiments with Intradex

In order to establish the correctness of the above finding, *i.e.* whether the protracted hypotension had caused the lack of any permanent life-saving effect of the plasma substitutes, a somewhat different way was chosen for the investigation of Intradex. Blood withdrawal was the same as above, but when the 60 mm Hg pressure level was attained and maintained for a few minutes, the plasma substitute was introduced immediately, in a volume corresponding to that of the lost blood. This was made to reveal an eventual difference between the action of saline and of the dextrans employed. Accordingly, in these experiments carotid pressure was measured also 3, 6, 12 and 22 hours after the administration of the materials. In the intervals the animals were covered with blankets and were kept lying on a cushion in their cages. Blood pressure was easy to measure in these animals even without anaesthesia. However, when the cat showed some resistance or pain reaction, a short anaesthesia was carried out by means of 10 to 40 mg Sodium-Evipan given intravenously. The fact was naturally taken into account that Evipan may sometimes induce a transient fall in blood pressure. After the 22-hour blood pressure recording,

the wounds of the animals were closed. Subsequently, the cats were replaced into their cages and survival was recorded for 48 hours under identical laboratory circumstances.

The Intradex used in our experiments was signed as Batch No. 54/047. It had an intrinsic viscosity of 0.31 at 35° C. The mean blood pressure values observed after Intradex administration are shown in Fig. 2, while the individual data are demonstrated in Tables II to IV. Statistical analysis is shown in Table V (test χ^2).

As seen in Fig. 2, mean blood pressure after the blood withdrawal was practically identical in the three series (51—53 mm Hg). This decreased

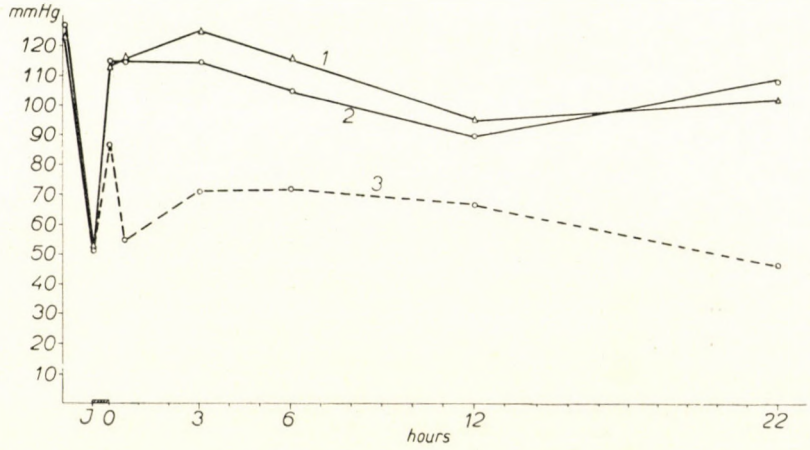

Fig. 2

pressure level was elevated by the different plasma substitutes to varying heights and for varying times. Physiological saline had the slightest effect (87 mm Hg). One half hour after the haemorrhage the pressure decreased to 55 mm Hg, while after 3, 6, 12 and 22 hours it was somewhat higher (62—75 mm Hg).

Much greater and more prolonged actions were observed with the dextran preparations. Immediately after the infusion, blood pressure was 27 to 28 mm Hg higher than in the saline controls.

One half hour, as well as three hours, after the infusion, blood pressure in the Plasmodex group was the same as immediately following the infusion. Though Intradex was somewhat more effective, the difference between the two groups was statistically insignificant and 6 and 12 hours after the infusion blood pressure was decreased to the same extent with both dextran preparations. Blood pressure after 12 hours was significantly higher in the dextran cats, than in the saline group (90—95 and 67 mm Hg, respectively). There-

after, both dextran types led to a slight elevation (108 to 102 mm Hg) which, however, did not reach the initial normal level (123—128 mm Hg).

The survival rate of these animals is shown in Tables II to IV. Sixty five per cent of the cats treated with physiological saline died within 24 hours, and 83 per cent in 48 hours. In the Plasmodex group, the 24-hour value was 33 per cent. This was unchanged after 48 hours. Only 21 per cent of the animals treated with Intradex died in the first 24 hours. This rate increased to 36 per cent by next day.

Table II

Effect of Plasmodex on blood pressure and survival of hypovolaemic cats. Hypotension maintained for a few minutes before giving Plasmodex

Date	Blood withdrawn ml	Initial	Before dextran	Blood pressure mm Hg after dextran						Survival	
				0′	1/2	3	6	12	22	24	48 h
July 3.	30.8	156	52	134	130	64	+	—	—	+	+
,, 17.	18.5	134	46	106	76	120	104	86	96	alive	alive
,, 19.	25.0	122	56	96	112	120	100	—	100	,,	,,
,, 25.	25.0	110	50	110	110	134	112	100	116	,,	,,
,, 31.	25.0	120	56	100	136	112	112	80	88	,,	,,
Aug. 7.	27.0	140	54	110	114	108	+	—	—	+	+
,, 8.	25.0	114	54	104	96	100	34	26	+	+	+
,, 14.	25.0	114	46	114	102	106	96	90	110	alive	alive
,, 16.	25.0	120	56	128	130	120	104	90	120	,,	,,
Sept. 3.	25.0	126	60	112	102	62	62	+	—	+	+
,, 4.	25.0	130	52	128	116	124	110	100	114	alive	alive
,, 5.	22.0	140	54	114	130	150	150	110	128	,,	,,
Oct. 21.	25.0	114	50	134	120	134	122	100	94	,,	,,
,, 22.	28.7	134	56	110	128	138	120	100	100	,,	,,
,, 23.	26.8	146	160	130	136	140	136	104	+	+	+
Mean value	25.2	128	53	115	115	115	105	90	108	5/15	5/15

All the above data indicate that both dextran preparations produced a greater survival rate after haemorrhagic shock than did physiological saline. In this respect no difference was found between the two preparations. Statistical analysis of the results with the χ^2 test as corrected by Yates revealed that the increase in the 24-hour survival in the Plasmodex group was statistically insignificant when compared to the saline group. On the other hand, the 48-hour survival was significant ($P = 0.02$). 24 hours' survival in the Intradex group was greater than in the saline group. This difference was significant statistically at the 5 per cent level, similarly as on the second day.

Table III

Effect of Intradex on blood pressure and survival of hypovolaemic cats. Hypotension maintained for a few minutes before giving Intradex

Date	Blood withdrawn ml	Initial	Blood pressure mm Hg							Survival	
			Before dextran	After dextran							
				0	1/2	3	6	12	22	24	48 h
Nov. 6.	33.3	102	60	110	142	114	122	122	46	+	+
,, 7.	26.8	—	56	114	116	104	102	76	106	alive	alive
,, 12.	25.0	102	54	112	100	116	108	80	106	,,	+
,, 13.	25.0	140	56	106	104	94	80	26	+	+	+
,, 14.	25.0	104	52	102	106	126	112	98	108	alive	alive
,, 18.	25.0	150	52	120	106	130	114	104	58	,,	,,
,, 19.	28.8	132	56	114	126	136	136	114	124	,,	,,
,, 20.	25.0	110	36	110	100	110	112	40	+	+	+
,, 21.	25.0	144	56	120	114	140	126	112	100	alive	alive
,, 25.	27.0	110	60	106	112	136	126	114	134	,,	,,
,, 26.	25.0	130	52	130	128	142	130	110	130	,,	,,
,, 27.	25.0	110	52	124	102	124	122	102	118	,,	,,
,, 28.	25.0	150	52	122	136	142	130	130	134	,,	,,
Dec. 3.	27.1	116	50	114	124	142	110	100	66	,,	+
Mean value	26.3	123	53	114	116	125	116	95	102	3/14	5/14

Haemodilution was followed by measuring the haemoglobin concentration. The results of these investigations are shown in Fig. 3.

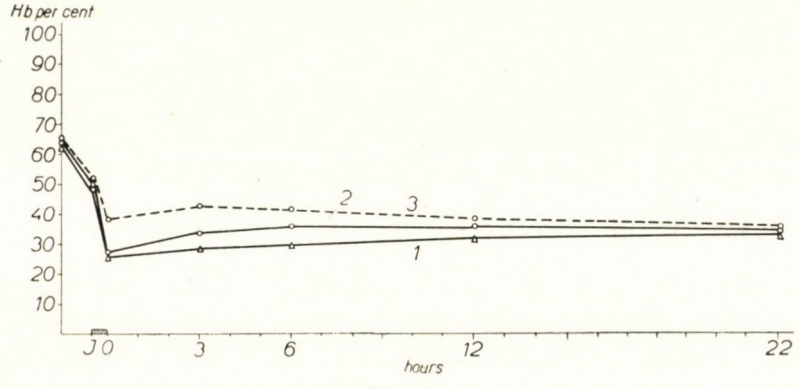

Fig. 3

The greatest haemodilution occurred always immediately after the infusion had been brought to an end. As it has been expected, physiological saline

Table IV

Effect of physiological saline on blood pressure and survival of hypovolaemic cats. Hypotension maintained for a few minutes before giving saline

Date	Blood withdrawn ml	Initial	Blood pressure mm Hg							Survival	
			Before dextran	After dextran							
				0	1/2	3	6	12	22	24	48 h
Apr. 5.	26.8	150	52	86	60	66	82	—	100	alive	+
,, 8.	25.0	136	58	94	50	+	—	—	—	+	+
,, 9.	21.0	104	40	86	62	100	108	—	98	alive	alive
,, 10.	30.6	146	40	86	70	62	56	—	+	+	+
,, 11.	28.7	120	48	98	48	+	—	—	—	+	+
May 2.	28.7	102	50	90	55	78	74	—	134	alive	alive
,, 3.	28.9	130	40	100	52	40	+	—	—	+	+
,, 6.	25.0	142	56	90	46	46	24	—	+	+	+
,, 7.	20.8	104	46	70	56	74	60	—	+	+	+
,, 8.	26.9	130	54	100	74	108	92	—	114	alive	alive
,, 23.	26.8	160	50	110	62	—	80	54	46	+	+
,, 28.	25.0	110	30	64	40	—	84	60	+	+	+
Sept. 14.	25.0	146	54	90	56	86	82	82	80	alive	+
,, 20.	25.0	120	60	80	72	52	60	54	+	+	+
Oct. 10.	26.5	134	58	94	54	94	82	90	50	alive	+
,, 14.	25.0	102	50	70	32	26	28	52	22	+	+
,, 15.	27.0	130	58	76	46	94	90	74	36	+	+
Mean value	26.0	127	51	87	55	71	72	67	75	11/17	14/17

Table V

Statistical evaluation	t	*P*
1. Dextraven, as compared to Plasmodex. Lethal bleeding volume	4.301	< 0.01
2. Plasmodex as compared to physiological saline. 6-hour blood pressure	3.87	< 0.01
3. Plasmodex as compared to physiological saline. 12-hour blood pressure	2.298	< 0.05
4. Plasmodex as compared to Intradex. 3-hour blood pressure	1.266	> 0.20
5. Plasmodex as compared to Intradex. 6-hour blood pressure	1.319	> 0.10
6. Plasmodex as compared to saline. 3-hour haemoglobin	4.051	< 0.01
7. Plasmodex as compared to saline. 6-hour haemoglobin	2.132	< 0.05
8. Intradex as compared to saline. 12-hour haemoglobin	1.468	> 0.10
9. Saline as compared to control. 4 hours' survival	9.207	< 0.01
10. Dextraven as compared to control. 24 hours' survival	3.926	< 0.05
11. Saline as compared to control. 24 hours' survival	3.133	< 0.10
12. Plasmodex as compared to saline. 24 hours' survival	2.09	> 0.10
13. Plasmodex as compared to saline. 48 hours' survival	6.036	< 0.02
14. Intradex as compared to saline. 24 hours' survival	4.18	< 0.05
15. Intradex as compared to saline. 48 hours' survival	5.210	< 0.05

produced the slightest haemodilution. In this respect, all dextran preparations behaved identically. After a transient decrease in the third hour, haemodilution in the saline group underwent a continuous increase so that the haemoglobin content after 22 hours was practically the same in both the saline and the dextran groups ; in the latter it continued further to augment, showing that the initial haemodilution was slowly subsiding. The disappearance of haemodilution was slower in the Intradex group than in the Plasmodex group. However, haemodilution after 6 hours was significantly greater even in the Plasmodex group than in the saline one, while 12 hours after the infusion the haemoglobin concentration was already the same as in the Intradex and the saline-treated animals.

Discussion

The above experiments indicate that, in the cat, withdrawal of about 25 ml/kg blood without administration of plasma substitute is not compatible with life (84.3 per cent mortality). This observation corroborates the results of other investigators (BAYLISS *et al.*, DUBROWSKI and PANASEWICZ [6]). Had the dextran preparations been administered within 15 minutes following the haemorrhage, they were capable of preventing the serious shock in two thirds of the animals. In this respect there was no difference in the life-saving effect of small and large molecular weight dextrans. However, when the hypotension had lasted one hour, the plasma substitutes achieved only temporary remission. 24 hours after the infusion, Dextraven still exhibited a statistically significant protective effect ($P = 0.05$), while the small molecular weight dextran failed to do so. Survival after 48 hours was not significantly ameliorated by the use of the dextran preparations.

Another interesting finding was the failure of the regulatory mechanism of the cat to maintain the blood pressure at the nearly normal level which had been reached immediately after the infusion of dextran preparations. The maximum fall in blood pressure was observed after 12 hours. After 22 hours the blood pressure level showed a slight increase to a level which, though higher than the 12-hour value, still did not reach the normal. This statement is probably valid also for other animal species, since PIRANI, JUSTER *et al.* [2] made similar observations on the dog.

In the present experiments no significant difference has been found between large and small molecular weight dextrans in their effect on blood pressure and survival rate. In this connection the question arises whether a dextran preparation with an even lower molecular weight would have a satisfactory therapeutical effect. According to the experience of American military surgeons in Korea [7], a dextran preparation with a molecular weight of 42 000 actually induced an improvement of the circulation, but this was

restricted to a few hours, due to the rapid elimination of this dextran type. SIMON [8], studying a Soviet dextran preparation with a similarly low molecular weight (viscosity = 0.15), found that the pressor effect of that preparation surpassed that of physiological saline only in the first three hours, while it completely failed to improve the survival rate. Accordingly, preparations of the above molecular weight and viscosity have no protracted effect. They may serve for increasing the plasma volume for a few hours, but thereafter transfusion of blood is needed.

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PHARMACOLOGY OF A NEW SPASMOLYTIC DRUG

By

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A new papaverine-analogue synthetized in the *Chinoin* laboratories has been investigated. The compound, known chemically as 1-(3′-4′-dimethoxyphenyl)-6-7-dimethoxy isoquinoline, bears the proprietary name Chinoparine.

Chinoparine showed a spasmolytic activity identical with that of papaverine, as tested on the coronary flow of the isolated cat heart, excised guinea pig lung, cat intestine *in situ* and rat uterus *in situ*. Chinoparine decreased blood pressure 30 per cent more than did papaverine.

Venous pressure was not affected by Chinoparine, while it was markedly elevated by papaverine.

The impairing effect on the cat heart *in situ*, as measured by means of a Henderson-type cardiometer, was considerably greater with papaverine than with Chinoparine.

Chinoparine proved to be half as toxic than papaverine when administered to rats intraperitoneally; and 2.8 times less toxic, when administered intravenously.

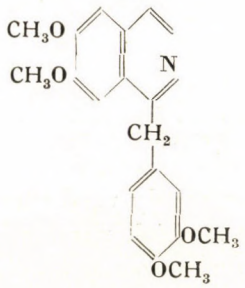
*

Papaverine is still one of the most commonly used spasmolytics. Sometimes, however, the drug exerts side effects which necessarily limit its use. Of these, the heart-impairing effect has been especially studied. Thus, HANZLIK [3], as well as ISSEKUTZ [5] observed in cold blooded animals that higher concentrations of papaverine were weakening the heart and evoked diastolic standstill. SAKUSSOW [9] demonstrated the negative inotropic, chronotropic, bathmotropic and dromotropic actions of papaverine on both the frog and the mammalian heart. The cardiac action of papaverine has been analysed in great detail by ELEK and KATZ [1], who found low concentrations of the drug to improve conduction in the dog heart, while higher concentrations to cause block, decrease myocardial excitability and to induce ventricular fibrillation or cardiac arrest. TARDOS [12] demonstrated that papaverine decreased the oxygen consumption of the cardiac muscle, an effect which he made responsible for the drug's depressive action on the contractility of the excised papillary muscle.

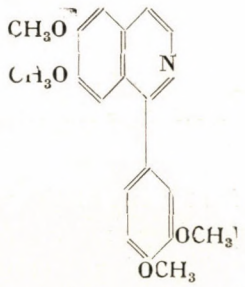
Therapeutic and toxic actions of spasmolytic drugs do not always go hand in hand. Thus, TSATSAS and FOURNEL [13] found that the chemically related 1-(2′-3′-dimethoxy phenyl)6-7-dimethoxy isoquinoline had a 50 per cent more advantageous therapeutic index than has papaverine. ISSEKUTZ [4] reported that the antispasmodic action of perparine was stronger than that of papaverine, while its toxicity was 2.4 times lower.

SLOTTA and HABERLAND [10] synthetized some papaverine analogues with an isoquinoline-phenyl ground structure instead of the isoquinoline-benzene present in papaverine. One of the compounds differing from papaverine only in the lack of the CH_2 group was 1-(3′-4′-dimethoxy-phenyl-)6-7-dimethoxy isoquinoline. This compound has not been investigated in detail as to its pharmacological actions ; ISSEKUTZ *et al.* [6] studied only the surface activity and spasmolytic action, which they found to be identical with those of papaverine.

Recently, this derivative has been synthetized again, in the Chinoin laboratories by BUKOSZA *et al.* The preparation with the proprietary name Chinoparine is closely related to the above-mentioned compound of TSATSAS and FOURNEL [13], from which it differs only in the position of the methyl groups. The spasmolytic activity of the 2-3-phenyl derivative was weaker than that of papaverine, while Chinoparine proved to be of identical if not of higher effectiveness.



Papaverine



Chinoparine

It commonly occurs that compounds inhibiting $BaCl_2$-induced spasms on excised organs are ineffective *in vivo*. For this reason, the present experiments were devoted mainly to investigating Chinoparine on intact animals.

Methods

The experiments were performed on cats under combined chloralose urethane 50 mg/kg and 400 mg/kg, respectively or urethane (800 mg/kg) anaesthesia. Arterial pressure in the left femoral artery was recorded by means of a mercury manometer. Venous pressure was measured through a polyethylene catheter inserted into the left jugular vein and connected to a Marey capsule. Motions of the duodenum were recorded by inserting into the duodenum a water-inflated balloon and connecting it to a Marey capsule. The top of the water column lay 80 to 120 mm above the intestine.

The drugs dissolved in physiological saline were injected into the right femoral vein.

In some experiments cardiac function was registered by means of a Henderson-type cardiometer. In these cases the chest was opened and artificial respiration was carried out.

The dilatating effect of Chinoparine on the coronary vessels was investigated on cat hearts isolated according to LANGENDORFF. The heart was perfused with oxygenated Locke solution at 37° C. Coronary flow was registered by means of an ordinate recorder.

Isolated guinea pig lungs served for investigating the bronchodilator effect of the drugs. For this purpose the technique of SOLLMANN [11] was used, as modified by L. ISSEKUTZ [7]. Through a cannula inserted into the trachea the lungs were perfused with oxygenated Locke solution at 37° C and at a perfusion pressure of 80 mm Hg. 25 to 30 small holes serving for the leakage of the perfusion fluid were made on the lung with a 20-gauge hypodermic syringe. Perfusion rate was measured by means of an ordinate recorder.

The method of ENGELHORN and SCHMIDT [2] was employed to measure the actions on the rat uterus *in situ*. The uterus horns explored under urethane anaesthesia (0.8 to 1.2 g/kg) were connected to an isotonic lever and the motions were registered kymographically. The uterine horns were enclosed into a glass vessel, in which Krebs phosphate solution was kept circulating at 37° C. The compounds were administered through a cannula inserted into the jugular vein.

Toxicity of the compounds was determined in the albino rat, both after intraperitoneal and intravenous administration.

Chinoparine, employed as its hydrochloride salt, was dissolved in physiological saline under warming. In all the experiments papaverine served for comparison.

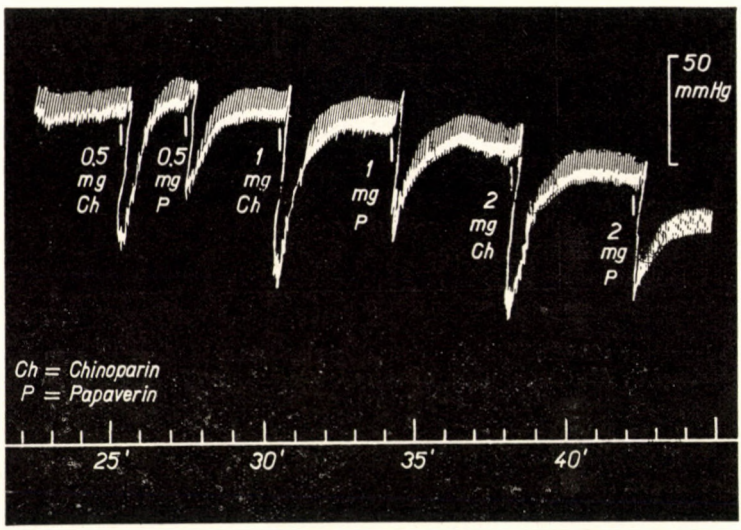


Fig. 1. Cat, 2000 g. Chloralose + urethane anaesthesia. Arterial blood pressure and time signal on the 0 line. Ch = Chinoparine, P = Papaverine

Results

1. *Arterial blood pressure*

0.5 to 2.0 mg/kg Chinoparine administered intravenously to the cat induced a greater fall in blood pressure than did identical doses of papaverine. However, when higher doses were employed, papaverine surpassed Chinoparine. The fall in blood pressure evoked by large doses of papaverine (3 to 10 mg/kg) was also of longer duration than that induced by Chinoparine. Fig. 2, which summarizes the results of these experiments, shows that for

evoking identical depressor responses, some 30 per cent less sufficed of Chinoparine than of papaverine.

2. *Venous pressure*

Fig. 3 demonstrates that the pressure in the jugular vein was hardly affected by 1.0 mg and 1.5 mg/kg Chinoparine, while identical doses of papaverine increased it considerably (30 and 32 mm water, respectively).

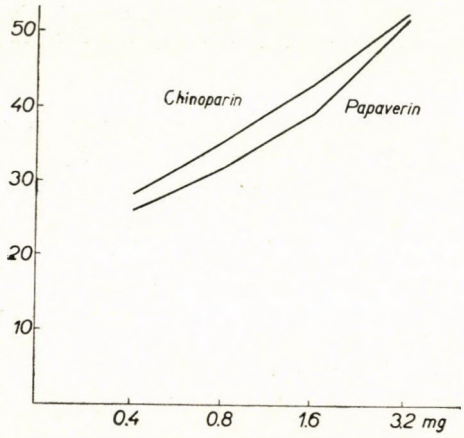


Fig. 2. Vasodepressor effect of Chinoparine and papaverine. Each value represents the mean from 14 experiments. Abscissa : doses in mg/kg. Ordinate : fall in blood pressure, mm Hg.

3. *Cat heart in situ*

In contrast with papaverine, Chinoparine impaired cardiac function only at very high dose levels, if at all. As Fig. 4 shows, 3 mg/kg Chinoparine had a positive inotropic and tonotropic action, while the same dose of papaverine a positive inotropic but negative tonotropic one. 6 mg/kg papaverine caused marked dilatation of the ventricles. 10 mg/kg Chinoparine exerted a transient negative inotropic effect which was quickly followed by a positive inotropic and tonotropic action. Identical doses of papaverine markedly depressed cardiac function and caused bradycardia, while Chinoparine did not influence heart frequency. These data, together with other similar observations, indicate that even large doses of Chinoparine hardly influence cardiac function ; they may eventually improve it. On the contrary, papaverine impairs the heart markedly. This might be responsible also for the finding that in large doses papaverine causes a greater and more protracted fall blood in pressure than does Chinoparine.

4. *Coronary flow*

No difference was found between Chinoparine and papaverine in their effect on the coronary flow in Langendorff hearts. Fig. 5 demonstrates the

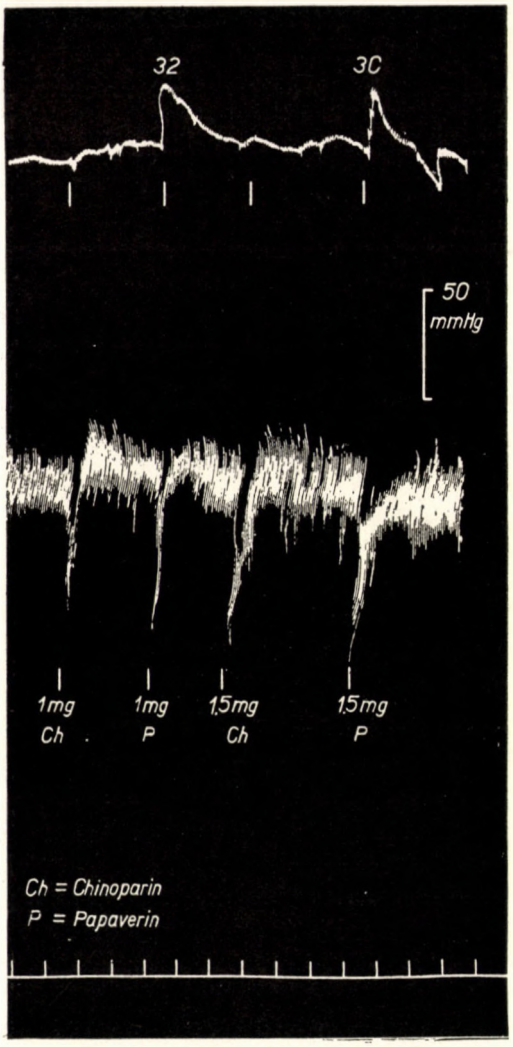


Fig. 3. Cat, 3400 g. Urethane + chloralose anaesthesia. Venous and arterial pressure. Time signal on the 0 line

action of 1 mg papaverine and 3 mg Chinoparine, respectively. The mean values in 15 experiments were,

1 mg papaverine : increase in the flow rate from 25.4 ml/min to 57.6 ml/min (+ 127 per cent),

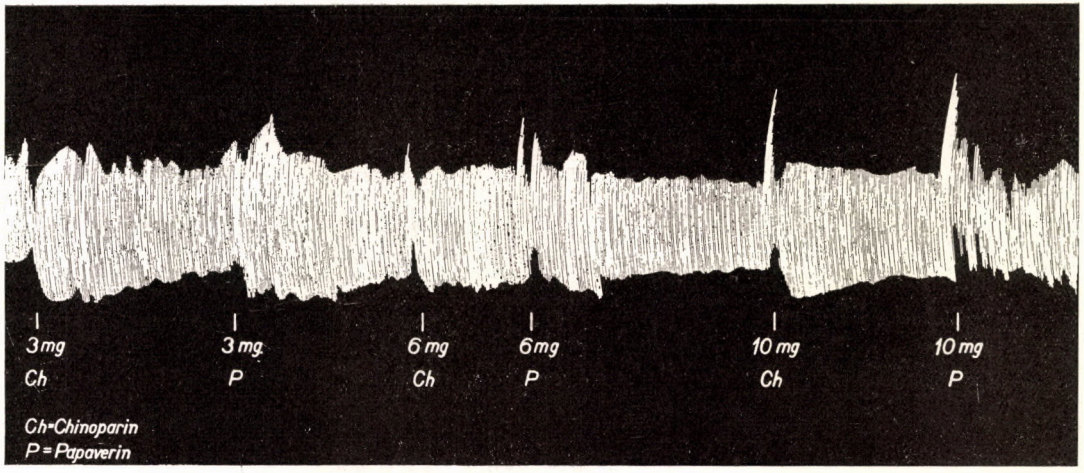


Fig. 4. Cat, 2500 g. Urethane + chloralose anaesthesia. Record of *Henderson* cardiometer. Upward deviations correspond to ventricular dilatation, downward deviations to reinforcement of the contractions

1 mg Chinoparine : increase in the flow rate from 24.8 ml/min to 57.2 ml/min (+ 131 per cent).

As seen, the two compounds dilated the coronary vessels to the same extent.

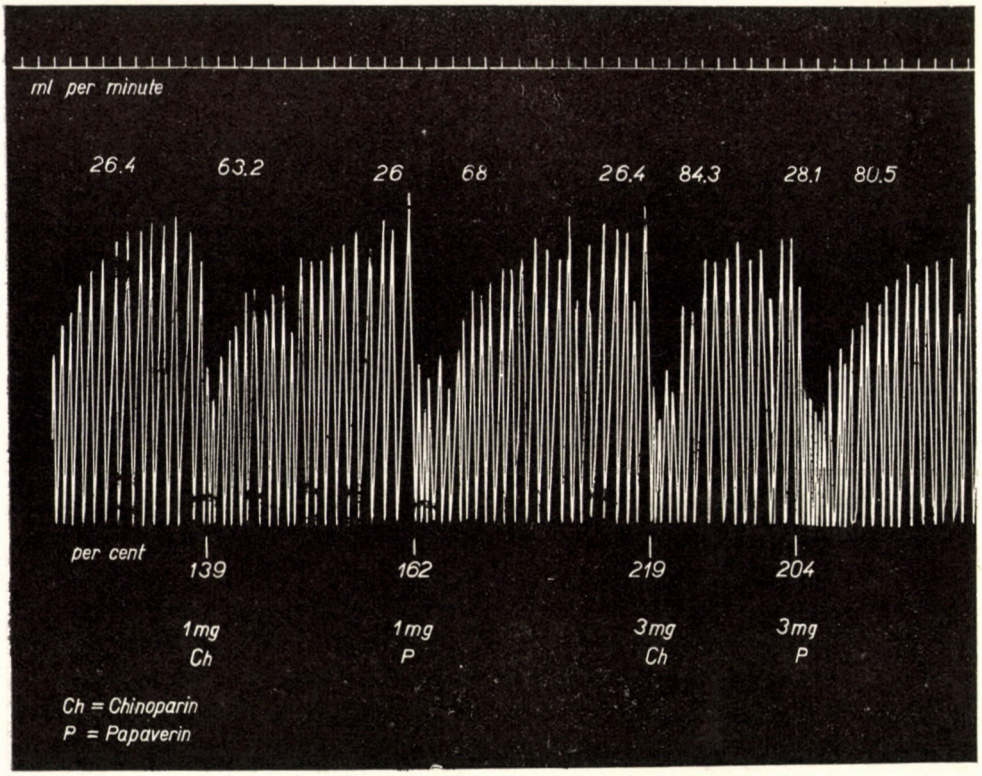


Fig. 5. Cat heart isolated according to LANGENDORFF. Coronary flow, as registered by an ordinate recorder. Top : time signal in minutes. Bottom : record of the ordinate recorder. Upper numbers : coronary flow, ml/min. Lower numbers : increase in coronary flow, expressed as per cent of initial value

5. *Isolated guinea pig lung*

The lungs were perfused with a Locke solution containing 20 μg/ml histamine. A typical experiment is shown in Fig. 6. During perfusion with histamine, the out-flow rate was 5.7 to 6.1 ml/min. 1 mg papaverine given into the cannula increased this value to 20.4 ml/min. 0.2 mg of any of papaverine and Chinoparine elevated the perfusion rate to 16.1 respectively 14.7 ml/min. All our similar experiments showed the two compounds to possess an essentially identical bronchodilating activity.

6. *Motility of the duodenum in situ*

Identical doses of papaverine and Chinoparine inhibited the motions of the cat duodenum for an identical period of time. Fig. 7 shows the effects of 0.1, 0.2, and 0.4 mg/kg Chinoparine and papaverine.

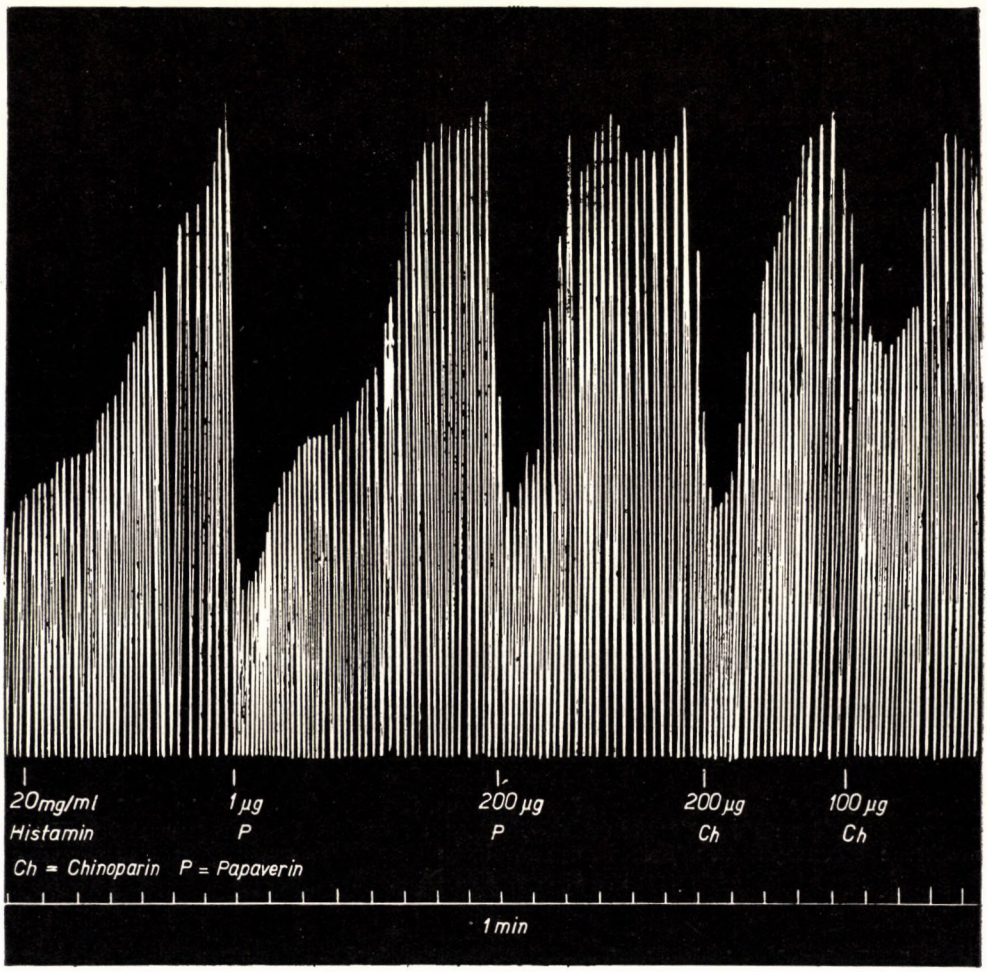


Fig. 6. Guinea pig lung, isolated according to SOLLMANN—L. ISSEKUTZ. Rate of flow through the bronchia registered by means of an ordinate recorder. Time signal in minutes. Starting from the sign at left, the perfusion fluid contained 20 µg/ml histamine

7. *Uterine motions in situ*

On the effect of 5 to 10 mg/kg Chinoparine or papaverine administered to rats by the intravenous route, uterine movements ceased for a certain period (Fig. 8). The compounds were identically effective.

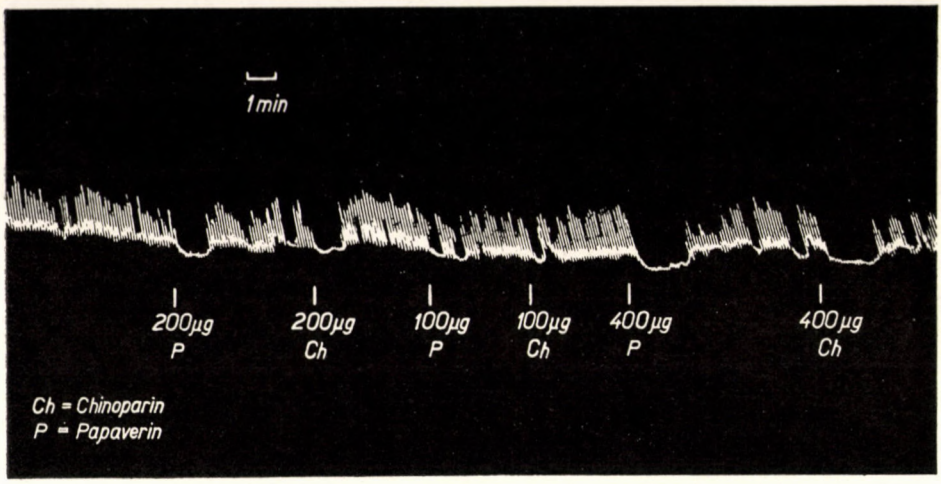


Fig. 7. Cat, 4200 g. Urethane anaesthesia. Motions of the duodenum *in situ*. Doses in mg/kg

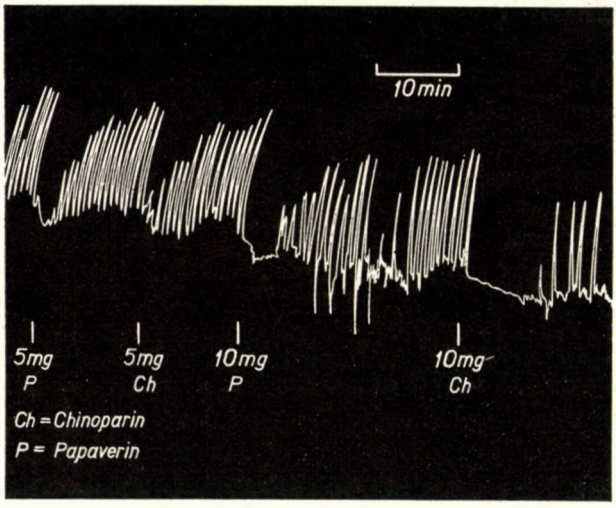


Fig. 8. Motions of rat uterus *in situ* (ENGELHORN—SCHMIDT). Doses in mg/kg.
P = Papaverine, Ch = Chinoparine

8. *Toxicity*

The toxicity of Chinoparine was compared with that of papaverine in the rat, on intravenous and intraperitoneal administration. In lethal doses both compounds caused deep narcosis. When the preparations were given intraperitoneally, the animals died of respiratory failure, while intravenous

administration rather led to cardiac arrest. The LD_{50} of papaverine was 26.25 mg/kg intravenously, that of Chinoparine, 73.33 mg/kg. On intraperitoneal administration the values were 107.0 mg/kg and 215.0 mg/kg. Accordingly, papaverine was found to be 2.0 to 2.8 times more toxic than Chinoparine.

Discussion

The above data showed Chinoparine to be equal in spasmolytic action to papaverine, as observed on the coronary vessels of the isolated cat heart, on the bronchia of guinea pigs, in the intestine *in situ* of the cat, and on the uterus *in situ* of the rat. The vasodepressor activity of Chinoparine was 30 per cent stronger than that of papaverine. In the rat, Chinoparine was half as toxic than papaverine, when administered intraperitoneally ; and 2.8 times less toxic, when given intravenously.

The toxicity of Chinoparine was decreased by the lack of the CH_2 group between the rings, a fact similarly observed with TSATSAS' and FOURNEL's papaverine-analogue [13]. As a rule, the absence of the CH_2 group seems to decrease the toxicity of the molecule, since KREITMAR [8] found the CH_2-free analogue of Eupaverine (compound No. 92) to be less toxic, but to exert a ten times greater spasmolytic activity, than the parent compound.

What feature can be accounted for the fact that Chinoparine is much less toxic than papaverine, especially when administered by the intravenous route? According to our findings, the heart-impairing effect of papaverine is considerably greater than that of Chinoparine. This was shown by the experiments on venous pressure and by those made with the cardiometer. These investigations revealed namely that Chinoparine failed to cause any marked dilatation of the ventricles, even at the 6 to 10 mg/kg dose level, while papaverine impaired the heart considerably. Most probably it is the heart-weakening action which causes large doses of papaverine to induce a greater and longer-lasting fall in blood pressure than that evoked by identical doses of Chinoparine. The reduced toxicity and the decreased heart-impairing effect of Chinoparine may prove of value, since papaverine is often being prescribed to patients with narrowed coronaries and with an affected myocardium. In such cases papaverine may be deleterious. Chinoparine, on the other hand, having an identical spasmolytic activity, might perhaps be tolerated in amounts considerably greater than papaverine.

Finally, our experiments brought evidence that the spasmolytic activity of a compound and its heart-impairing toxic action are not closely connected, a fact offering the opportunity of synthetizing new, more active and less toxic derivatives.

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RECHERCHES SUR QUELQUES PROPRIÉTÉS PHARMACODYNAMIQUES DE L'HYDROXYZINE (ATARAX)

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Dans une première série d'expériences, on a étudié l'action de l'hydroxyzine administrée par voie intraveineuse, dans des doses variant entre 5 et 36 mg/kilocorps, sur les modifications tensionnelles et électrocardiographiques produites par l'excitation de l'hypothalamus et d'autres structures végétatives centrales. Les expériences ont été effectuées sur 8 chats et 4 chiens. On a excité électriquement (2—10 V) 45 points différents situés dans les régions basales du cerveau, par des électrodes mono- et bipolaires, dont l'emplacement a été vérifié anatomiquement. Quatorze de ces points ont produit, après l'excitation, un effet cardio-vasculaire. L'adrénalino-sécrétion déclanchée par l'excitation hypothalamique a été supprimée, dans quelques expériences, par une section médullaire pratiquée au niveau C_7—D_1 ou D_2—D_3.

On a obtenu les résultats suivants :

1. L'hydroxyzine diminue, jusqu'à suppression presque totale, les effets hyper- et hypotensifs produits par l'excitation de l'hypothalamus et d'autres structures végétatives centrales.

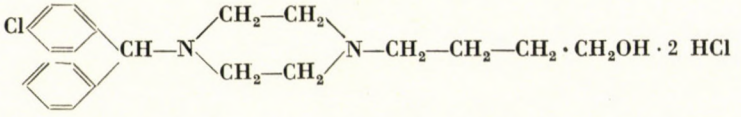
2. L'hydroxyzine a une influence plus réduite sur l'effet hypotensif qui précède ou succède l'effet hypertensif de l'excitation hypothalamique, de même que sur l'adrénalino-sécrétion déclanchée par l'excitation hypothalamique ou rhinoencéphalique.

Dans une deuxième série d'expériences on a étudié, sur 9 chats et 6 chiens curarisés, l'action de l'hydroxyzine sur l'électrocardiogramme, enregistré dans les dérivations standard et la précordiale de l'apex. On a constaté que l'hydroxyzine (5—10 mg/kg. i. v.) provoque l'augmentation de l'amplitude de l'onde T ou la positivation d'une onde T négative, ce qui traduit une favorisation de la répolarisation cardiaque. Ces modifications ne sont pas en rapport avec les modifications tensionnelles produites par l'hydroxyzine.

Dans une troisième série expérimentale on a étudié l'action de l'hydroxyzine sur le coeur isolé de grenouille. Les résultats ont montré que les grandes doses d'hydroxyzine ont une action inotrope négative. L'hydroxyzine diminue, jusqu'à suppression totale, l'effet inotrope négative de l'acétylcholine et du potassium. Par contre, l'hydroxyzine n'a aucune action sur l'effet inotrope positif de l'adrénaline et du calcium.

*

L'hydroxyzine (Atarax, UCB 4492) est un dérivé de la pipérazine, synthétisé dans les dernières années et qui répond à la formule suivante :



L'hydroxyzine présente une toxicité très réduite et possède de nombreuses actions pharmacodynamiques [9] dont les plus importantes sont : l'action antihistaminique, analgésique, antispasmodique, anesthésique locale, antiémétisante, éosinopénique, antiphlogistique et vasodilatatrice. Introduite par voie intraveineuse, l'hydroxyzine produit une chute brusque de la pression

sanguine, qui revient rapidement aus niveau initial. L'hydroxyzine ne possède pas une action ganglioplégique [9].

Dans les derniers temps, l'hydroxyzine, qui manifeste un remarquable effet neuro-sédatif, est utilisée de plus en plus fréquemment dans le traitement des affections psychiques. Mais, bien que les indications cliniques de l'hydroxyzine ont été — en général — précisées, on ne connaît presque rien sur le lieu et le mécanisme d'action de cette substance dans le système nerveux central. Ainsi, LA BARRE [1] affirme — étant donné que l'hydroxyzine diminue l'influence de l'hypoglycémie insulinique sur les centres hypothalamiques — que la substance diminue l'excitabilité des régions de la base du cerveau.

Ayant en vue la participation active de l'hypothalamus tant dans la régulation des fonctions végétatives, que dans les processus psychiques, nous avons étudié l'influence de l'hydroxyzine sur cette région du système nerveux central. Nous avons utilisé comme indicateur les modifications cardio-vasculaires produites par l'excitation de l'hypothalamus. Nous avons exploré aussi parallèlement quelques points situés dans le rhinencéphale, dans la formation réticulée mésencéphalique, dans le thalamus et le sous-thalamus.

Au cours de ces recherches, nous avons remarqué aussi que la substance manifeste une action propre sur le coeur, action qui paraissait être indépendante de celle exercée par l'hydroxyzine sur la tension artérielle. Ce fait nous a incité à faire des études spéciales sur l'action cardiaque de l'hydroxyzine. Dans la littérature, nous n'avons trouvé que deux travaux où on fait mention d'une pareille action : CAMPO—RODRIGUEZ [4] met en évidence, dans le coeur du crapaud, un chronotropisme négatif et un inotropisme positif à des concentrations faibles et modérées de l'hydroxyzine et un effet toxique aux hautes concentrations, tandis que WEYNE et ROUSSEL [13] n'ont pu observer aucun effet de l'hydroxyzine sur l'électrocardiogramme chez l'homme.

Méthode

La première série d'expériences a été effectuée sur 8 chats et 4 chiens curarisés par voie intramusculaire avec 2 mg/kilocorps de curare ; dans deux cas, on a ajouté 7 ctg/kilocorps de chloralose. On a pratiqué la respiration artificielle. Pour supprimer l'adrénalino-sécrétion produite par la stimulation hypothalamique, on a sectionné dans quelques cas la moelle épinière au niveau C_7—D_1 ou D_2—D_3, 5—6 heures avant l'expérimentation. Pour l'excitation électrique de l'hypothalamus, on a utilisé des électrodes de cuivre émaillées ; l'isolation électrique était supprimée à la pointe des électrodes sur une longueur de 1 mm. On a utilisé des électrodes monopolaires et bipolaires. Les électrodes monopolaires avaient un diamètre de 0,3 mm. Les électrodes bipolaires étaient constituées par deux fils solidarisés avec une solution de plexiglass et dont les pointes étaient situées à environ 1 mm l'une de l'autre ; un fil avait le diamètre de 0,3 mm et l'autre, 0,1 mm. Pour l'excitation électrique, on a utilisé un courant alternatif de 2—10 V, appliqué pendant 10—15 secondes. Dans quelques cas, on a utilisé un courant rectangulaire, de 10—50 Hz, la durée de chaque pouls étant de 0,5 millisecondes. On a enregistré la pression sanguine au niveau de l'artère carotide primitive ou de l'artère fémorale, par la méthode LUDWIG. On a enregistré toujours l'électrocardiogramme, avant et après la stimulation, et parfois, aussi pendant la stimulation ; on a utilisé dans ce but un électroencéphalographe SCHWARZER à quatre canaux. On a enregistré les dérivations standard

et la précordiale de l'apex, en utilisant comme électrodes de dérivation des aiguilles implantées sous la peau de l'animal. Après l'expérience, le cerveau était fixé dans une solution de formol, parfois après une perfusion préalable avec une solution saline physiologique et ensuite avec du formol. La position de la pointe des électrodes a été vérifiée anatomiquement ; pour le chat on a utilisé l'atlas de WINKLER et POTER [14]. L'hydroxyzine hydrochlorique a été administrée par voie intraveineuse, à la dose de 5—30 mg/kilocorps, en solution de 2% et 20%.

Dans une deuxième série expérimentale, effectuée sur 9 chats et 6 chiens, nous avons étudié l'action de l'hydroxyzine sur l'électrocardiogramme (ECG). Les animaux ont été curarisés avec 2 mg/kilocorps de curare, injectée intramusculaire. On a enregistré les trois dérivations standard et la précordiale de l'apex ; parallèlement, on a enregistré toujours la pression sanguine au niveau de l'artère carotide primitive par la méthode de LUDWIG. L'hydroxyzine, en solution de 2% et 20%, a été injectée par la voie intraveineuse, en doses fractionnées — 5, 10 et 20 mg/kilocorps.

Dans une troisième série expérimentale, comprenant 26 expériences, nous avons étudié l'action de l'hydroxyzine sur le coeur de grenouille (Rana aesculenta, Rana temporaria), isolé sur une canule STRAUB et perfusé avec du ser RINGER. De même, dans ces expériences, nous avons étudié l'action de l'hydroxyzine sur l'effet cardiaque de l'acétalcholine (Ach), de l'adrénaline (Adr), du potassium (K) et du calcium (Ca), ces substances étant introduites dans la canule après avoir introduit l'hydroxyzine, selon la méthode de DANIELOPOLU [5].

Résultats

A) *Action de l'hydroxyzine sur les effets cardio-vasculaire produits par l'excitation électrique des structures végétatives centrales*

Nous avons excité 45 points divers situés dans l'hypothalamus et autres structures végétatives centrales. Nous avons obtenu des effets hyper- ou hypotensifs et électrocardiographiques seulement à l'excitation de quatorze de ces points, et c'est sur ces effets que nous avons étudié l'action de l'hydroxyzine. L'effet presseur produit par l'excitation de ces points a varié — par rapport à la région excitée et aux paramètres du courant stimulant — entre 0,6—19 cm Hg. L'hydroxyzine, administrée par voie intraveineuse, diminue beaucoup — jusqu'à la suppression presque totale — la réponse hypertensive de l'excitation des structures végétatives centrales (fig. 1—3). L'action de la substance est, en général, proportionnelle à la dose injectée. Ainsi, la dose de 10 mg hydroxyzine/kilocorps diminue la réponse hypertensive de 25—40%, la dose de 18—22 mg/kilocorps — de 67% environ, la dose de 27—30 mg/kilocorps — de 81% environ et la dose de 36 mg/kilocorps diminue l'effet presseur de l'excitation hypothalamique de 83—85%. Nous n'avons remarqué aucune relation entre la valeur initiale de l'hypertension produite par la stimulation de l'hypothalamus et l'action déprimante de l'hydroxyzine. La substance a été administrée en doses fractionnées (5—10 mg/kilocorps) afin que son effet hypotensif soit fugitif. Mais même quand nous avions administré une dose forte et unique (20 mg/kilocorps) et qu'il s'était produit une hypotension artérielle durable, celle-ci n'a pas influencé le degré de l'inhibition de la réponse hypertensive produite par l'excitation de la substance grise péri-aqueductulaire.

Dans les cas où l'excitation hypothalamique ou rhinencéphalique déclenche secondairement une adrénalino-sécrétion (que nous avons évitée dans la majorité des expériences par une section spinale au niveau C_7—D_1 ou D_2—D_3)

qui produit un deuxième crochet hypertensif, avec une pente descendente très lente, nous avons remarqué que l'hydroxyzine influence beaucoup moins cette adrénalino-sécrétion (fig. 1).

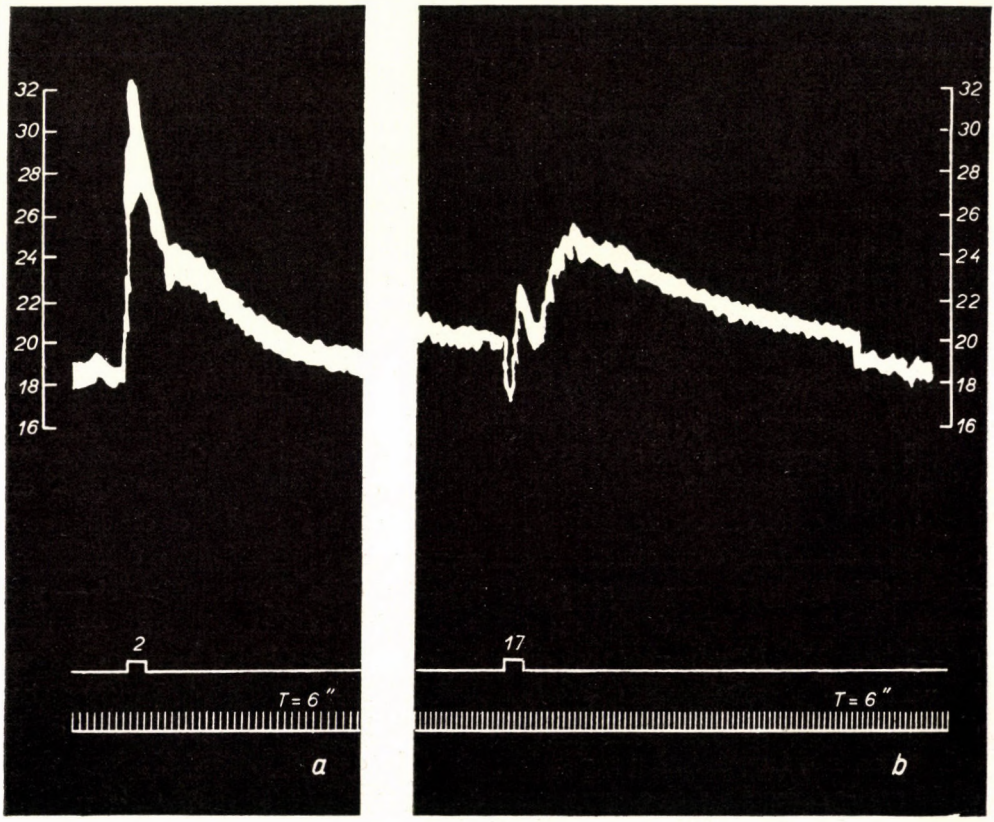


Fig. 1. — Chien no. 17.
a—2 : Excitation de l'électrode no. 5, ∼, 50 Hz, 10 v. durée = 15 secondes
b — Après 46 mg/kilocorps d'hydroxyzine, administrée i. v., en doses fractionnées à 9 mg/kilocorps
17 : même excitation qu'à A—2

Dans un seul cas l'excitation électrique des structures végétatives centrales a produit une légère hypotension. Après l'administration de l'hydroxyzine, cet effet disparaît (fig. 4).

Pour pouvoir interpréter nos résultats, nous avons considéré nécessaire d'étudier l'influence de l'hydroxyzine sur l'effet hypertensif de l'adrénaline introduite dans la veine. Nous avons remarqué qu'après l'hydroxyzine l'hypertension adrénalinique diminue en général de 8—19% par rapport à la valeur

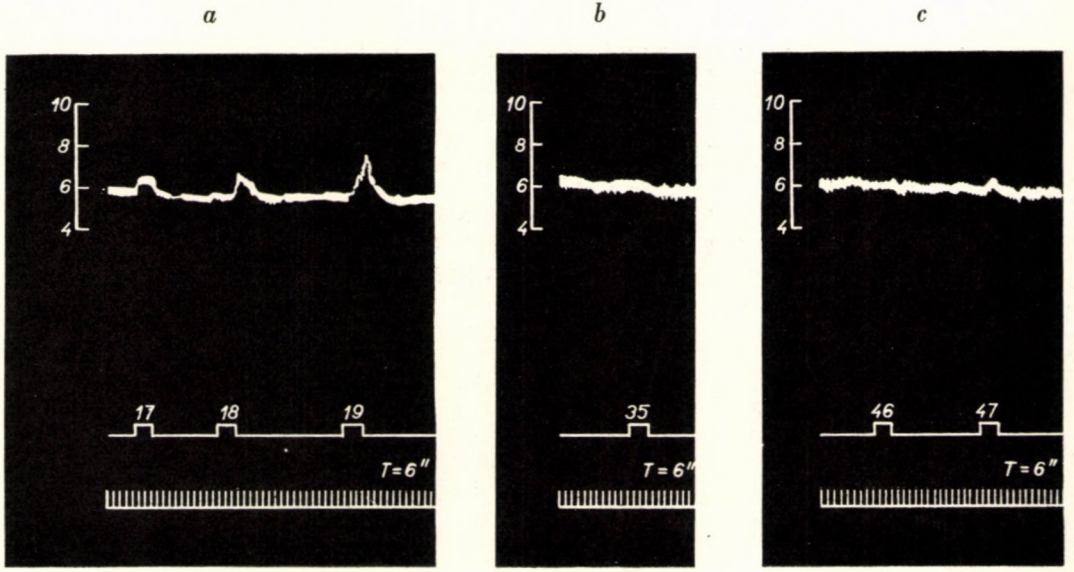


Fig. 2. — Chat no. 4.

a — 17 : excitation de l'électrode no. 1, ∼, 50 Hz, 2 v. durée = 15 secondes

— 18 : idem, avec 4 v.

— 19 : idem, avec 4 v.

b — après 5 mg/kilocorps d'hydroxyzine i. v.

— 35 : même excitation qu'à *a* — 17

c — Aprés encore 10 mg/kilocorps d'hydroxyzine i. v. (dose totale = 15 mg/kilocorps)

— 46 : même excitation qu'à *a* — 18

— 47 : même excitation qu'à *a* — 19

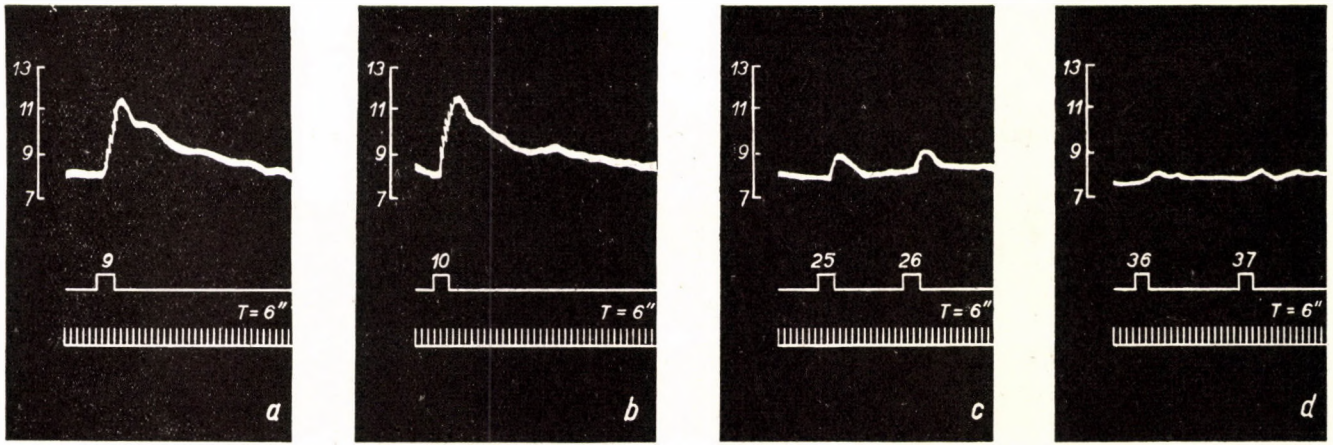


Fig. 3. — Chat no. 2. Section médullaire D_2—D_3 (sous ether) 5 heures avant l'expérience
a — 9 : excitation de l'électrode no. 1, ∼, 50 Hz, 5 v. durée = 15 secondes
b — 10 : excitation de l'électrode no. 2, idem, 4 v.
c — après 10 mg/kilocorps d'hydroxyzine i. v.
— 25 : même excitation qu'à *a* — 9 (mais avec 4 v.)
— 26 : même excitation qu'à *b* — 10
d — Après encore 10 mg/kilocorps d'hydroxyzine i. v., administrée en deux reprises à 5 mg/kilocorps (dose totale = 20 mg/kilocorps).
— 36 : même excitation qu'à *a* — 9 et *c* — 25
— 37 : même excitation qu'à *b* — 10 et *c* — 26

initiale. La diminution est plus grande et atteint un maximum de 43% quand l'adrénaline est administrée immédiatement après l'injection de l'hydroxyzine.

Enfin, nous devons ajouter que l'hydroxyzine (doses totales = 17,5 mg et 22 mg/kilocorps) n'a pas modifié, dans nos expériences, que très peu les effets cardio-vasculaires produits par l'excitation du bout central ou périphérique des nerfs vagues, de même que les réflexes cardio-vasculaires déclenchés par l'occlusion temporaire des deux artères carotides primitives.

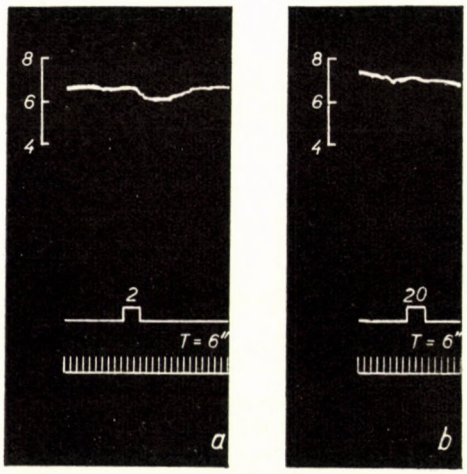


Fig. 4. — Chat no. 5. Section médullaire C_7—D_1 (sous ether), 7 heures avant l'expérience.
a — 2 : excitation de l'électrode no. 1, ∼, 50 Hz, 4 v. durée = 15 secondes.
b — Après 10 mg/kilocorps d'hydroxyzine i. v., administrée en deux reprises à 5 mg/kilocorps.
— 20 : même excitation qu'à *a* — 2.

Les modifications électrocardiographiques produites par l'excitation de l'hypothalamus sont beaucoup moins influencées par l'hydroxyzine et elles persistent, parfois, même alors que l'effet presseur consécutif à l'excitation hypothalamique disparaît sous l'action de l'hydroxyzine (fig. 5). Nous avons remarqué aussi qu'il n'existe pas toujours un parallélisme parfait entre l'action de l'hydroxyzine sur les effets vasculaires de l'excitation hypothalamique et l'action sur les effets cardiaques de la même excitation.

Dans le tableau ci-dessous nous présentons les données fournies par le contrôle anatomique de l'emplacement des électrodes.

Obs. On a obtenu une hypertension à l'excitation de tous ces points, exceptant l'électrode No. 1 du chat No. 5 qui a provoqué une hypotension artérielle.

Tableau

Animal	Électrode	Siège
1	2	3
Chien No. 16	No. 3, bipolaire	Dans la corne d'Ammon, sur une section vertico-frontale passant à 2 mm en arrière de la commissure blanche antérieure et par les noyaux antérieurs du thalamus.
Chien No. 16	No. 5, bipolaire	Dans la région hypothalamique, médial par rapport au corps de Luys droit, sur une section vertico-frontale passant immédiatement en arrière du N. reuniens et par les noyaux du tuber.
Chien No. 17	No. 4, bipolaire	A 2 mm latéral droit et plus bas que l'aquéducte, médial par rapport au noyau rouge, sur une section vertico-frontale passant immédiatement en arrière des corps mamilaires.
Chien No. 17	No. 5, bipolaire	Dans l'extrémité médiale, juxta-hypothalamique, de la région periforme droite, sur une section vertico-frontale passant par la région infundibulo-tubérienne et par le tiers antérieur du thalamus.
Chien No. 18	No. 1, bipolaire	Dans la région sous-thalamique, dans le corps de Luys droit, sur une section vertico-frontale passant à 0,5 mm en arrière de la tige pituitare.
Chien No. 18	No. 2, bipolaire	Dans l'espace perforé postérieur, sur une section vertico-frontale passant immédiatement en arrière des corps mamilaires.
Chien No. 18	No. 3, bipolaire	Même section. L'électrode se trouve dans le thalamus ventro-latéral, immédiatement au-dessus de la capsule interne droite.
Chien No. 18	No. 4, bipolaire	Dans la substance grise périaquéductulaire, sur la ligne médiane, sur une section vertico-frontale passant par les tubercules quadrijumeaux antérieurs et par le pied du pédoncule cérébral.
Chien No. 18	No. 5, bipolaire	Dans le plancher du III-e ventricule, sur la ligne médiane (région tubéroinfundibulaire) ; même section vertico-frontale que l'électrode no. 1.
Chien No. 18	No. 6, bipolaire	Dans l'hypothalamus antérieur, dans la région préoptique, sous la commissure blanche antérieure.
Chat No. 2	No. 1, monopolaire	Dans le noyau central du thalamus, au niveau de la commissure moyenne, à 1,5 mm paramédial, en droite. Section XII *Winkler—Poter* (W. P.)
Chat No. 2	No. 2, monopolaire	Dans le noyau infundibulaire médial droit, juxta-médian. Section X W. P.
Chat No. 4	No. 1, monopolaire	Dans l'hypothalamus antérieur, dans la région préoptique, paramédian droit. Section V W. P.
Chat No. 5	No. 1, monopolaire	Electrode située immédiatement en haut du corps mamilaire droit, à 1 mm en dehors de la ligne médiane, sous le noyau hypothalamique médial. Section XIV W. P.

B) *Action de l'hydroxyzine sur l'ECG*

La modification électrocardiographique la plus constante observée sous l'action de l'hydroxyzine est l'augmentation de l'amplitude de l'onde T.

Cet effet de la substance est très net surtout chez les animaux qui présentent au commencement de l'expérience une onde T négative ; après l'injection d'hydroxyzine, l'onde T devient positive. Cette action de l'hydroxyzine est

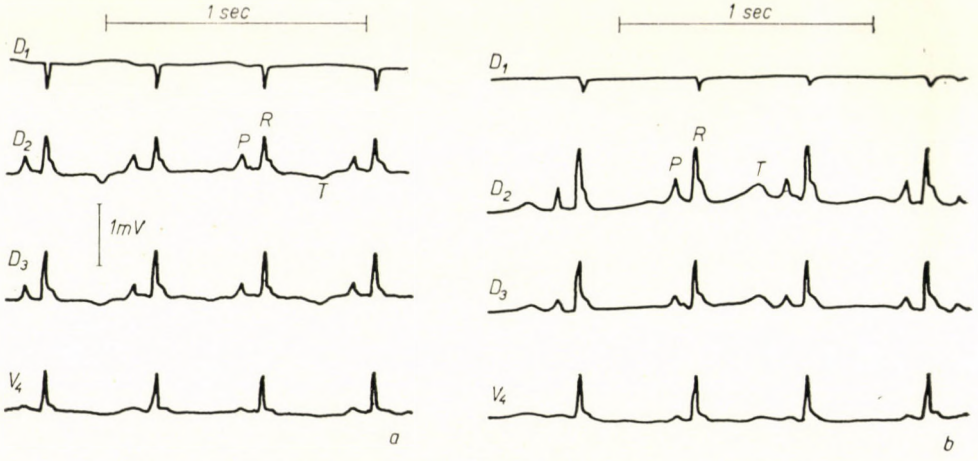


Fig. 5. — Chat no. 4. L'animal a reçu déjà 2 × 5 mg/kilocorps d'hydroxyzine i. v. T. A. revenue au niveau initial.
a — ECG : T(—) en D_2, D_3 et CR_4 et T(+) en D_1.
Rythme = 144/1 min.
b — ECG après l'excitation de l'électrode no. 1 (voyez fig. 3), ∼, 50 Hz, 4 v., 15 secondes. T. A. ne se modifie pas. L'onde T devient positive dans toutes les dérivations.
Rythme = 133/minute.

additive de sorte qu'un animal qui présente initialement une onde T négative, après 3—4 heures d'expérience (temps pendant lequel on a injecté des doses répétées d'hydroxyzine) présente constamment une onde T positive.

Les autres modifications électrocardiographiques produites par l'hydroxyzine sont moins constantes ; de même, les modifications du rythme cardiaque qui peut rester inchangé, il s'accélère ou se ralentit, mais dans une mesure assez réduite.

Nous devons remarquer que les modifications électrocardiographiques observées par nous, bien qu'elles apparaissent pendant la chute tensionnelle produite par l'hydroxyzine, n'ont aucune relation avec celle-là, puisqu'elles persistent longtemps après le retour de la pression sanguine à la valeur initiale.

Pour illustrer nos observations nous présentons un exemple :

Chat No. 7, 3,3 kg. Curare 2 mg/kilocorps i. m. + 1 mg/kilocorps i. v. Respiration artificielle 75 cc air (fig. 6).

a — ECG avant l'injection de l'hydroxyzine. Segment ST sousdénivelé dans D_2, D_3 et CR_4 ; onde T négative dans les mêmes dérivations. Rythme cardiaque = 133/1′. T. A. = 8 cm Hg.

b — Après 5 mg/kilocorps hydroxyzine i. v. La tension artérielle diminue avec 2 cm Hg et revient ensuite lentement au niveau initial. ECG enregistrée après le retour de la T. A. à la normale : segment ST isoélectrique dans toutes les dérivations ; onde T positive en D_2, D_3, CR_4. Rythme = 112/1′.

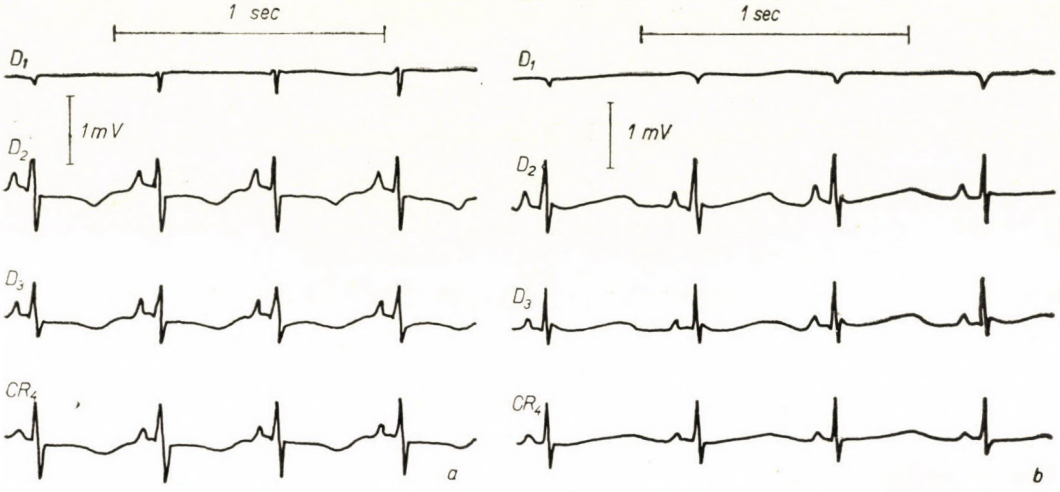


Fig. 6. — Action de l'hydroxyzine sur l'ECG du chat.
a — ECG avant l'hydroxyzine.
b — après 5 mg/kg. hydroxyzine i. v.

C) *Action de l'hydroxyzine sur le coeur isolé et perfusé*

1°. L'étude de l'action propre de l'hydroxyzine (3 expériences) sur les contractions cardiaques, nous a montré que les doses faibles (0,1 μg, 1 μg) ne modifient pas la fonction contractile du coeur ; à partir de la dose de 5 μg, on constate un effet inotrope négatif, qui est très marqué à la dose de 50 μg (fig. 7).

2°. Administrée avant l'Ach (7 expériences) et le potassium (6 expériences), l'hydroxyzine empêche leur action inotrope négative (fig. 8, 9).

3°. Administrée avant l'Adr (4 expériences) et le calcium (6 expériences), l'hydroxyzine n'a aucune influence sur leur action inotrope positive.

Discussion

Nos recherches démontrent que l'hydroxyzine diminue les effets cardio-vasculaires produits par l'excitation électrique directe de l'hypothalamus.

Dans nos expériences, l'hydroxyzine aurait pu exercer son action tant sur l'organe effecteur que sur les points suivants de la voie efférente excitée :

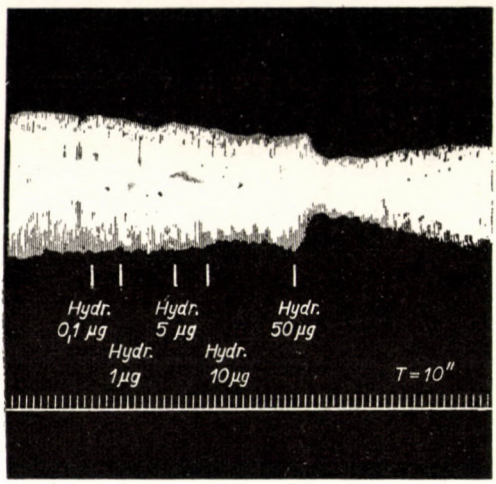


Fig. 7. — Action propre de l'hydroxyzine sur le coeur isolé de grenouille

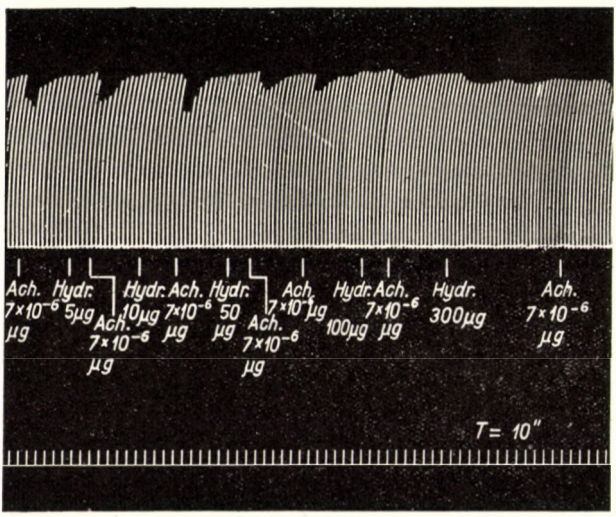


Fig. 8. — Suppression de l'effet inotrope négatif de l'acétylcholine, par l'hydroxyzine

centre nerveux hypothalamique ou autre structure végétative centrale, fibres nerveuses efférentes, synapses ganglionnaires.

Ayant en vue les faits suivants :

— l'hydroxyzine administrée par la voie intraveineuse n'a pas une action ganglioplégique (9) ;

— l'hydroxyzine n'a pas une action de blocage de la conduction sur la fibre nerveuse (10) ;

— l'hydroxyzine empêche dans une mesure assez réduite l'action hypertensive de l'adrénaline (Levis *et al.* [9] ; Reuse [11, 12] ; nos recherches) ;

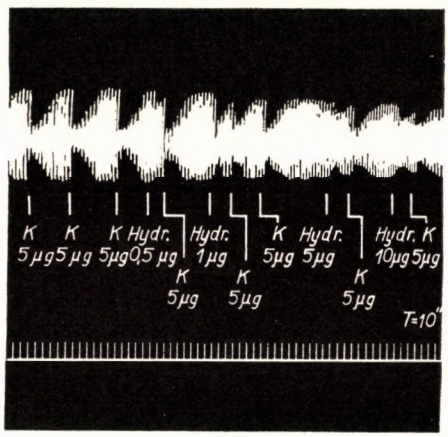


Fig. 9. — Diminution de l'effet inotrope négatif du potassium, par l'hydroxyzine

— l'hydroxyzine ne modifie qu'assez peu les réflexes hypertensifs sino-carotidiens et les effets cardio-vasculaires de l'excitation du bout central et périphérique du pneumogastrique (Reuse [11, 12] ; nos recherches) ;

— l'hydroxyzine ne modifie pas l'hypertension produite par l'excitation du bout périphérique du sympathique qu'à de très grandes doses qui suppriment l'action de l'adrénaline (Reuse [11] ; Donnet *et al.* [6]) ;

— l'hydroxyzine n'est pas sympathicolytique et parasympathicolytique (Reuse [11]) ;

nous croyons que nous pouvons conclure que l'hydroxyzine diminue l'excitabilité des centres cardio-vasculaires situés dans l'hypothalamus et diminue aussi l'excitabilité des autres structures végétatives centrales que nous avons excitées.

Nous ajoutons que Bovet [3], en étudiant l'action de l'hydroxyzine chez le lapin, en doses de 10—20 mg/kilocorps, conclue que la substance augmente légèrement le seuil de l'excitabilité des structures sous-corticales.

Les effets de l'hydroxyzine ont été semblables, quelle que soit la structure végétative centrale excitée.

En ce qui concerne la similitude des effets de l'hydroxyzine sur l'hypothalamus et le rhinencéphale, nous croyons que ce fait pourrait être expliqué par les vastes connexions anatomiques qui existent entre ces deux régions, surtour par le système du trigone.

A l'excitation de la formation réticulée nous avons probablement excité les centres végétatifs situés dans cette région ou la voie descendente de l'hypothalamus (faisceau de SCHÜTZ).

En ce qui concerne l'effet égal de l'hydroxyzine sur les différents points du diencéphale (hypothalamus, sous-thalamus, thalamus), nous devons remarquer que les auteurs qui ont étudié les effets végétatifs produits par l'excitation de cette région n'ont pu faire aucune distinction entre les sous-divisions du diencéphale ; ces auteurs n'ont pas trouvé des points spécifiques, mais des zones de représentation végétative (HESS ; RANSON ; BEATTIE, GRASTYÁN *et al.* [8]).

Tous ces faits nous mènent à la conclusion que l'hydroxyzine diminue l'excitabilité des voies végétatives plurisynaptiques et des structures végétatives centrales ; d'autre part, elle a une influence très peu marquée sur les voies végétatives plus courtes (l'hydroxyzine a une action faible sur les réflexes presseurs sinocarotidiens et les effets cardio-vasculaires de l'excitation vagale) de même que sur les effecteurs vasculaires.

Les doses d'hydroxyzine utilisées par nous ont modifiée la pression sanguine seulement pour quelques dizaines de secondes, immédiatement après l'administration. Ce fait démontre que les mécanismes élémentaires qui règlent le niveau de la tension artérielle ne présentent plus aucune modification à quelques minutes après l'administration de la substance.

Dans la deuxième série d'expériences, nous avons vu que l'hydroxyzine a la propriété d'augmenter l'amplitude de l'onde T de l'ECG et de positiver une onde T négative. Dans l'interprétation vectorielle de l'ECG, la présence d'une onde T négative sur les dérivations inscrites par nous, traduit un trouble de répolarisation, prédominant surtout au niveau de la région apexienne qui est la plus sensibles de tout le coeur aux diverses influences. La positivation de l'onde T sous l'influence de l'hydroxyzine démontre que la substance est capable d'améliorer le trouble de répolarisation. L'hydroxyzine favorise donc la phase de répolarisation cardiaque. L'augmentation de l'amplitude d'une onde T, positive auparavant, mais applatie, a — selon nous — la même signification. Nous devons remarquer que l'action de l'hydroxyzine sur l'ECG est très ressemblante aux effets électrocardiographiques de l'excitation sympathique (FOGELSON [7]), étudiés en détail par l'un de nous [2] dans des recherches antérieures.

Cet effet sympathique n'est pas une conséquence de l'hypotension produite par l'hydroxyzine, puisqu'il persiste aussi après le retour de la pression sanguine au niveau initial. C'est pourquoi, croyons-nous, l'hydroxyzine ne supprime qu'avec difficulté l'effet sympathique cardiaque de l'excitation hypothalamique. Le fait que l'hydroxyzine produit des effets différents sur les vaisseaux et sur le coeur pourrait expliquer la discordance qu'on observe parfois entre l'action de l'hydroxyzine sur les effets vasculaires de l'excitation hypothalamique et son action sur les effets cardiaques de la même excitation.

En ce qui concerne l'action sur le coeur isolé, l'hydroxyzine ne présente des effets manifestes qu'aux grandes doses. Mais, elle a la propriété de diminuer et même de supprimer l'effet inotrope négatif de l'Ach et du K, sans influencer l'effet inotrope positif de l'Adr et du Ca. L'hydroxyzine a donc une action anti-acétylcholinique et anti-potassique. Peut-être qu'ainsi s'explique l'effet de sens sympathique qu'on observe sur l'ECG après l'administration d'hydroxyzine.

Il est important de souligner que les doses d'hydroxyzine qui influencent les phénomènes électriques cardiaques, donc les phénomènes métaboliques — en dernière instance, ont un effet très peu manifeste sur le rythme cardiaque.

Il serait, peut-être, utile d'étudier l'action de l'hydroxyzine chez les malades présentant des troubles d'irrigation myocardique.

Conclusions

A) En ce qui concerne l'action sur les structures végétatives centrales :

1. L'hydroxyzine diminue jusqu'à suppression presque totale les effets hyper- et hypotensifs produits par l'excitation de l'hypothalamus et d'autres structures végétatives centrales.

2. L'hydroxyzine a une influence plus réduite sur l'effet hypotensif qui précède ou succède l'effet hypertensif de l'excitation hypothalamique, de même que sur l'adrénaline-sécrétion déclenchée par l'excitation hypothalamique ou rhinencéphalique.

3. L'hydroxyzine influence probablement les voies nerveuses plurisympathiques.

B) En ce qui concerne l'action cardiaque :

1. L'hydroxyzine provoque l'augmentation de l'amplitude de l'onde T de l'electrocardiogramme ou la positivation d'une onde T négative. L'hydroxyzine favorise donc la répolarisation cardiaque. Cet effet est indépendant des modifications tensionnelles produites par l'hydroxyzine.

2. L'hydroxyzine provoque des modifications inconstantes du rythme cardiaque : celui-ci peut s'accélérer ou se ralentir, ou bien il peut rester inchangé.

3. Les doses fortes d'hydroxyzine ont une action inotrope négative sur le coeur de grenouille isolé.

4. L'hydroxyzine diminue, jusqu'à suppression totale, l'effet cardiaque inotrope négatif de l'acétalcholine et du potassium.

5. L'hydroxyzine n'a aucune influence — même dans de grandes doses — sur l'effet inotrope positif de l'adrénaline et du calcium.

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ACTA PHYSIOLOGICA

Том XV. вып. 2

РЕЗЮМЕ

КРОВООБРАЩЕНИЕ И ОБМЕН ВЕЩЕСТВ ГОЛОВЫ У СОБАК В ИШЕМИЧЕСКОМ ШОКЕ

А. Г. Б. КОВАЧ, П. Ш. РОХЕЙМ, М. ИРАНЬИ, Э. ЧЕРХАТИ, ДЬ. ГОСТОНЬИ и Э. КОВАЧ

Авторы исследовали у собак при ишемическом шоке кровообращение и обмен веществ головы.

Они установили, что при шоке кровообращение головы уменьшается, сопротивление головных сосудов остается без изменения, а в то же самое время сопротивление в системном кровообращении повышается. Фракция минутного объема кровообращения головы в шоке сигнификантно выше нормального.

При уменьшающемся кровообращении головы повышается артерио-венозная разница O_2 сильнее, чем это исчисляется на основе снижения кровообращения. Следовательно, прием O_2 в голове в условиях шока значительно повышается, за исключением терминального состояния, при котором он значительно ниже нормального приема. Вместе со снижением терминального потребления O_2 уменьшается также и прием глюкозы в голове.

РЕГИСТРАЦИЯ ТОКОВ ДЕЙСТВИЯ НА МАГНЕТОФОНЕ И ИХ ВЫПРЯМЛЕНИЕ ГЕРМАНИЕВЫМ ДИОДОМ

Я. ПОРСАС и Ф. САБО

Авторы описывают методику, с помощью которой токи действия нервов можно регистрировать на магнетофоне и затем при передачи на осциллоскоп сфотографировать.

Шум можно устранить и получить «монофазные» токи действия при помощи германиевого диода с изменяемым предварительным напряжением, включенным между выходом усилителя и осциллоскопом.

Методика авторов предоставляет также возможность анализировать весьма тонкие волокна.

МИНУТНЫЙ ОБЪЕМ И КРОВООБРАЩЕНИЕ ПОЧЕК ПРИ ОСТРОЙ ГИПОТОНИИ

П. БАЛИНТ, Е. КИШ и Я. ШТУРЦ

Авторы в большой серии опытов исследовали на наркотизированных собаках величины кровяного давления, минутного объема, кровообращения почек, клубочковой фильтрации и диуреза, с одной стороны, при нормальном уровне кровяного давления, а с другой, в острых гипотонических состояних, возникавших в связи с опытами.

Исследование гемодинамики почек проводилось отчасти путем непосредственного измерения количества вытекающей из почечной вены крови, а отчасти на основе классической техники клиренса.

Авторы установили, что минутный объем и непосредственно измеренное количество почечного кровотока практически уменьшаются параллельно с кровяным давлением, значит, при гипотонии исчисленные общепериферическое и почечное сопротивления не меняются. В соответствии с этим так наз. почечная фракция кровообращения остается без изменения.

На основе техники клиренса проводились сообщенные в литературе опыты, согласно результатам которых предпочтительно повышается сопротивление почек. При олигурии низкие величины клиренса являются техническим следствием самой олигурии, и на взгляд авторов, в таком состоянии из значений клиренса нельзя делать выводов относительно почечного кровотока.

ДЕЙСТВИЕ ВЗЯТИЯ НЕБОЛЬШИХ КОЛИЧЕСТВ КРОВИ НА МИНУТНЫЙ ОБЪЕМ И НА КРОВООБРАЩЕНИЕ ПОЧЕК

П. БАЛИНТ, Е. КИШ и Я. ШТУРЦ

Авторы вызывали путем постепенного взятия небольших количеств крови, у наркотизированных собак олигемию причем кровяное давление уменьшалось лишь в незначительной степени. В олигемическом состоянии измерялись минутный объем, количество почечного кровотока, клубочковая фильтрация, и выделение натрия.

Измерение почечного кровотока, как и клубочковой фильтрации проводились путем непосредственного измерения вытекающей из почечной вены крови, также как и на основе классической техники клиренса. Согласно установлениям авторов непосредственное измерение показывает, что абсолютные величины РБФ вследствие взятия крови уменьшаются, однако, уменьшение пропорционально уменьшению кровяного давления, и следовательно, почечное сопротивление не меняется. Подобным образом почечная фракция минутного объема также остается без изменения. На основе техники клиренса следовало бы ожидать выраженную почечную ишемию, повышение почечного сопротивления и сильное снижение почечной фракции минутного объема.

Авторы устанавливают, что олигемия приводит к снижению выделения соли и воды, и низкие величины клиренса являются техническим следствием олигурии.

Значит, в олигемическом состоянии не состоится диверсии крови в ущерб почек и в пользу других органов.

ИССЛЕДОВАНИЕ ФУНКЦИИ КОРЫ НАДПОЧЕЧНИКОВ В СОСТОЯНИИ РЕГЕНЕРАЦИИ

П. ВЕЙС, Л. ХОРВАТ, Т. КАДАШ, П. КЁВЕШ и Л. РИТТЕР

Авторы исследовали у крыс с удаленным мозговым слоем надпочечников диастолическое кровяное давление и подробно анализировали в процессе регенерации кортикального слоя надпочечников выделение кортикостероидов. *Скелтон* описал, что у таких животных развивается гипертония (он регистрировал только систолическое кровяное давление) и предполагал, что повышение кровяного давления обусловливается перепроизводством минералокортикоидов. Результаты авторов следующие :

1. У животных с удаленным мозговым слоем надпочечников повышается не только систолическое, но и диастолическое кровяные давления.
2. Секреция гормона : кортикостерона, выделяемый у крыс в самом большом количестве, после начального колебания впоследствии уменьшается.
3. Секреция алдостерона не повышается.
4. Повышенной секреции прочих кортикостероидов также не удалось выявить.
5. Из сказанного кажется вероятным, что в патогенезе гипертонии Скелтона кортикостероиды не играют значительной роли.

О КОМПЕНСАТОРНОЙ ФУНКЦИИ АФФЕРЕНТНОЙ СИСТЕМЫ ПОЧЕЧНЫХ ПОЛОСТЕЙ

ДЬ. АДАМ, Х. БОДАНСКИ, Э. МАТЬЮШ, И. МЕСАРОШ и К. НАДЬ

Авторы выявили в своих опытах, проведенных на собаках методом интероцептивных условных рефлексов почечной лоханки, что в случае одностороннего экспериментального гидронефроза, или одностороннего удаления почки, полостная система другой почки показывает повышенную деятельность афферентного возбуждения. Интероцептивная система неповрежденной почки компенсирует этим путем уменьшенную афферентную импульсацию.

ИССЛЕДОВАНИЕ ЗАДЕРЖИВАЮЩЕГО РОСТ ДЕЙСТВИЯ НЕКОТОРЫХ ПРОИЗВОДНЫХ ТИОПРОПИОНОВОЙ КИСЛОТЫ НА КЛЕТОЧНЫХ КУЛЬТУРАХ ГЕЛА

И. ФЕХЕР, А. ЛЕНДЬЕЛ, И. НАС, В. ШЕЛМЕЦИ и Я. БОРВЕНДЕГ

В прежних работах авторы выявили, что тиопропионовая кислота является эффективным антагонистом метионина, цистина и глютаминовой кислоты. В настоящих опытах они получили некоторые тиоэфиры тиопропионовой кислоты (S-метил) пропионовая кислота, (S-этил) пропионовая кислота, (S-пропил) пропионовая кислота, (S-CH_2COONa) пропионовая кислота (S-$C_3H_4O_2Na$) пропионовая кислота Na и исследовали действие последних на клеточных культурах Гела. Они установили, что вышеприведенные производные задерживают рост культур, подобно этионину и метионсульфоксимину. Задерживание отчасти удалось отражать метионином и глютаминовой кислотой. Эффективность производных повышается по мере роста длины алкилирующей цепи. Соединения типа дикарбоновой кислоты были неэффективными.

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CIRCULATION AND METABOLISM IN THE HEAD OF THE DOG IN ISCHAEMIC SHOCK

By

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(Received June 17, 1958)

Head circulation and metabolism have been investigated in dogs in ischaemic shock. It was found that in shock head circulation diminishes, vascular resistance in the head remains unchanged, while in the torso it increases. The head fraction of the minute volume is significantly greater in shock than under normal conditions.

With the decrease of head circulation the arterio-venous oxygen difference in the head grows more than it would follow from the decrease in the rate of blood flow. Thus the oxygen consumption in the head increases decidedly in shock, except at the final stage when it is much lower than normal. Simultaneously with the terminal drop in oxygen consumption there is a decrease in glucose consumption in the head.

*

In the development of shock and the generalization of the changes involved, injury to the central nervous system plays a decisive role. As previously demonstrated by Kovách, Róheim, Irányi, Kiss and Antal [1], the isolated perfusion of the head exerts a beneficial effect on the course of both ischaemic and haemorrhagic shock. There are many data in the literature to prove the occurrence in shock of a biochemical injury to cerebral tissue, where significant changes are brought about in macroerg phosphate metabolism [2, 3, 4], acetylcholine content [5], oxygen consumption *in vitro* [6, 7, 8].

Information is, however, lacking as regards the metabolism of the head or brain of the animal in shock. Blalock [9] and also Tcherkassova and Merezhinsky [30] studied the arteriovenous oxygen difference in the head during shock but since none of these authors measured the blood flow in the head, they could not determine the oxygen consumption in that area. From the increase in the arterio-venous oxygen difference both Blalock and Tscherkassova concluded that there was a decrease in blood flow in the head.

The purpose of the present investigations was to establish the circulatory and metabolic changes occurring *in vivo*, in the head of dogs in shock and to compare these with the circulatory and biochemical alterations brought about in the whole organism.

Methods

The experiments were carried out on 13 dogs in shock and 11 controls, each of 10 to 12 kg weight, under chloralose anaesthesia (0.1 g/kg). Shock was induced by applying a tourniquet to the two hind limbs for 5 hours [10].

As in our previous studies, circulation between head and torso was maintained only through the two carotids and the two jugular veins. The spinal cord and the vagosympathetic were naturally left intact [11, 12]. Head circulation was constantly measured by means of a rotameter. The uptake or release of oxyxen, glucose, lactic acid and inorganic phosphate were determined by simultaneously taking blood from the carotid and the jugular vein. Oxygen was determined by the method of ISSEKUTZ, JR. *et al.* [13], glucose by SOMOGYI's [14], lactic acid by BARKER and SUMMERSON's [15], inorganic phosphate by FISKE and SUBBAROW's [16] method, and the haemoglobin content by a *Zeiss* haemometer. Minute volume was determined on the basis of the FICK principle ; the total peripheral resistance, or that of the head area, by the ratio of mean arterial pressure and the quantity of perfusing blood.

Statistical calculations were made on the basis of STUDENT's "*t*" test [17].

Results

a) *Haemodynamic and biochemical changes in the blood of dogs in ischaemic shock*

The following haemodynamic conditions were observed in dogs in ischaemic shock (Table I).

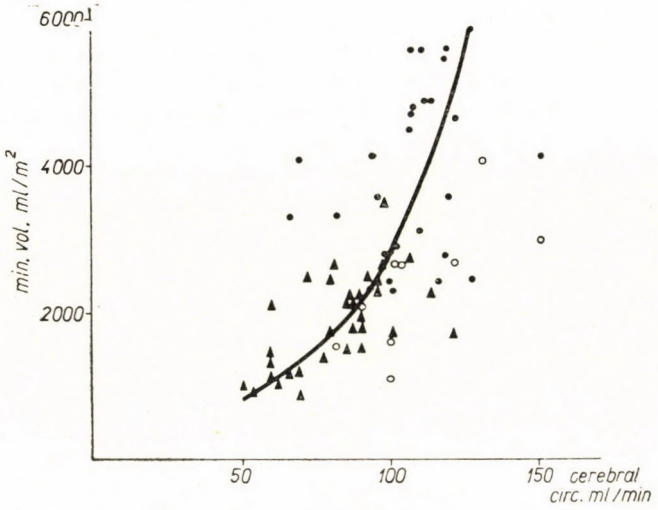

Fig. 1. Conditions in head circulation of dogs in ischaemic shock, as a function of minute volume

Abscissa : head circulation ml/minute.
Ordinate : minute volume/m² body surface.
● = normal values
○ = after 5 hours' ligation
▲ = in ischaemic shock

In *arterial blood pressure* there was no significant change during ligation but it decreased decidedly and oscillated around a mean of 70—80 mm Hg after the tourniquet had been released. In the terminal stage blood pressure decreased further and in the last 15 minutes averaged 45 mm Hg (Table I).

The *haemoglobin* content of the blood increased sharply after taking off

the tourniquet and rose to a maximum of 18.4 g per 100 ml, as a sign of pronounced haemoconcentration ($t = 5.672$, $P < 0.01$).

The *minute volume* began to fall significantly during the ligation ($t = 2.534$, $P < 0.05$) and continued to do so after the release (Table I). Total peripheral resistance increased under ligation and increased still more following its release, then stabilized at above the normal value (Fig. 1).

Oxygen consumption showed a consistent drop with the development of shock ($t = 4.762$, $P < 0.02$).

The *glucose* content of arterial blood increased slightly during ligation and remained augmented to the end, not reaching the limit of statistical significance.

The *inorganic phosphate* content of arterial plasma increased significantly during ligation and following its release. From the initial 3.5 mg per 100 ml it rose to 9 mg per 100 ml after release and remained high up to the final state of shock ($t = 5.763$, $P < 0.001$).

The *lactic acid* content of arterial blood rose during ligation from the initial value of 17.4 mg to 33 mg per 100 ml. After release of the tourniquet it increased to 50.7 mg per 100 ml ($t = 5.342$, $P < 0.001$).

b) *Circulation and metabolism of the head in dogs in ischaemic shock.*

The amount of blood perfusing the head was 108 ml/min in our normal control. This value did not change practically until the end of the tourniquet period, when 105 ml of blood perfused the head per minute. Immediately after release, conformable to our previous experiments [1], this volume increased for a short time but after a few minutes a decrease set in ($t = 3.008$, $P < 0.01$). The vascular resistance in the head decreased slightly following the release of the tourniquet but this change did not reach the limit of significance (Table I).

The head fraction of minute volume already doubled during ligation ($t = 4.632$, $P < 0.001$), to remain considerably higher than normal up to the final stage ($t = 4.4278$, $P < 0.001$). The decline in minute volume does not imply a drop in head circulation ; in fact, the amount of blood perfusing the head was percentually increased (Table I).

Oxygen consumption of the head. The arterio-venous oxygen difference did not change during ligation but it rose immediately after the tourniquet had been released and, instead of the normal mean difference of 7.2 volume per cent, it moved at about 12 per cent until the terminal stage. This increase was highly significant statistically ($t = 5.195$, $P < 0.001$). The arterio-venous oxygen difference increased parallel with the decrease of head circulation. In the terminal stage, in spite of a further drop in head circulation, the arterio-venous oxygen difference did not increase.

In the period of ligation the oxygen consumption of the head was normal. After release it rose from the initial value of 7.65 ml/min to 10 ml/min. This

Table I

Circulation, metabolism and biochemical changes in the blood of dogs in ischaemic shock (averages for 13 experiments)

	Normal	I Before release	II	III	IV	V	VI Before death	VII Death	Statistical difference
			After release						
			10′	30′	60′	180′	30′—60′	5′—15	
Mean arterial blood pressure mm/Hg	117 ±12	104 ±11	72 ±7	77 ±4	74 ±7	71 ±9	66 ±3	45 ±12	
Haemoglobin, per cent	13.3 ±0.3	14.4 ±0.5	15.8 ±0.8	16.5 ±0.5	16.6 ±1.2	18.4 ±0.7	17.0 ±0.5	17.2 ±0.5	
Minute volume, ml/min	2380 ±240	1260 ±210	1010 ±180	1050 ±100	780 ±200	670 ±30	770 ±105	— —	Normal-shock III. $t = 3.574$, $P < 0.01$
Peripheral resistance	0.049 ±0.003	0.084 ±0.002	0.071 ±0.003	0.073 ±0.005	0.095 ±0.002	0.106 ±0.003	0.085 ±0.003	—	
Peripheral A—V O_2 difference vol. per cent	5.30 ±0.12	8.30 ±0.20	9.20 ±0.15	9.40 ±0.20	11.60 ±0.32	12.20 ±0.30	11.70 ±0.18	11.50 ±0.50	
O_2 consumption ml/min	127 ±21	105 ±14	93 ±5	99 ±3	90 ±8	82 ±7	87 ±5	74 ±6	
Amount of blood perfusing head ml/min	108 ±8	105 ±10	82 ±8	86 ±7	83 ±10	74 ±10	74 ±4	43 ±5	Normal-shock III. $t = 3.008$, $P < 0.01$
Vascular resistance in head	1.06 ±0.05	1.00 ±0.02	0.90 ±0.04	0.90 ±0.05	0.90 ±0.02	1.00 ±0.04	0.92 ±0.04	1.12 ±0.05	
Minute volume fraction of head perfusion	4.20 ±0.20	8.30 ±0.50	8.10 ±0.32	8.20 ±0.50	10.70 ±0.40	11.20 ±0.20	9.30 ±0.05	6.20 ±0.12	Normal-shock III. before release $t = 4.4278$, $P < 0.001$, $t = 4.632$; $P < 0.001$

Head A—V O_2 difference	7.60 ±0.20	7.70 ±0.41	11.0 ±0.38	11.90 ±0.54	10.80 ±0.40	12.70 ±0.42	11.90 ±0.55	12.0 ±0.55	
Head O_2 consumption, ml/min	7.50 ±0.15	8.10 ±0.30	9.0 ±0.35	10.0 ±0.52	9.60 ±0.50	9.30 ±0.54	8.0 ±0.50	5.20 ±0.45	Normal-shock III—IV. — terminal $t = 2.988$; $P < 0.01$ $t = 2.805$; $P < 0.01$
Head fraction of body O_2 consumption	6.70 ±0.20	7.70 ±1.20	9.62 ±0.82	10.10 ±0.74	10.60 ±0.85	11.30 ±0.40	9.20 ±0.30	6.50 ±0.11	
Arterial glucose mg per 100 ml	70 ±8,5	90 ±5	84 ±10	82 ±8	71 ±14	80 ±12	81 ±10	82 ±8	
Head A—V glucose diff.	8.61 ±0.35	9.42 ±0.42	13.40 ±0.54	11.60 ±0.30	10.0 ±0.40	12.90 ±0.20	12.90 ±0.22	10.0 ±0.40	
Head glucose uptake mg/min	9.30 ±0.30	9.90 ±0.11	11.0 ±0.24	10.0 ±0.15	8.20 ±0.34	9.50 ±0.32	9.50 ±0.14	4.30 ±0.22	Normal-shock terminal $t = 3.646$; $P < 0.01$
Art. blood inorganic phosphate mg per 100 ml	3.50 ±0.35	5.40 ±0.50	8.10 ±0.80	9.0 ±0.70	8.90 ±0.70	8.70 ±0.62	9.50 ±0.70	10.40 ±0.72	
Art. blood lactic acid mg per 100 ml	—0.06 ±0.002	+0.04 ±0.02	+1.07 ±0.24	+0.91 ±0.20	+0.49 ±0.20	+0.53 ±0.11	±1.54 ±0.62	+1.87 ±0.50	
	17.10 ±2.50	33 ±5	52 ±7	50 ±7	51 ±5	—	48 ±8	54 ±5	
Head lactic acid A—V mg per 100 ml	—2.70 ±0.41	—2.33 0.30	+6.65 ±0.82	—0.10 ±0.05	81 ±0.08	—	2.27 ±0.50	0.30 ±0.12	

rise was statistically significant ($t = 2.988$, $P < 0.01$). Parallel with the increasing severity of shock, the oxygen consumption again decreased, to a preterminal 8.0 ml/min and a terminal 5.2 ml/min. As compared with the initial value, the decrease was statistically significant ($t = 2.805$, $P < 0.01$) (Table I).

The fraction of total oxygen consumption falling to the head increased considerably while the tourniquet was in its place and was significantly higher than normal during the whole course of shock ($t = 5.221$, $P < 0.001$).

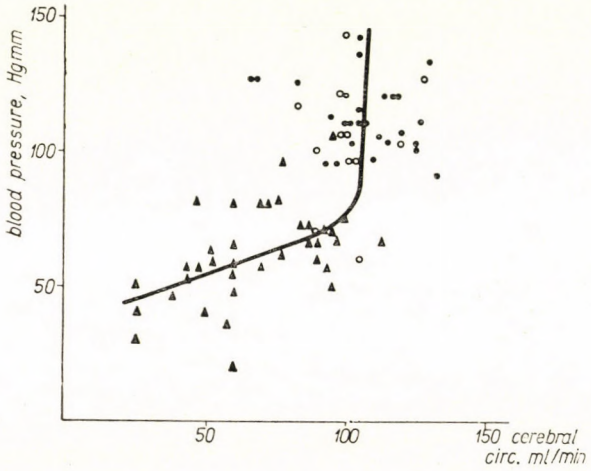

Fig. 2. Head circulation of dogs in ischaemic shock as a function of blood pressure
Abscissa : head circulation ml/minute.
Ordinate : blood pressure.
● = normal values
○ = at end of 5 hours' ligation
▲ = in ischaemic shock

Glucose uptake of the head. The arterio-venous glucose difference rose from an initial 8.6 mg to 9.4 mg per 100 ml during ligation and to 13.4 mg per 100 ml after its release. It remained higher than normal during the whole course of shock ; differences of 10—12.9 mg per 100 ml were observed.

Glucose uptake rose from 9 mg/min to 11 mg/min after release, in spite of a simultaneous lowering of head circulation, then changed in the same direction as head circulation. Terminally, glucose uptake dropped to 4.3mg/min. Compared with the initial value, this difference was statistically significant ($t = 3.645$, $P < 0.01$) (Table I).

The *arterio-venous difference in inorganic phosphate* of the head is normally around zero. This difference became considerable after releasing the tourniquet, reaching 1.07 mg per 100 ml, then for one or two hours it gradually decreased, though quite to the end there remained an arterio-venous difference.

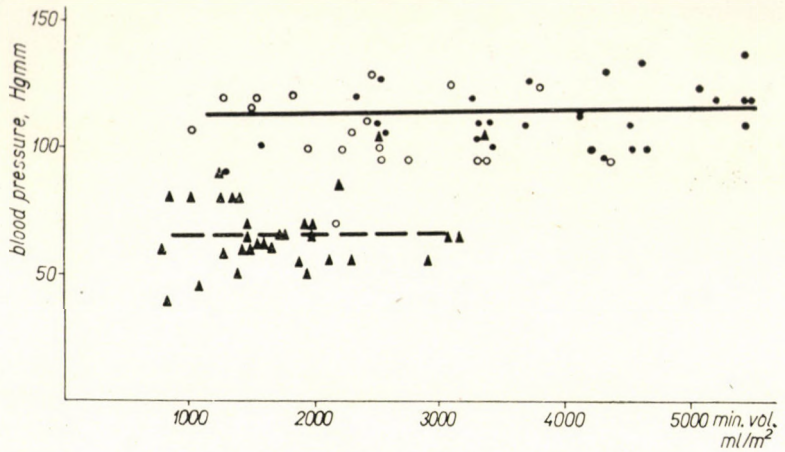

Fig. 3. Minute volume in dogs in ischaemic shock as a function of blood pressure
Abscissa : minute volume/m² body surface.
Ordinate : mean arterial pressure in mm Hg.
● = normal
○ = at the end of 5 hours' ligation
▲ = in shock

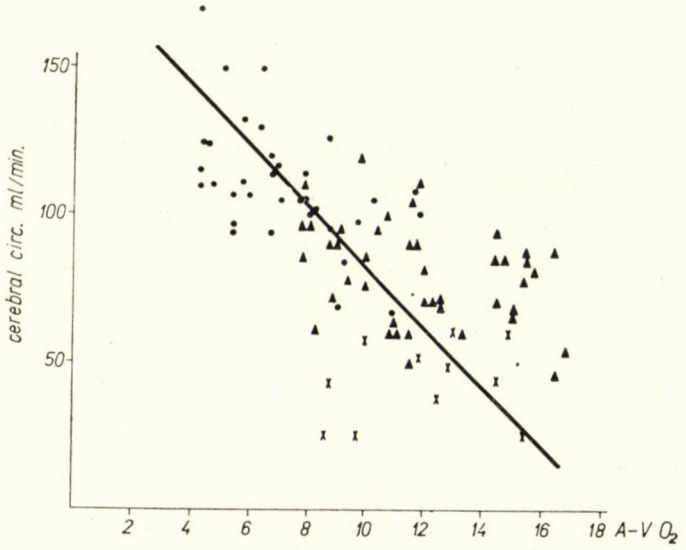

Fig. 4. Arterio- venous oxygen difference in heads of dogs in ischaemic shock ; different head circulation values
Abscissa : arterio-venous oxygen (external jugular carotid).
Ordinate : blood pressure in head, ml/minute.
● = normal
○ = shock
▲ = in the terminal stage of shock

Preterminally and in the final stage the difference again rose considerably. On calculating the uptake of phosphate in the head it was found that after the tourniquet had been released the head constantly took up inorganic phosphate from the plasma. The increase in phosphate uptake was statistically highly significant ($t = 8.570$, $P < 0.01$).

The *arterio-venous difference in lactic acid in the head* is normally about 2.5 to 3.5 mg per 100 ml, the venous blood content being that much greater than the arterial. During ligation this did not change significantly, but immediately after the release marked lactic acid uptake occurred. Half an hour after the limbs had been set free there again began a release of lactic acid which constantly increased quite to the terminal period when it ceased and in half of the cases uptake was again to be observed.

C) *Normal control experiments*

In our normal control experiments (on 11 dogs), in addition to all the operations performed on the animals in shock (excepting the tourniquet on the two hind limbs), we investigated the head circulation, oxygen, glucose, inorganic phosphate and lactic acid uptake. The measurements were begun 4 hours after binding the animals to the table, as in the ischaemic experiments, and were controlled for 4 hours. Anaesthesia, the binding and the operations themselves did not cause changes comparable to those in shock and, except for slight oscillation, no significant alterations were observed (Table II).

Discussion

It was found that in ischaemic shock the amount of blood perfusing the head decreases considerably, oxygen consumption of the head during the course of shock is higher than the normal, except at the final stage when, in spite of a weakening of the circulation, the arterio-venous oxygen difference no longer increases, hence terminally oxygen consumption in the head diminishes.

The decrease in minute volume being more marked than the decrease in head circulation, in shock the fraction of the minute volume falling to the head is greater. The peripheral resistance of the head does not change, in fact it slightly decreases just when a considerable rise occurs in total peripheral resistance. From a certain aspect this does not agree with the results of POUPA's X-ray studies [18, 19] nor with those obtained by means of the cerebral window method by JOURDAN [20], who found vasoconstriction in shock. The results of POUPA relate only to the contraction of the internal carotid artery ; they throw no light on the circulation of the whole head or the brain, for it is known that, as regards cerebral blood supply in the dog, the signif-

Table II

Circulation and metabolism of control dogs bound for 8 hours (average for 11 experiments)

	I	II	III	IV
		Time elapsed after binding dogs to table		
	4^h—$4^h30'$	5^h—$5^h30'$	6^h—$6^h30'$	7^h—$7^h30'$
Arterial blood pressure, mm/Hg	117 ±12	102 ±8	113 ±11	111 ±17
Haemoglobin, per cent	13.3 ±0.3	13.2 ±0.5	13.1 ±0.4	12.9 ±0.5
Minute volume ml/min	2380 ±240	2540 ±170	2420 ±180	2560 ±210
Peripheral resistance	0.049 ±0.003	0.042 ±0.002	0.047 ±0.004	0.045 ±0.004
Peripheral A—V O_2 difference vol. per cent	5.30 ±0.12	5.20 ±0.15	5.90 ±0.20	4.80 ±0.12
O_2 consumption ml/min	127 ±21	130 ±18	143 ±12	125 ±14
Amount of blood perfusing head ml/min	108 ±8	110 ±12	113 ±14	110 ±12
Head resistance	1.06 ±0.05	1.03 ±0.08	1.00 ±0.07	1.00 ±0.08
Fraction of minute vol. perfusing head	4.20 ±0.20	4.10 ±0.25	4.50 ±0.18	4.30 ±0.18
Head A—V O_2 difference	7.60 ±0.20	6.90 ±0.20	7.30 ±0.25	6.70 ±0.32
Head O_2 consumption ml/min	7.50 ±0.15	7.80 ±0.22	8.30 ±0.18	7.60 ±0.25
Head fraction of body O_2 consumption	6.70 ±0.20	7.30 ±0.32	6.30 ±0.15	6.10 ±0.20
Head glucose uptake	9.30 ±0.30	8.80 ±0.20	11.60 ±0.35	10.20 ±0.28
Inorganic phosphate A—V mg per 100 ml	—0.06 ±0.002	+0.06 ±0.01	+0.03 ±0.002	—0.03 ±0.005
Arterial blood lactic acid mg per 100 ml	17.10 ±2.50	18.40 ±3.50	18.10 ±1.80	17.60 ±2.70
Head lactic acid A—V mg per 100 ml	—2.70 ±0.41	—5.50 ±0.72	—1.60 ±0.34	—3.30 ±0.27

icance of the internal carotid is subordinate to the vertebral and orbital arteries. It is naturally a problem what part of the head circulation measured *in vivo* falls to the central nervous system and how much to the other areas ; furthermore, how the blood is distributed throughout the brain under conditions of shock, especially considering that under certain conditions changes in the distribution of blood may take place [21, 22].

When head circulation is plotted in a graph according to minute volume, resp. arterial blood pressure, as may be seen in Figs. 1 and 2, it may be stated that head circulation diminishes to a smaller extent but parallel with the decrease in minute volume, though arterial blood pressure, varying between 100 and 180 mm Hg, does not affect the circulation of the head ; at lower pressure levels, however, even a small decrease in blood pressure causes a sharp decline in head circulation. In Fig. 3, arterial blood pressure is shown as a function of minute volume. It may be seen that normally, and in the animals before releasing the tourniquet, blood pressure does not fall in spite of a drop in minute volume. In shock, however, with a similar low minute volume lower blood pressure values are registered. In spite of this, the peripheral resistance is higher than normal in shock.

In shock, the more satisfactory circulation in the head as compared with the other organs may be attributed to regulating mechanisms. One of the possibilities for the maintenance of head circulation is the known adrenalinaemia in shock. It has previously been demonstrated that, though adrenaline causes direct vasoconstriction, it nevertheless augments the circulation of the head by reflectory vasodilation brought on from the periphery [23].

As already mentioned, according to our previous experiments perfusion of the head with normal blood affords a certain improvement of the shock [1]. From this and the observation that in ischaemic shock circulation in the brain does not decrease to a critical value, so that grave stagnating hypoxia does not occur before the terminal stage, it follows that in the generalization of shock, in addition to the hypoxic injury to the nervous system, an important role must be attributed to the humoral factors released in the torso.

As brain circulation diminishes less than might be expected from the increase in the arterio-venous oxygen difference, we must conclude — in contradiction to the findings of BLALOCK [9] — that in shock the arterio-venous difference is not a reliable quantitative measure of head circulation. It is a question to what extent the two follow a parallel course in other pathological conditions.

Numerous experiments have been made *in vitro* to clear the question of oxygen consumption of the brain in shock. In cats in haemorrhagic shock, BECHER and CRAIG [7] found no change in the oxygen consumption of the cerebral cortex, the kidneys, liver or heart muscle. In the brain homogenizate from cats in severe haemorrhagic shock ROSENTHAL, SHENKIN and DRABKIN [24]

found no change in oxygen consumption. WILHELMI, RUSSEL and LONG [8] observed a terminal decrease in some rats subjected to haemorrhagic shock, though in others they obtained normal values. KOVÁCH, FONYÓ, VITTAY and POGÁTSA [6] found no decrease in oxygen consumption *in vitro*, in brain slices from rats ; in fact, a statistically significant rise in oxygen consumption was apparent.

From results *in vitro* it is naturally difficult to draw conclusions on the oxygen consumption of the brain *in vivo*. The medium is changed in experiments *in vitro* and, in consequence, some of the substances lose much of their activity. This may give a falsification of the conditions *in vivo*. According to the present findings, the oxygen requirements of brain tissue does not diminish in shock but inclines to augment and this continues quite until the final stage. In that period, in spite of a further weakening in the circulation, the arterio-venous oxygen difference does not continue to rise. In consequence of arterial anoxia, the arterio-venous oxygen difference increases not only in proportion to the slowing down of the circulation, as may be seen from Fig. 4. The increase is greater than the decline in flow. It is a question what role stagnating hypoxia may play in this phenomenon. In normal cats, HIRSCH, KRENKEL, SCHNEIDER and SCHNELLBÄCHER [25] found a linear correlation between brain circulation and the change in arterio-venous oxygen difference. Hence, in spite of the changing circulation, oxygen consumption remains constant. On the basis of these data stagnating hypoxia cannot be considered responsible for the increase in oxygen consumption in shock, and this must be attributed to other factors. MUUS and HARDENBERG [26] and COOK, JENSEN and SOUTH [27], found that the oxygen consumption of liver slices of normal rats incubated in the serum of animals in shock showed a rise of 25 to 40 per cent. This means that some humoral factor circulates in the blood of animals in shock which augments the oxidative processes of liver tissue. It is possible that the same factor is responsible for the increased oxygen consumption found, *in vivo* in the brain of animals in shock. Since in this stage oxygen consumption is increased and macroerg phosphate synthesis is decreased in the brain [31], the possibility of a change in oxidative phosphorylation in shock may be considered. Terminally, the arterio-venous oxygen difference diminishes ; in other conditions this would be a sign of a decline in oxygen requirement. In this case, however, the arterial blood is not any more fully saturated with oxygen, so that this terminal phenomenon is but a secondary consequence of arterial hypoxia. It is interesting that in shock in spite of a decline in oxygen consumption throughout the whole organism, the oxygen consumption of the brain tissue increases, which may in part be explained by the circulatory conditions being there more favourable.

The increase in head oxygen consumption may also be attributed to hock adrenalinaemia, inasmuch as KING, SOKOLOFF and WECHSLER [28]

have shown that on administering adrenaline cerebral oxygen consumption increases.

In shock the glucose consumption *in vitro* of non-injured muscle (diaphragm) decreases. This may be due to the hexokinase inhibition [29]. Inhibition of the glucose consumption of brain tissue *in vitro*, or a diminution of its hexokinase activity were not observed [6].

According to our present results, in shock the glucose consumption of brain tissue *in vivo* increases slightly, but it is interesting to note that at the end there is a considerable diminution, parallel with the decrease in oxygen consumption. The glucose consumption before releasing the tourniquet amounts to 80 per cent of the oxygen consumption. After release this value gradually declines and in severe shock the glucose loss is only 66 per cent of the value of oxygen consumption.

The increase in lactic acid and inorganic PO_4 uptake recorded after releasing the tourniquet is probably related to the great quantity of these substances liberated in the ligated limbs and then invading the extracellular space of the head from the arterial blood. In reality, some time after the equilibration of the differences in concentration, uptake of the materials in question becomes markedly less but quite to the end there is a difference between the lactic acid and inorganic phosphate consumption, so that this is not purely a question of an equilibrium being established.

*

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RECORDING OF ACTION POTENTIALS ON MAGNETIC TAPE AND THEIR RECTIFICATION BY GERMANIUM DIODE

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A method has been devised by which nervous action currents can be recorded on magnetic tape and thence transmitted to an oscilloscope, facilitating photographic recording. Noise can be eliminated and "monophasic" action currents can be obtained by placing a biased diode between the amplifier output and the oscilloscope. The method facilitates precise fibre analysis.

*

When recording action potentials of nerves, the following difficulties are usually encountered.

1. Continuous photography or continuous listening to sounds from a loudspeaker is costly, and tiresome.

2. The so-called valve noise is around 2 to 4 μV, even with the best devices.

3. The monophasic lead *in situ* requires that the nerve be lesioned at the site of one electrode. Thereby conduction is interrupted and the responses (respiratory, circulatory, *etc.)* to the impulses transmitted by the fibre cannot be observed.

In the following we shall outline a method, by which the above difficulties may be overcome.

1

Continuous photography, as well as visual and auditory observation are greatly facilitated by the use of a magnetic tape recorder, because the phenomena recorded on the tape can be played back and photographed at will. Exact records can be made from experiments, including experiments lasting several hours. In the literature available we have found only one report of this type of recording [1], but the author, ZIPF, did not describe the apparatus he had used. The method developed by us is shown in Fig. 1, where the following signs are used.

I. Recording electrode.

II. Alternating current differential amplifier.

III. Switch box.

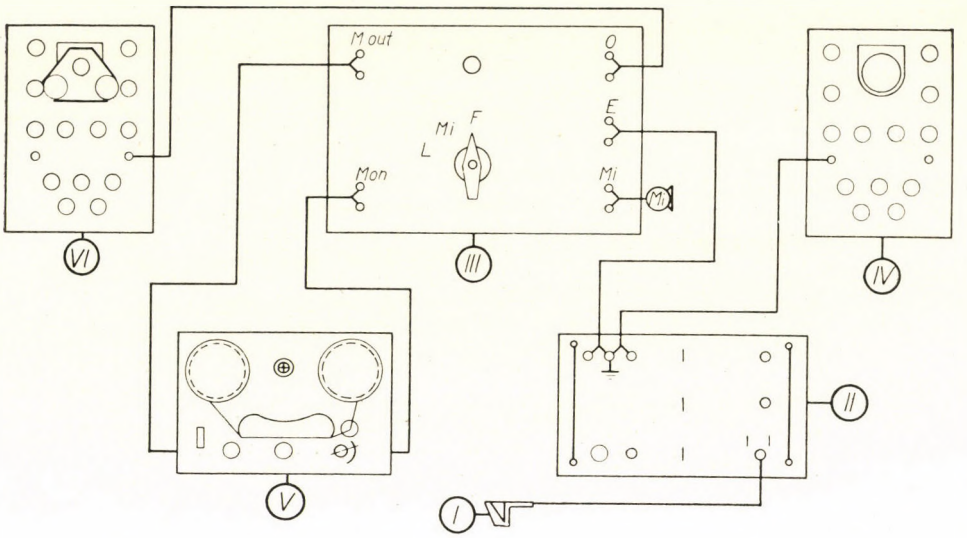

Fig. 1

IV. Oscilloscope, for continuous observation *(Orion*, Type 1538).

V. Tape recorder (Type RS 86—001/A).

VI. Oscilloscope, with photographic adapter *(Orion*, Type 1538 and *Orion*, Type 1578/I).

The signals from the recording electrode (I) are amplified by an amplifier (II), reach the oscilloscope (IV), where they are further amplified and the switch box (III). Thence the impulses are conducted into the recording oscilloscope (VI) and the tape recorder (V), if the central switch is in position *F*. Should any change occur, or if some experimental intervention takes place, the central switch is turned to position *Mi*, and the observation can be spoken into a microphone, and thus recorded on the magnetic tape. When the switch

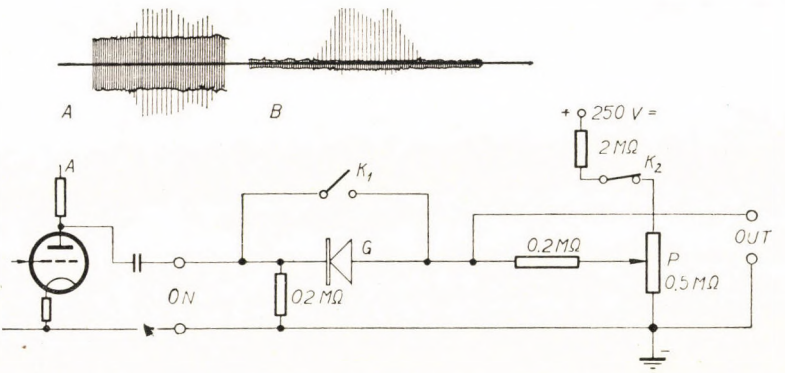

Fig. 2

is in position *L*, the phenomena observe dreach, through the switch *M* out, the oscilloscopes, and we may control visually what we are photographing in the other oscilloscope. In this way experiments lasting several hours can be recorded, and the phenomena played back from the tape can be recorded by film, almost with perfection.

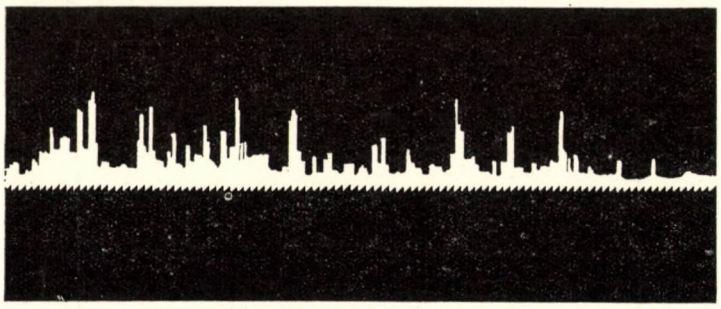

Fig. 3. Action currents from a nerve fibre about 0.05 mm in diameter, supplying the ear of a rat. In response to heating, chiefly the slow-conducting fibres of low amplitude are activated. Occasionally, discharges from "pain fibres" of higher amplitude occur. Time signal, 0.1 sec

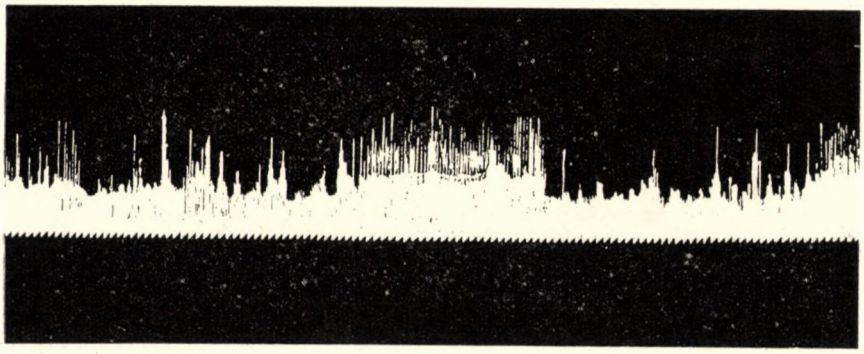

Fig. 4. Action currents in response to touching the skin, in the neurogram of the rat saphenous nerve. Even a film rate of 9 cm/sec is insufficient for a precise analysis of the single action currents, because the lead was made from a fibre thicker than 0.1 mm

The sound frequency tape recorder, because of its distortion, is not suitable for slow wave shape analysis but eminently so for counting fast impulses and for a perfect reproduction of the size of impulses.

The difficulties outlined in points 2 and 3 may be overcome simultaneously, by a noise clipping stage (Fig. 2). This consists of a germanium diode (*G*) so adjusted as to permit the flow of current only when it exceeds the voltage set by the potenciometer P. For instance, if 5 V noise impulses reach the diode from the amplifier and the potenciometer is set to 6 V, no noise signals

will pass through the diode. Thus, the part above 6 V of the useful signals (action potentials) may further be amplified to the full size of the oscilloscope screen and the signal/noise ratio can be improved to meet the requirement.

The noise clipper stage is connected between the output of the amplifier and the oscilloscope or the tape recorder. With switch K_1 off, the full curve A will pass, but if K_1 is on and K_2 is off, and the potenciometer is ad-

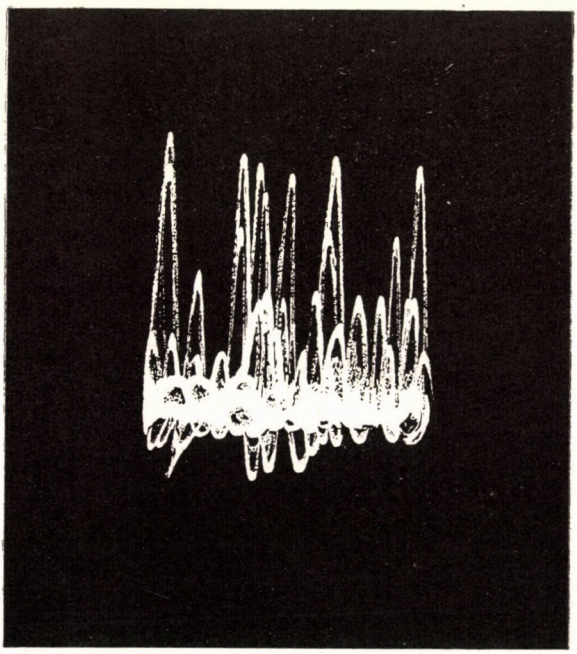

Fig. 5. Detailed analysis of the action currents shown in *Fig. 4.* Biphasic recording, rectified on playing back by germanium diode. In response to touch, fibres of 4 different amplitudes are activated, with those of lower amplitude preponderating. This explains the relatively thick "base line" 9 cm/sec on the record. (Photographed with "Exa", 1 : 2.9, 1/25 sec)

justed to the adequate voltage, curve B will be obtained with half of the oscillations, because the diode conducts in one direction only. This arrangement has the following advantages.

a) The signal/noise ratio can be adjusted at will.

b) When using a loudspeaker for observation, the oscillation developing owing to acoustic feedback of the amplifier can be eliminated.

c) The continuous sizzling noise is greatly reduced, the impulses sound clear from the loudspeaker and the changes can be heard much better, facilitating the work of the observer.

We present several figures to illustrate the usefulness of the method. Fig. 3 shows that in response to heating chiefly the small-amplitude, slow

conducting fibres are activated, while at times discharges from single fast-conducting, *i. e.* higher amplitude, "pain fibres" occur. The impulses obtained by heating correspond in every respect to the findings reported by DODT [2], according to whom in response to a burning sensation the fast-conducting "pain fibres" and the cold fibres of lower amplitude are activated. Fig. 4 shows the action currents in response to touching the skin, in the electroneurogram of the saphenous nerve. The detailed analysis of the electroneurogram is illustrated in Fig. 5, where it can be seen that fibres of four different amplitudes are activated by touching the skin.

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CARDIAC OUTPUT AND RENAL BLOOD FLOW IN ACUTE HYPOTENSION

By

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In extensive experiments on anaesthesized dogs, blood pressure, cardiac output, renal blood flow, glomerular filtration rate, sodium and water output has been investigated at normal blood pressure levels and during acute hypotensive states.

Renal haemodynamics were analysed partly by direct determination of the renal venous outflow and partly on ground of the classic clearance technique.

It has been found that the cardiac output and the directly measured renal blood flow decrease practically in direct proportion with the drop in blood pressure, so that the calculated total peripheral and renal vascular resistance do not change in hypotension. Correspondingly, there is no change in the so-called renal fraction of cardiac output, either.

The results published in the literature, according to which during hypotension there is a preferential increase in renal resistance, were all obtained by the clearance technique. In oliguria the low clearance is a technical consequence of the oliguria itself and in this condition no conclusions should be drawn from the clearance values as to the rate of renal blood flow.

*

According to the general view, circulatory failure, whether, acute or chronic, is associated with a significant decrease in renal blood flow (RBF), glomerular filtration rate (GFR) and salt and water excretion. BULL [5] described a disproportionately great reduction in renal blood flow on the application of a venous tourniquet on the limbs further in the various forms of shock, in dehydration, orthostasis, congestive heart failure and muscular work. CORCORAN and PAGE [6] found in the dog an extremely low RBF during post-haemorrhagic hypotension. According to PHILLIPS, DOLE, HAMILTON, EMERSON, ARCHIBALD and VAN SLYKE [16], renal blood flow may cease completely following major blood losses, even with a considerable arterial pressure (e. g. 90 mm Hg). LAUSON, BRADLEY and COURAND found after injury extremely low RBF values in man [13]. KERPEL-FRONIUS, VARGA, KUN and VÖNÖCZKY [12] reported that in dehydrated and undernourished infants the renal haemodynamics were disproportionately reduced, as compared to the systemic circulation. GÖMÖRI, ROMHÁNYI, FÖLDI and SZABÓ [8] demonstrated in the dog dehydrated by pyloric ligation a disproportionate decrease in renal blood flow.

In the above conditions marked oliguria, or anuria is a characteristic feature. STUDY and SHIPLEY [23] observed when stimulating the renal nerves

that during the oliguric period the RBF values computed from the clearances were considerably lower than those obtained by the direct determination of renal venous outflow by means of a rotameter. A similar phenomenon has been observed by SELKURT [18, 19] in oliguria following a temporary clamping of the renal artery, as well as in haemorrhagic shock BÁLINT, FEKETE, HAJDÚ, LÁSZLÓ and PINTÉR [1] observed the same phenomenon in the dog during posthaemorrhagic hypotension, and BÁLINT, KISS and SZALAY [3] in oliguria induced by water deprivation.

In the experiments mentioned in the first paragraph, renal blood flow was determined by the classic clearance technique. It has been suggested that the low RBF might be a technical consequence of oliguria in which state the clearances cannot be relied upon in assessing renal blood flow. BÁLINT [2], in posthaemorrhagic hypotension, BÁLINT and STURCZ [4] in dehydration induced by pyloric ligation, found that the reduction in renal venous outflow, as determined by direct measurement, was by far not so great as it was suggested by the results of the clearance tests. Thus, in the conditions mentioned above, even if there occurs a reduction in renal blood flow, the reduction is not so great as to explain an almost complete renal ischaemia.

It follows from the above that the view according to which the renal portion of the cardiac output, the so-called renal fraction, would decrease significantly, should be revised. LAUSON *et al.* [13], KERPEL *et al.* [12], as well as TAKÁCS and KÁLLAY [24] determined the renal blood flow by the clearance technique, in addition to estimating the cardiac output. SELKURT [19] determined the renal blood flow directly in haemorrhagic shock and, comparing his data with the values for cardiac output reported by WIGGERS and MIDDLETON [28], stated that the renal fraction was reduced. In our own investigations we found that, if the renal blood flow was determined by direct measurement, very little or no decrease in the renal fraction of cardiac output took place in posthaemorrhagic hypotension and in dehydration following pyloric ligation.

Next, we endeavoured to elucidate the relation of cardiac output to renal blood flow in a number of normal dogs, as well as in dogs in the state of "acute hypotension". "Normal" dogs should be understood to mean the animals prepared for surgery as described under "Methods", with the arterial blood pressure above the accepted normal level of 100 to 110 mm Hg. The term "acute hypotension" denotes the state when blood pressure dropped spontaneously, or, more correctly, without any experimental treatment intended to cause a drop in blood pressure. It is well-known that in some anaesthesized and operated dogs arterial pressure decreases even when no considerable loss of blood has occurred. It is not the aim of the present report to discuss the definition and pathomechanism of shock, and for this reason is WIGGERS' [27] term acute hypotension used to denote the phenomenon when the blood pres-

sure of the anaesthesized and operated animal decreases without oligaemia. The living conditions of the animal before experiment, uncontrollable experimental fractors, as well as the untoward consequences of the replacement of blood taken for analysis by the blood of another animal may undoubtedly all play a role in it.

Methods

Mongrel dogs of both sexes, weighing 10 to 20 kg each, were used, mostly animals that had been kept at our Institute for not less than a week. The number of the experimental dogs totalled 61. Most of the animals were deprived of food for 16 hours and of water for 2 to 14 hours before the experiment, but previously were allowed water *ad libitum*. In a few cases fasting and water deprivation lasted 48 to 72 hours. This factor by itself having caused no hypotension, the data for these dogs have been included in the evaluation.

Surgical procedure. General anaesthesia was induced by the slow intravenous injection of 0.1 g/kg of chloralose, in the form of a 1 per cent solution. The appropriate veins and arteries were cannulated in order to administer the various infusions, to measure blood pressure and to take samples of blood. The left kidney was exposed from a lumbar incision and the renal vein was connected with the external jugular vein by means of a plastic tube. A T-extension in the plastic tube made it possible to make direct measurements of renal venous outflow and to take samples of renal venous blood for analysis. The maximum duration of ischaemia during surgery was 3 minutes. Urine was collected by means of an ureter catheter introduced up to the renal pelvis. The plasma inulin and PAH concentrations for the determination of clearance and extraction were provided for by giving a priming dose (0.15 g/kg of inulin and 0.015 g/kg of PAH, dissolved in 50 ml of 0.9 per cent NaCl solution) and a maintenance dose (1.5 per cent inulin and 0.15 per cent PAH, dissolved in 0.9 per cent saline, infused at a rate of about 1 ml/minute throughout the experiment).

The actual experiment began about 20 to 30 minutes after the surgical intervention had been completed and the maintenance infusion had been started. Urine was collected in 15-minute periods, by the end of which blood was taken for analysis and the cardiac output was determined. Renal blood flow was measured directly, 2 or 3 times during the 15-minute periods. The volume of blood taken for analysis was around 50 ml, and this was replaced by the infusion of an equal volume of dog's blood. In most cases two such periods were registered. If blood pressure dropped during the second period, or was low in the first one already, in some cases more than two "hypotensive" periods were analysed. The total number of periods studied in the 61 animals was 139.

Technique of determinations. Arterial blood pressure was measured in the femoral artery, by means of a mercury manometer. Cardiac output was determined by the Evans blue dilution method of HAMILTON, MOORE, KINSMAN and SPURLING [10]. Renal blood flow was measured directly, by means of a graded cylinder and stop watch. The inulin concentration of plasma and urine was estimated according to HARRISON [11], the PAH concentration by the technique of SMITH, FINKELSTEIN, ALIMINOSA, CRAWFORD and GRABER [22]. Sodium concentration was determined in a *Zeiss* flame-photometer, the haematocrit was estimated by the copper sulphate method of PHILLIPS, VAN SLYKE, HAMILTON, DOLE, EMERSON and ARCHIBALD [17].

Abbreviations, calculations. In the Figures and Tables the arterial pressure is given in mm Hg, the cardiac output in litre/minute, the renal blood flow (RBF), glomerular filtration rate (GFR) and minute diuresis in ml/minute, sodium excretion in μaeq/minute. RBF_{dir} means the directly determined renal blood flow, and $RPF.E_{in}$ is the inulin clearance computed from the RBF_{dir}, haematocrit and E_{in}. $RBF_{C/E}$ means the renal blood flow as computed by the classic clearance technique, emphasizing that the PAH clearance was divided in every case by the PAH extraction, *i. e.* we computed not the so-called effective, but the so-called true RBF.

Total peripheral resistance (TPR) and renal vascular resistance (R) were computed by dividing the arterial pressure by the cardiac output in ml/min, resp. the renal blood flow. It seemed suitable to present the reciprocals of these values (1/TPR and 1/R), thus an increase in the numerical value of the ratio means a decrease in resistance and vice versa. $1/R_{dir}$ means that RBF was determined by direct measurement, $1/R_{C/E}$ means that it was estimated on the basis of clearance and extraction. The percentage ratio of renal blood flow and cardiac out-

put represents the so-called renal fraction (RF) of the cardiac output. According to the methods of RBF determination employed, we shall speak of RF_{dir} and $RF_{C/E}$ values.

Statistical analysis was made by FISHER's *t*-test [7].

Results

The results are presented in Figs. 1 to 5 and in Table I. In each Figure blood pressure is shown on the abscissa. In Table I are shown the means for the single blood pressure ranges, with standard deviation and number of cases.

Fig. 1 shows the correlation between blood pressure and cardiac output. The diagonal lines represent the total peripheral resistance (TPR), the points falling in the same line correspond to identical TPR values. Line 3 of Table I shows that the mean cardiac output falling to the single blood pressure ranges decreased rather proportionately. The 1/TPR values indicate that in the range of 111 to 160 mm Hg, considered to be normal, and in the very low range of 30 to 70 mm Hg the total peripheral resistance was identical whereas

Table I

Cardiac output and renal function in normal blood pressure and in "acute" hypotension

	I	II	III	IV	V	VI
1. Blood pressure	30—50	51—70	71—90	91—110	111—130	131—160
2. Blood pressure, mean	42±7	60±5	84±6	103±6	122±4	143±9
3. Cardiac output	1.16±0.48	1.39±0.66	1.77±1.06	2.24±0.93	3.13±1.42	3.60±1.35
4. 1/TPR	27.6±10.8	26.0±2.3	21.4±13.7	21.9±8.2	25.8±12.0	25.1±9.1
5. RBF_{dir}	135±66	235±83	299±99	443±141	535±201	528±126
6. $RBF_{C/E}$	2±5	29±49	74±100	308±267	498±236	508±252
7. $1/R_{dir}$	3.16±1.40	3.88±1.31	3.56±1.19	4.26±1.29	4.45±1.67	3.69±0.78
8. $1/R_{C/E}$	0.04±0.10	0.46±0.82	0.88±1.19	2.89±2.46	4.10±2.00	3.45±1.62
9. RF_{dir}	11.7±3.2	19.0±14.3	18.3±6.7	19.7±7.9	19.3±7.0	18.6±8.5
10. $RF_{C/E}$	0.1±0.3	3.4±7.9	4.9±7.1	14.9±14.5	15.8±8.2	16.3±9.6
11. $RPF \cdot E_{in}$	10±10	25±18	28±20	47±32	69±44	71±35
12. C_{in}	0±1	2±4	19±40	29±23	49±22	54±25
13. V/min	0.02±0.06	0.03±0.05	0.24±0.46	0.51±0.49	0.71±0.37	0.98±0.26
14. Na excr./min	1±6	4±6	11±11	51±66	77±95	139±123
15. E_{in}	6±12	18±8	16±7	18±9	21±10	21±8
16. E_{PAH}	36±21	51±20	48±13	55±12	61±13	63±12
17. Haematocrit	39	40	41	40	41	39
18. Number of cases	8—11	7—8	18—21	33—38	30—36	17

it was somewhat (not significantly) higher in the blood pressure range of from 71 to 110 mm Hg. Thus, in this form of hypotension TPR was essentially unchanged.

In Fig. 2 is shown the correlation between RBF and arterial pressure. On the left side are the values obtained by direct measurement, on the right those computed from clearance and extraction. The diagonals represent the renal vascular resistance. The points falling in the same line correspond to identical R values. As it can be seen, RBF_{dir} decreased nearly parallel with

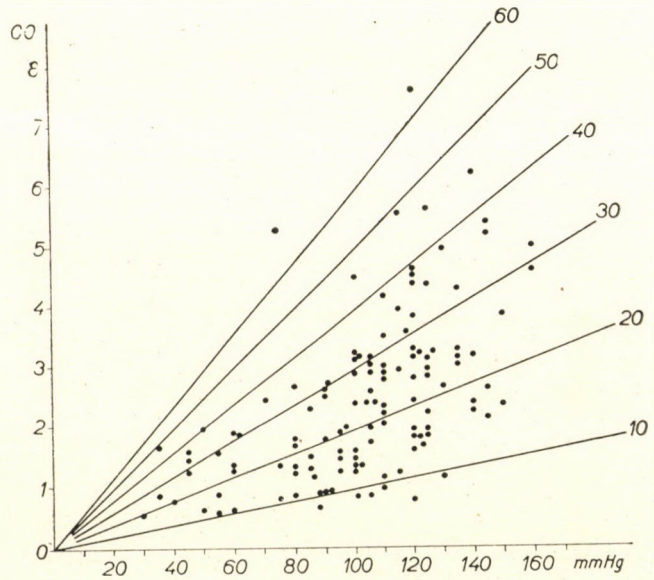

Fig. 1. Relation of cardiac output to blood pressure. The diagonals correspond to the reciprocals of total peripheral resistance (1/TPR)

the fall in blood pressure, whereas $RBF_{C/E}$ was greatly reduced, often to O at lower blood pressure levels. This is reflected also by the mean REF values in Table I. The $1/R_{dir}$ values (reciprocal renal vascular resistance computed on grounds of direct measurement) have the highest numerical value in column V., decrase slightly in column VI. and decrease gradually from columns IV. to I. The difference between the single columns is not significant. In contrast with this, the value of $1/R_{C/E}$ (reciprocal renal vascular resistance computed from the clearance) was markedly reduced at lower blood pressures, as a natural result of the similar reduction in $RBF_{C/E}$.

In Fig. 3 are shown the values for RF at different blood pressure levels, on the left side on grounds of RBF_{dir} and on the right side of $RBF_{C/E}$. Except in the lowest blood pressure range, the numerical value of RF_{dir} was un-

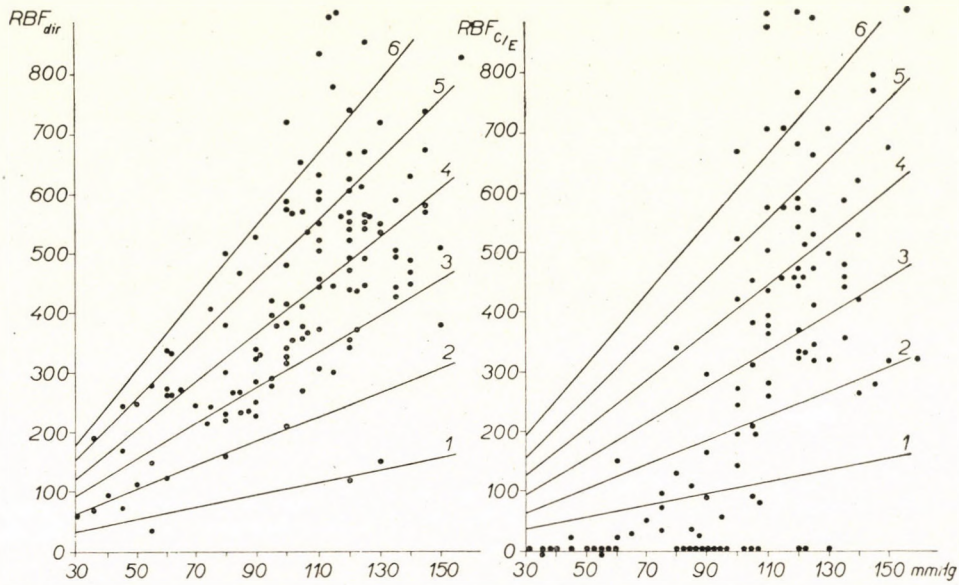

Fig. 2. Relation of renal blood flow to blood pressure. Left side : renal blood flow as determined by direct measurement. Right side : renal blood flow, computed from PAH clearance and extraction. The diagonals correspond to the reciprocals of renal resistance (1/R)

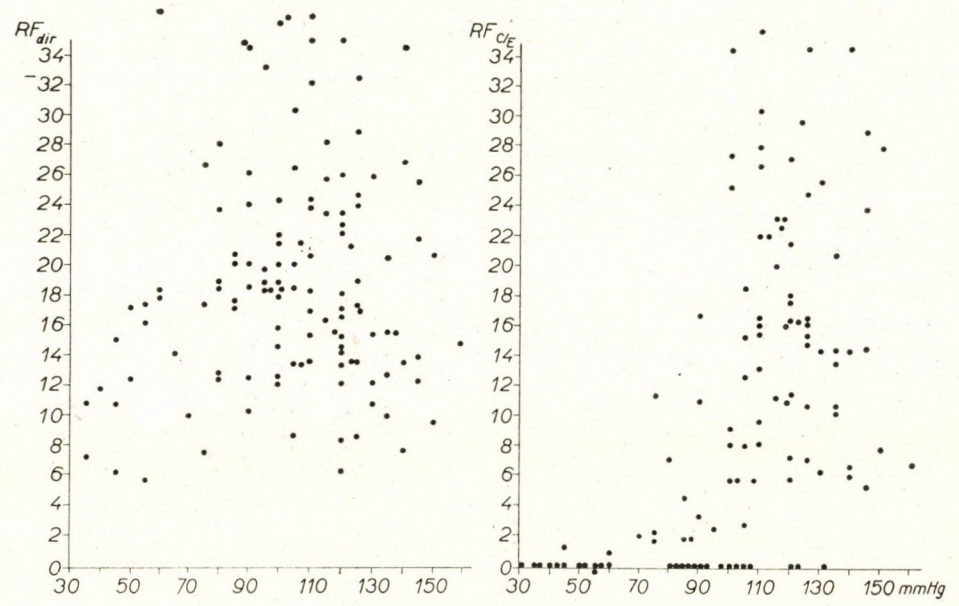

Fig. 3. Relation of the renal fraction of cardiac output to blood pressure. On the left : directly measured renal blood flow. On the right : renal blood flow computed from PAH extraction and extraction

changed, indicating that the renal fraction of cardiac output was the same in the whole blood pressure range of from 50 to 160 mm Hg. In contrast with this, the value of $RF_{C/E}$ decreased sharply and was very low below the blood pressure level of 90 mm Hg.

Fig. 4 illustrates the correlation between GFR and blood pressure. On the left side is given the RPF, as computed on grounds of the haematocrit value from the directly measured RPF. This was then multiplied by the inulin extraction for the corresponding period (RPF. E_{in}). On the right side, the

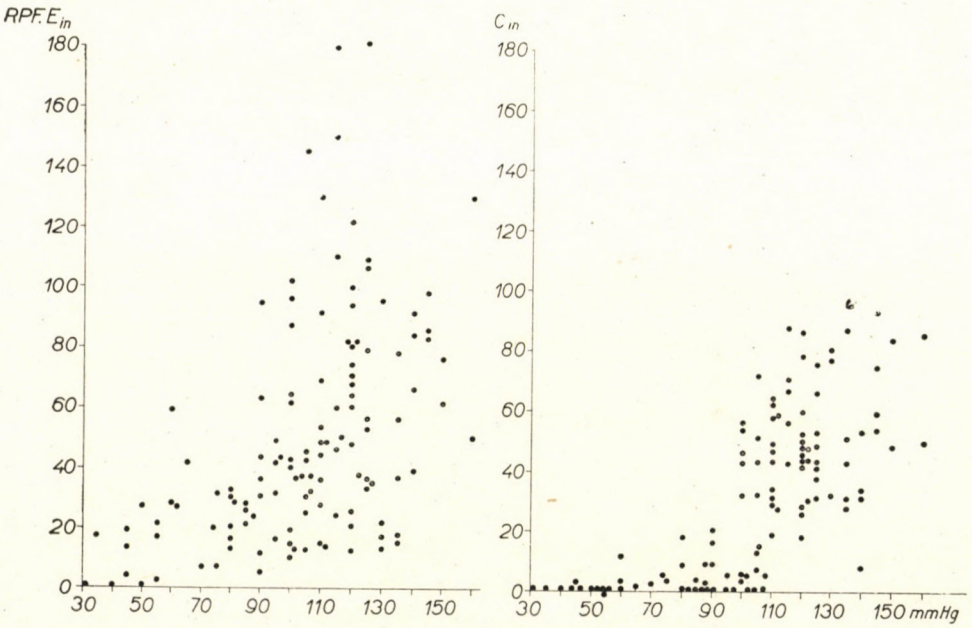

Fig. 4. Relation of glomerular filtration rate to blood pressure. On the left : on the basis of renal blood flow, measured directly, haematocrit and inulin extraction. On the right : classic urinary clearance

inulin clearance (C_{in}) determined by the classic technique is visible. Fig. 4, as well as lines 11 and 12 in Table I, indicate that, as determined by direct measurement, GFR decreased slowly, but was appreciable even at very low blood pressures. In the blood pressure range of from 50 to 160 mm Hg the directly determined inulin clearance decreased practically in proportion to the reduction in RBF_{dir}, as the haematocrit value did not change and E_{in} was also practically unchanged. The low RBF (measured directly) at the lowest blood pressures (30 to 50 mm Hg) was a result of a simultaneous reduction in RBF and E_{in}. The inulin clearance values shown on the right side of Fig. 4 decreased steeply with the decline of blood pressure and were negligibly low below the 70 mm Hg pressure level.

Fig. 5 shows how diuresis (left side) and sodium excretion (right side) depend on blood pressure. Above a pressure of 100 mm Hg, sodium excretion and water diuresis are variable, but at lower blood pressure levels complete anuria is common and the mean excretion rates (Table I) are correspondingly low.

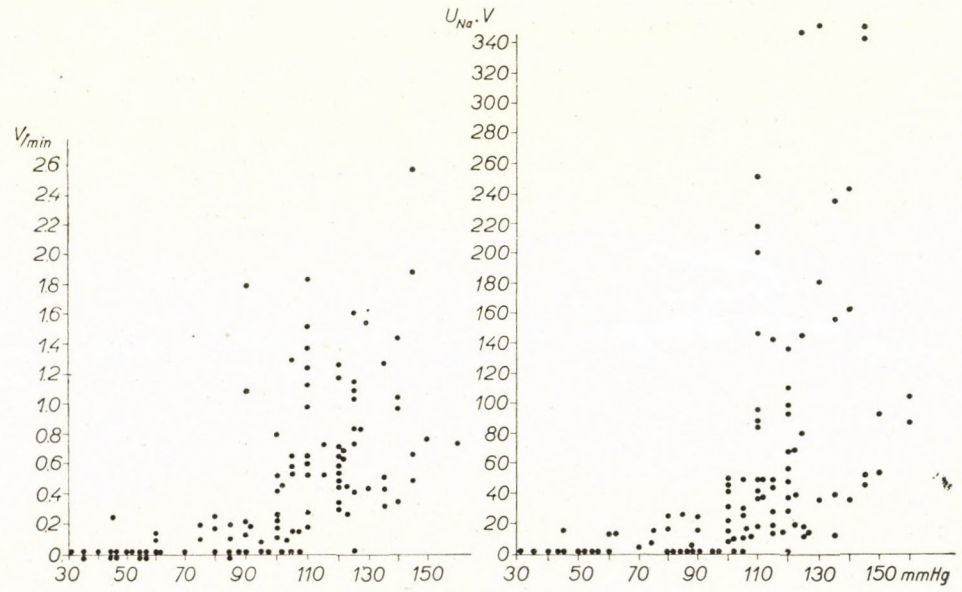

Fig. 5. Relation of diuresis (ml/min) and sodium excretion (μaeq/min) to blood pressure

Discussion

The most important problem to be discussed is whether the view put forward in the literature that in hypotensive states the decrease in renal blood flow is disproportionate to the reduction in blood pressure and cardiac output, is still tenable. To facilitate evaluation, we present Fig. 6, showing the cardiac output, resistances, and renal function at various blood pressure levels, on the basis of the means of all data.

The decrease of cardiac output goes parallel with the decrease of blood pressure. It is thought that a decrease in venous backflow is responsible for the fall in cardiac output and the drop in arterial blood pressure is a secondary sequel to it.

The value of 1/TPR shows the numerical relation of cardiac output to arterial pressure, at different levels of blood pressure. This ratio is usually accepted as being the measure of the so-called total peripheral resistance. It would nevertheless be erroneous to draw conclusions as to a constriction or

dilatation of arterioles directly from this numerical value. As it has been pointed out by GREEN, LEWIS, NICKERSON and HELLER [9], the ratio is influenced, besides the condition of the arterioles, also by the passive dilatation of blood vessels and by the opening up of new capillary beds. Accordingly, we consider this ratio merely a numerical expression of the relation of a given blood pressure to cardiac output.

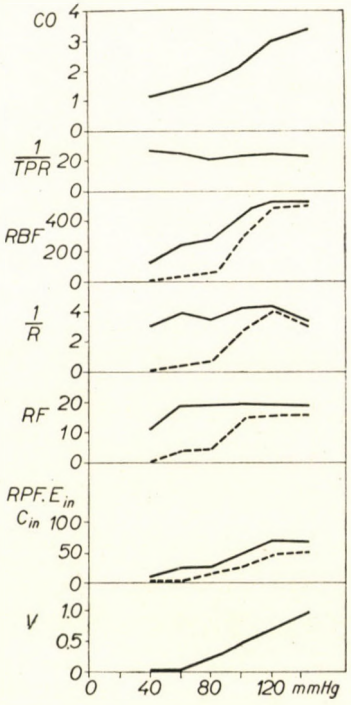

Fig. 6. Relation of cardiac output and of renal function to blood pressure. The curves have been plotted from the means of the points belonging to the single blood pressure values

In the two highest blood pressure ranges the curve for RBF_{dir} runs horizontally, then with the drop in blood pressure declines gradually. There is no significant difference between the numerical value of $1/R_{dir}$ at the different blood pressure levels, but there is no doubt that $1/R_{dir}$ decreases gradually at the levels lower than 111 to 130 mm Hg, considered normal. The considerations about TPR apply even more to the so-called renal resistance. We believe that from the ratio RBF to blood pressure no direct conclusion can be drawn as to a constriction or dilatation of renal blood vessels. As it has been pointed out also by WHITTAKER and WINTON [26], the viscosity of blood, too, may influence the value of blood pressure/RBF. In this case we again restrict ourselves to stating that the numerical value of the so-called renal resistance ex-

presses the relation of blood pressure to renal blood flow. The increase in the value of the ratio blood pressure/RBF with the lowering of blood pressure does not necessarily indicate that the vessels had constricted, because an increase in the viscosity of blood or a re-arrangement of intrarenal circulation may also effect this increase.

The curve for RF_{dir} (the renal fraction of cardiac output) is in full agreement with those outlined above. This curve runs low exclusively in the lowest blood pressure range, and is seen to maintain the same height between 51 and 160 mm Hg. This means that in this form of hypotension cardiac output and RBF decrease proportionately, so that there is no preferential reduction in renal blood flow.

Should we derive the values for renal circulation from the PAH clearance and extraction ($RBF_{C/E}$, $1/R_{C/E}$ and $RF_{C/E}$) we would arrive at quite the opposite conclusions. The value of renal blood flow computed from the clearance decreases sharply with the decline of blood pressure. The same applies to the values for reciprocal resistance and for the renal fraction of the cardiac output. In the two highest blood pressure ranges there is no significant difference between the values determined directly and those computed on the basis of clearance and extraction, but below 110 mm Hg (columns IV to I.) the difference is highly significant.

If there is a discrepancy between the directly measured RBF value and that determined from the clearance, there is little doubt as to which of them is reliable. Obviously, it is the directly measured value that has to be accepted. PHILLIPS *et al.* [16] pointed out that in hypotensive states the rate of PAH extraction is constant, even at very low clearances, and they concluded that C_{PAH} is a correct measure of renal plasma flow even in marked ischaemia. We agree with the first part of this statement, because line 15 of Table I shows that E_{PAH} decreases but slightly with the drop in blood pressure. Our data, however, suggest that, despite the comparatively small decrease in E_{PAH}, no reliable conclusions can be drawn from the PAH clearance in the hypotensive period.

We should be cautious when evaluating the so-called directly measured GFR ($RPF.E_{in}$). The haematocrit value can be determined precisely and thus the determination of RPF_{dir} is just as reliable as that of the RBF_{dir}. However, for methodical reasons E_{in} cannot correctly be determined. Let us suppose for example that the inulin concentration of arterial plasma is 50 mg per 100 ml and that of renal venous plasma 40 mg per 100 ml, then E_{in} equals 0.2. Should the analytical estimation yield 48 resp. 42 mg for these values, extraction were as low as 0.12 and the computed GFR only 60 per cent of the value we would have obtained by calculating with an E_{in} of 0.2. This is the cause of the wide scattering of the results in these tests and it is for this reason that the curve for $RPF.E_{in}$ may serve qualitative evaluation only. At any rate, it

is clear that the decline of the curve lags behind the changes in the C_{in} values determined by the classic clearance technique. At a low blood pressure the clearance is negligibly low.

What explains the unreliability of clearance data in hypotension? CORCORAN and PAGE [6], as well as LAUSON *et al.* [13] suggested that in hypotension the tubular extraction of PAH (diodrast) is greatly hindered and the tubular epithelium becomes permeable for inulin. Experience has shown that in many a hypotensive period there is no decrease whatever in E_{PAH} and E_{in}, as compared with the controls, and yet the clearances are unreliable. A look at Fig. 5, and the right side of Figs. 2 to 4 makes it obvious that there the single points are arranged similarly. Thus, the clearance is undeterminably low in the periods when diuresis is low. This confirms the view that oliguria is responsible for the low clearance. And since the absolute values for RBF_{dir} and $RPF.E_{in}$ also decrease with the fall of arterial pressure, our data do not discredit the view accepted by many authors (SMITH, [21], SELKURT, [20]) that a very slight, hardly determinable decrease in filtration may result in a considerable reduction of water and sodium excretion. As it has been shown by O'CONNOR [15] for the unanaesthesized dog, however, arterial blood pressure must be regarded as one of the most important determinants of sodium (and water) excretion. When it rises, sodium and water excretion increase. The present methods do not make it possible to determine the extent to which the changes of GFR play a role in that process. We therefore think that hypotension is responsible for the extreme oliguria or the anuria, a technical consequence of which is the extreme lowness of the clearances.

We have found in this form of hypotension, which was designated by the term "acute hypotension", that both the total peripheral and the renal resistance increase very slightly and run parallel until blood pressure has fallen to 50 mm Hg. This means that the renal fraction of cardiac output does not change. Consequently, like it has been shown for posthaemorrhagic hypotension and for severe dehydration [2, 4], there is no preferential shutting-off of renal blood flow in this form of hypotension, and there seems to be no justification for the claim that the blood would be diverted to organs momentarily more vital. In our opinion, the contradiction in the literature is due to the fact that renal blood flow was determined by the clearance technique.

Let us now consider briefly the reliability of our method. The explanted kidney of the anaesthesized animal is undoubtedly not equivalent to the organ *in situ* of the conscious animal. Most of the published evidence has been derived from experiments on anaesthesized animals. If we want to determine renal venous outflow directly, we have to expose the renal vein. The method we described does not seem to be less "gentle" that the technique of SELKURT [18] in which a metal tube is introduced through the jugular vein and the heart into the inferior vena cava and is ligated into the renal vein under manual

control through a laparotomy wound. The only datum suggesting that our kidneys suffer functional lesion is that even during the control period PAH extraction is comparatively low (61—63), lower than the values obtained by venous catheterization of kidneys *in situ*. With SELKURT's technique [19], PAH extraction in the control period is 73, *i. e.* lower than the means for the kidney *in situ*. We do not think that the slightly lower E_{PAH} would seriously influence the direct determination of RBF and the conclusions drawn from it.

It may be asked whether in hypotension some kind of a shunt mechanism is functioning in the kidney. We agree with MAXWELL, BREED and SMITH [14], in that the presence of a shunt of the TRUETA type [25] would lead to an extreme reduction in inulin extraction. Our experiments having not demonstrated any appreciable drop in E_{in}, we do not think that a shunt would be involved in the process, but our findings neither confirm, nor disprove the involvement of a shunt mechanism.

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INFLUENCE OF NONSHOCKING HAEMORRHAGE ON CARDIAC OUTPUT AND RENAL BLOOD FLOW

By

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Oliguria has been induced in anaesthesized dogs by gradually withdrawing small volumes of blood. Blood pressure decreased only slightly. In the oligaemic state the cardiac output, renal blood flow, glomerular filtration rate and sodium-water excretion were measured.

Renal blood flow and glomerular filtration rate were determined both by direct measurement and by the classic clearance technique. It was found that according to the results of direct measurement the absolute value of RBF decreased in response to bleeding, but the decrease was proportionate to the drop in blood pressure. This means that renal resistance did not change. The renal fraction of cardiac output also remained unchanged. At the same time the results obtained by the clearance technique suggested the presence of marked renal ischaemia, an increase in renal resistance and a considerable reduction in the renal fraction.

It may, accordingly, be stated that oligaemia causes a reduction in salt and water excretion and that the low clearance values are a technical consequence of oliguria.

Thus, in the oligaemic state no diversion of blood takes place, at the expense of the kidney and in favour of other organs.

*

There is ample experimental and clinical evidence to show that a reduction in the volume of circulating blood and of the extracellular space leads to a decrease in sodium and water output. (For the literature the reader should consult references [4, 5, 6, 7, 8, 15] and [17]. The role of the kidney in this process is not clear. One team considers a reduction in the glomerular filtration rate, *i. e.* a decrease in haemodynamics to be of primary significance, the other team an increase of tubular Na reabsorption. The simplest way to reduce plasma volume is to draw blood. This, however, by causing a drop in blood pressure, leads to profound circulatory alterations. For this reason attempts have been made to lessen the plasma volume by taking so little blood that blood pressure did not change substantially. In this way the extracellular space was reduced without altering the osmotic relations, *i. e.* isoosmotic hypovolaemia was produced.

LOMBARDO, EISENBERG, OLIVER, VIAR, EDDELMAN and HARRISON [14] reported that in man following the withdrawal of small volumes of blood salt and water excretion changed, but the glomerular filtration rate did not. YOUMANS and HUCKINS [19] found a decrease in filtration and in sodium excretion following minor blood loss in the dog. Similar results have been reported by WATSON, GULLIXSON, RENNIE and YOUMANS [18]. HEINEMANN, SMYTHE

and MARKS [13] have shown that renal blood flow, as determined by the clearance technique, decreased after withdrawal of moderate volumes of blood. GOODYER and JAEGER [12] reduced blood pressure by 20 mm Hg by taking blood from asaesthesized dogs and noted that in response to the reduction sodium excretion decreased significantly while filtration hardly decreased. A decrease in blood pressure similar to that produced by hexamethonium blockade did not influence sodium excretion and renal blood flow. From this the above authors concluded that it is not the decrease in blood pressure, but the reduction in the blood volume which is responsible for the changes mentioned. ZUIDEMA, CLARKE, REEVES, GAUER and HENRY [20] found in the dog a significant decrease in diuresis in response to a reduction of the calculated blood volume by 10 to 30 per cent.

In the above-mentioned investigations renal haemodynamics were studied by the clearance technique. In our earlier reports it has been pointed out that in post-haemorrhagic hypotension [1], acute hypotension [2], and in the first phase of haemorrhagic shock [3], the reduction in renal blood flow, as determined by direct measurement, is by far not so great as that suggested by the results obtained by the clearance technique in the same experiments. At the same time, the so-called renal fraction of cardiac output (RF), as determined on the basis of direct RBF measurement, remains practically unchanged in hypotension, whereas the results of the clearance tests indicate that the kidney is practically ischaemic and renal vasoconstriction has diverted the blood to other organs. In the present series of experiments we have investigated the effect of repeated withdrawal of small volumes of blood on the cardiac output, renal blood flow (RBF), glomerular filtration rate (GFR), as well as on the excretion of Na and water. We have compared the values yielded by the direct determination of RBF with those obtained by the classic clearance technique.

Methods

A total of 11 experiments was carried out on mongrel dogs of both sexes. Preparation for surgery, the determination of the data for cardiac output and renal haemodynamics were carried out exactly as described previously [2]. Urine was collected in 15-minute periods and blood for analysis was taken by the end of each period. Apart from these samples a volume of blood was withdrawn to cause a total blood loss amounting to 0.5 per cent of the body weight. Immediately after blood sampling was the next 15-minute period begun, during which the blood volume was already reduced. (No blood was transfused in these type experiments). At the end of the second 15-minute period again a volume of blood equalling 0.5 per cent of the body weight was withdrawn and this procedure was repeated three times. In the Figures and in the Table, column I. means the control period ; column II. illustrates the changes following a blood loss amounting to 0.5 per cent of the body weight ; columns III., IV., and V., those after losses of blood corresponding to 1.0, 1.5 and 2.0 per cent of the body weight, respectively.

The abbreviations and calculations are exactly the same as those used in our previous report [2].

Results

The data obtained in experiments are presented in Figs. 1 to 7, the means (with s. d.) in Table I. In each figure the single points represent the results of a certain experimental period and the columns the means of these points.

As it is visible in Fig. 1, blood pressure decreased continuously in response to the gradual loss of blood, but its mean was still as high as 90 mm Hg after the loss of 1.5 per cent. In two experiments in period IV., and in two

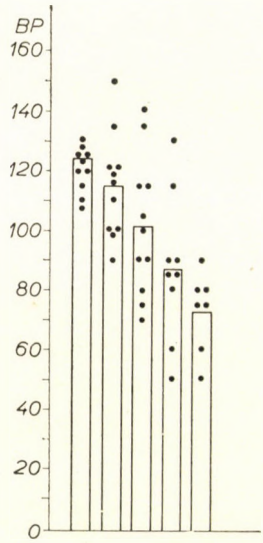

Fig. 1. Changes in arterial pressure in response to repeated bleedings. The single points indicate the single experimental periods, the columns show the means. Column I. : control period. Columns II. to V. : after the loss of blood corresponding to 0.5, 1.0, 1.5 and 2.0 per cent of the body weight, respectively

others in period V., blood pressure was so low that it was unreasonable to continue the experiment. Fig. 2 shows the values of the cardiac output (CO) and reciprocal total peripheral resistance (1/TPR). The cardiac output decreased gradually, while the total peripheral resistance increased slightly.

In Fig. 3 we find the directly measured values of the renal blood flow (RBF_{dir}) on the left side, and those determined on the basis of the PAH clearance and extraction ($RBF_{C/E}$) on the right. It is obvious that the reduction in RBF was much more marked by the clearance technique.

Fig. 4 illustrates the relation of blood pressure to RBF. On the left we present the reciprocal of the renal resistance, as determined on the basis of direct RBF measurement ($1/R_{dir}$). On the right are seen the values computed from $RBF_{C/E}$ ($1/R_{C/E}$). It is clear that despite the continuous decrease in blood volume and blood pressure, renal vascular resistance did not change, *i. e.*

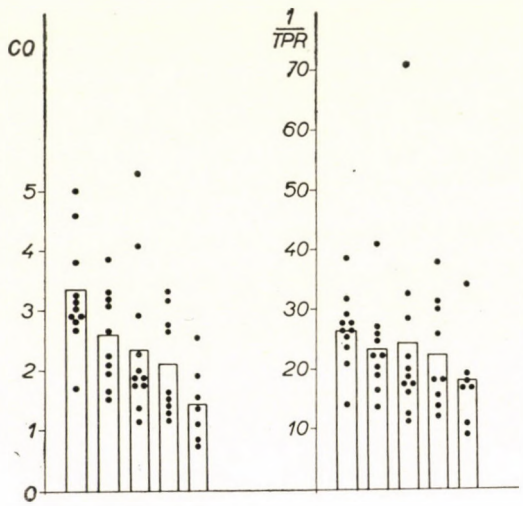

Fig. 2. Cardiac output and reciprocal peripheral resistance in gradual bleeding. Signs as in *Fig. 1*

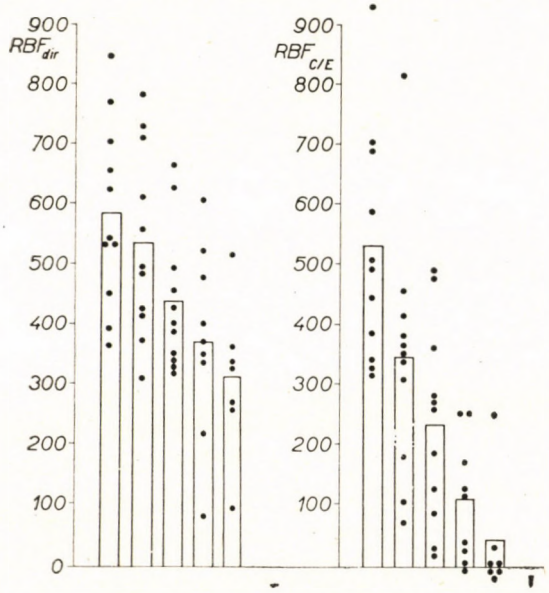

Fig. 3. Renal blood flow (measured directly and computed from PAN clearance and extraction, respectively) in gradual bleeding. Signs as in *Fig. 1*

Table I

Changes in cardiac output and renal function in response to gradual loss of blood

	I	II	III	IV	V
1. Total volume of bleeding, in percentage of body weight	—	0.5	1.0	1.5	2.0
2. Mean blood pressure	124±14	115±17	101±23	87±25	73±14
3. Cardiac output	3.28±0.91	2.58±0.79	2.36±1.27	2.09±0.87	1.42±0.65
4. 1/TPR	26.3±6.2	22.9±7.4	24.0±16.6	22.1±9.1	17.8±8.1
5. RBF_{dir}	584±154	536±157	434±119	372±159	307±128
6. $RBF_{C/E}$	532±224	344±201	233±166	107±100	40±93
7. $1/R_{dir}$	4.76±1.35	4.77±1.53	4.43±1.59	4.40±2.19	4.41±2.03
8. $1/R_{C/E}$	4.35±1.88	3.09±1.87	2.32±1.70	1.09±0.98	0.53±1.24
9. RF_{dir}	19.0±6.4	21.9±8.3	22.2±10.4	19.7±11.9	23.7±11.8
10. $RF_{C/E}$	18.1±9.8	16.3±10.2	10.9±14.9	5.4±6.1	1.9±3.7
11. $RPF \cdot E_{in}$	57±57	47±37	43±32	43±38	32±20
12. C_{in}	49±16	37±17	19±14	8±10	2±6
13. V/min	0.83±0.42	0.49±0.30	0.29±0.25	0.12±0.10	0.03±0.06
14. Na excr./min	108±120	40±40	16±16	6±6	1±3
15. E_{in}	17±10	15±12	14±9	20±13	19±12
16. E_{PAH}	64±10	67±10	62±10	69±13	57±18
17. Number of cases	11	11	11	9	7

the true RBF decreased in exactly the same measure as was expectable on ground of POISEUILLE's law, with unchanged vascular calibre and viscosity, as a result of a drop in blood pressure. The clearance results indicated an excessive increase in renal vascular resistance. In Fig. 5 are shown the values for the renal fraction of cardiac output, on the left as determined from the directly measured RBF (RF_{dir}), and on the right as computed from the RBF shown by the clearance and extraction results. ($RF_{C/E}$). After the above it was only natural that the directly measured, *i. e.* the true renal fraction was unchanged, while the clearance suggested an extreme reduction in the renal fraction.

On the left in Fig. 6 the inulin clearance was computed by multiplying the directly measured renal plasma flow by the inulin extraction rate ($RPF.E_{in}$) whereas on the right the results of the classic clearance tests (C_{in}) are presented. Determination by the direct technique showed a moderate decrease in the inulin clearance, the classic technique suggested a much more marked decrease.

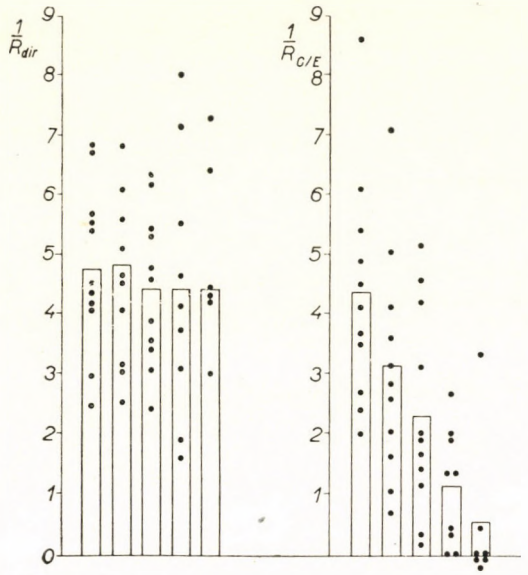

Fig. 4. Reciprocal renal vascular resistance (as determined on grounds of RBF_{dir} and $RBF_{C/E}$ resp.) in gradual bleeding. Signs as in *Fig. 1*

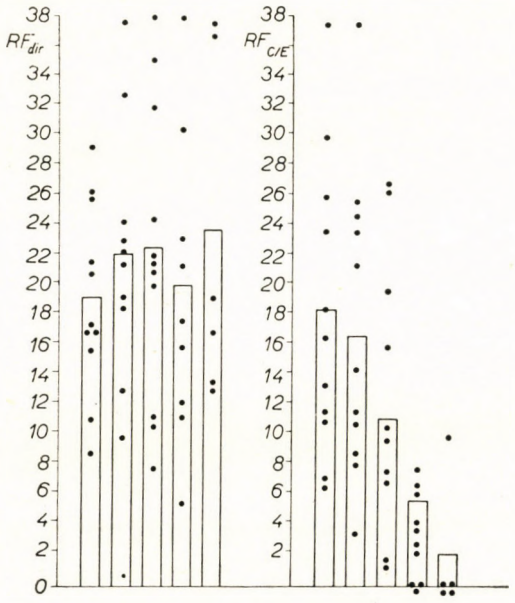

Fig. 5. The renal fraction of cardiac output (on grounds of RBF_{dir} and $RBF_{C/E}$) in gradual bleeding. Signs as in *Fig. 1*

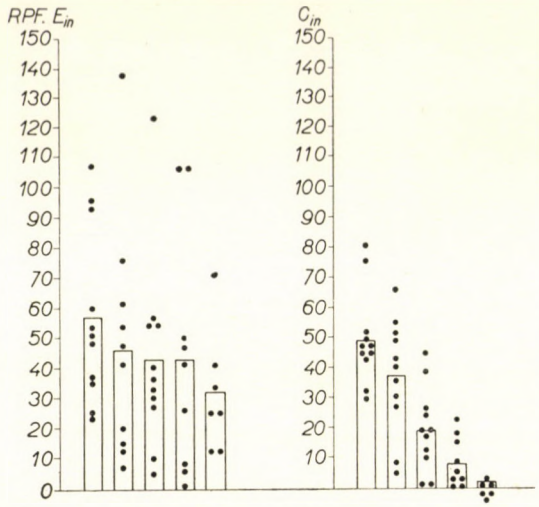

Fig. 6. Glomerular filtration rate (as computed from RBF_{in}, haematocrit and inulin extraction, and from the classic inulin clearance, respectively) in gradual bleeding. Signs as in *Fig. 1*

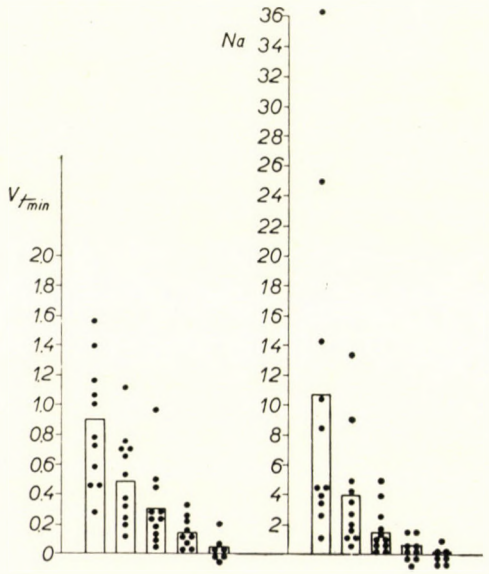

Fig. 7. Diuresis (V/min) and sodium excretion (μeeq/min) in gradual bleeding. Signs as in *Fig. 1*

Finally, Fig. 7 shows the diuresis per minute and the rate of sodium excretion, which roughly corresponds to diuresis.

Table I contains the means for the corresponding columns, together with their dispersion ranges.

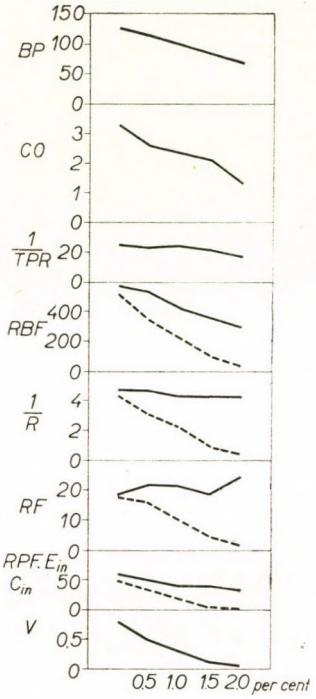

Fig. 8. Changes in cardiac output and renal function in response to gradual bleeding. The curves have been plotted on the basis of the means belonging to the single grades of blood loss

Discussion

As to the evaluation and reliability of the methods employed, we refer to what has been elaborated in the previous report [2].

In column I of Table I there is no difference between the renal blood flow values obtained by direct determination (RBF_{dir}) and those obtained from the clearance and extraction ($RBF_{C/E}$); in column II the difference borders significance and in columns III to V it is highly significant. The same applies to the 1/R, RF, $RPF.E_{in}$ and C_{in} values, determined by the two methods. We wish to emphasize once again that if a discrepancy occurs between the RBF_{dir} and $RBF_{C/E}$ values, it is the former that can be trusted. Accordingly, in experiments of this type no conclusion as to the RBF can be drawn from the clearance, even if extraction, too, is taken into consideration.

The same applies to the GFR. As we have already pointed out [2], the error of E_{in} determination is great, owing to technical difficulties. To this may be ascribed the wide variability of the single values, which forbid everything but a qualitative evaluation. Inulin clearance (filtration, according to the generally accepted view) is still demonstrable, even in cases in which on the basis of clearance the filtration seems to have ceased.

If we consider the diuresis values in Fig 7 and compare them with the values on the right side of Figs. 3 to 6, it will be conspicuous that in the various Figures the points are arranged in a roughly similar manner. In other words, the values computed from the clearance and extraction become immeasurably low in such periods in which diuresis is low. And because in these periods the values yielded by the direct determination of renal blood flow are higher, we see confirmed our earlier view [2] that the low clearance is a technical consequence of oliguria.

It is another question whether the decrease in the absolute values of renal haemodynamics (RBF_{dir}, RPF_{dir}. E_{in}) might not be the cause of the reduction in diuresis. According to certain schools, salt and water diuresis is controlled in a decisive way through the control of the filtered load, and a slight, methodologically almost inaccessible, decrease in filtration may lead to a significant reduction in salt and water diuresis, even with an unchanged tubular sodium reabsorption (SELKURT [16]). If we adhere to this view, we may ascribe the reduction in diuresis to a decrease in both the directly measured RBF and the absolute value of GFR. More recently, however, there is more and more evidence that the tubular factors, too, have a decisive role to play. FARREL, ROSNAGLE and RAUSCHKOLB [9], bleeding dogs through the adrenal vein, found an increase in the aldosterone content of plasma. According to GOODKIND, BALL and DAVIS [11], repeated minor haemorrhages in the dog lead to an increase of aldosterone output, with the clearances unchanged. Similar results have been obtained in man by FINE, MEISELAS and AUERBACH [10], who found that after blood withdrawal the creatinine clearance was unchanged, sodium excretion was reduced and the output of aldosterone increased.

We have found that in the dog repeated, minor haemorrhages induce oliguria. As determined by direct measurement, the decrease in RBF is proportionate to the drop in blood pressure, thus the calculated renal vascular resistance and the renal fraction of cardiac output do not change. Under these experimental conditions the oliguria is not associated with renal vasoconstriction, but, of course, renal blood flow and the absolute value of the glomerular filtration rate decrease. According to our view, it is partly a reduction in haemodynamics and partly an increase in tubular sodium reabsorption which leads to a decrease in sodium excretion, to which oliguria is a secondary sequel.

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UNTERSUCHUNGEN ÜBER DIE FUNKTION DER NEBENNIERENRINDE BEI REGENERATION*

Von

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Die Hypertonie, die an Ratten während der Regeneration der demedullierten Nebenniere auftritt, führt zur Erhöhung nicht nur des systolischen, sondern auch des diastolischen Druckes. Nach initialen Schwankungen nimmt die Corticosteronsekretion ab. Eine erhöhte Ausscheidung anderer Steroide, inklusive Aldosteron, konnte nicht nachgewiesen werden.

*

Skelton [1, 2, 3, 4] beschrieb die Hypertonie von unilateral nephrektomisierten juvenilen Ratten, bei denen eine Nebenniere entfernt und die andere demediullert wurde. Die Hypertonie tritt nur auf falls die Tiere Kochsalzlösung als Trinkflüßigkeit erhalten. Seiner Annahme nach wird diese Hypertonie durch einen Überschuß von Mineralocorticoiden verursacht, die im Laufe der Regeneration der demedullierten Nebenniere produziert werden. Außer dieser theoretischen Folgerung liegt eine ausführliche Analyse dieses Problems noch nicht vor. Zur Klärung dieser Frage haben wir die Corticoidsekretion der regenerierenden Nebenniere untersucht. Diese Versuche, sowie einige neue Angaben über das Verhalten des Blutdruckes unter den obenerwähnten Umständen sind in dieser Arbeit enthalten.

Methodik

Die Versuche wurden an 60—80 g schweren männlichen Wistar-Ratten durchgeführt. Die Operationen erfolgten nach der Methode von Skelton [1, 2]. Als Trinkflüssigkeit erhielten die Tiere physiologische Kochsalzlösung *ad libitum*. Die Wichtigkeit dieses Umstandes wird auch durch einige eigene Erfahrungen unterstützt. Der Blutdruck wurde zum Teil nach dem Verfahren von Kersten [5], in anderen Versuchen nach der von Rózsa, Gáti und Weisz [6] eingeführten Methode gemessen. Wie die Kerstensche Methode, benutzt auch diese letztere das photoelektrische Prinzip, ermöglicht jedoch — durch Einschaltung eines Differenzialverstärkers — eine genauere Erfassung des systolischen Druckes, und ist sogar auch für die unblutige Messung des diastolischen Druckes geeignet. Mit dieser Methode ist es nämlich möglich, die Geschwindigkeit zu messen, mit welcher das Blut in die Extremität nach Ablassen der Abklemmung hineinströmt. Nach den obenerwähnten Autoren wird als diastolischer Druck derjenige niedrigste Manschettendruck betrachtet, unterhalb dessen sich die Füllungsgeschwindigkeit schon nicht mehr erhöht.

* Ein Teil dieser Arbeit, der sich auf die Corticosteron- und Aldosteron-Analyse des venösen Nebennierenblutes bezieht, wurde am Kongreß der Ungarischen Physiologischen Gesellschaft (4—6. Juli 1958) vorgetragen.

Bei einem Teil der Tiere haben wir auch histologische Untersuchungen durchgeführt. Zwecks histochemischer Auswertung wurden die Nebennieren mit Sudan gefärbt, und für Nachweis doppelbrechenden Fettes unter Polarisationsoptik ungefärbt untersucht.

Zur Prüfung der Hormonausscheidung der Nebennierenrinde wurde nach VOGT [7] aus der V. renalis Blut entnommen, und die Corticosteroide mit der Methode von BUSH [8] bestimmt. Dieses Verfahren besteht aus Extraktion mit Aethylazetat, Reinigung und nachfolgender Chromatographie in Toluol : Methanol : Wasser (4 : 3 : 1). Das Chromatogramm wurde mit Tetrazoliumblau entwickelt und die Flecke mit Hilfe von Standardpräparaten identifiziert. Nach Eluierung wurde die quantitative Bestimmung einzelner Komponenten photometrisch durchgeführt.

Die fluorometrischen Untersuchungen erfolgten nach der Methode von SWEAT [9].

Ergebnisse

Da über den diastolischen Blutdruck von Ratten mit SKELTONscher Hypertonie keine Literaturangabe vorliegt, haben wir auch diese Frage untersucht. Abb. 1 zeigt die nach der Methode von RÓZSA, GÁTI und WEISZ [6] erhaltenen systolischen und diastolischen Druckwerte einiger Tiere.

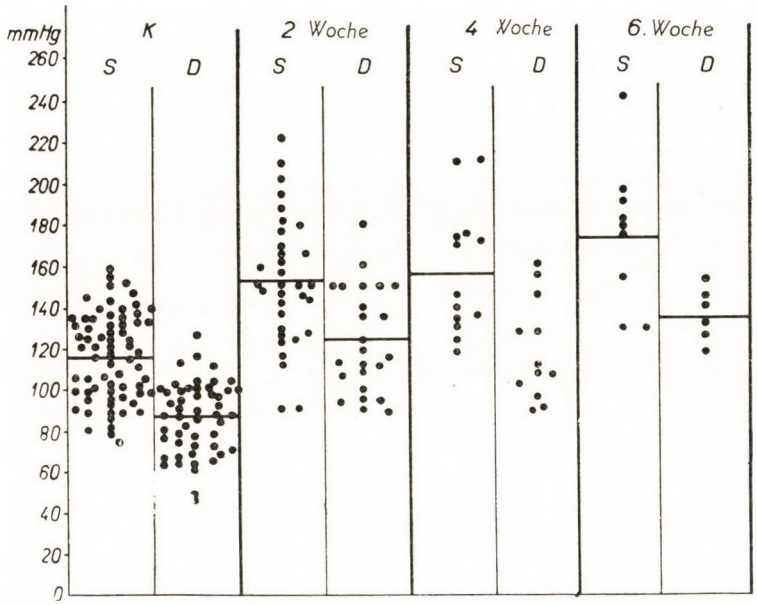


Abb. 1. Systolischer und diastolischer Druck bei Kontrollratten (*K*) und bei operierten Tieren in verschiedenen Zeitabständen nach der Operation. Sämtliche Gruppen zeigen eine signifikante Differenz zu den Kontrollen ($P = 0{,}01$). Bei zwei Ratten, die in der zweiten Woche einen niedrigen systolischen Blutdruck aufwiesen, konnte der diastolische Druck aus technischen Gründen nicht gemessen werden, was den Durchschnitt offensichtlich nach oben verschieb

Wie aus der Abbildung hervorgeht, liegen die systolischen Blutdruckwerte schon zwei Wochen nach der Operation signifikant höher als die der Kontrollen. Am Ende der sechsten Woche erhöht sich der Blutdruck noch wei-

ter. Auffällig ist die Tatsache, daß die Tiere in ihrem Verhalten nicht völlig miteinander übereinstimmen, und obwohl der Blutdruck im Durchschnitt höher liegt, als der der Kontrollen, finden wir auch nach sechs Wochen noch Tiere mit normalem Blutdruck. Auf ähnliche Beobachtungen können wir auch aus den Bemerkungen von GROSS [10] folgern. Weiterhin zeigt Abb. 1, daß sich auch der diastolische Druck in signifikanter Weise erhöht. Im allgemeinen ist es zu bemerken, daß in allen solchen Fällen, wenn ein Anstieg des systolischen Drucks gefunden wurde, auch der diastolische Druck höher als bei den Kontrollen lag.

Bei den operierten Tieren haben wir auch die histologischen Kontrollen routinemässig durchgeführt, deren Ergebnisse mit den Angaben von SKELTON [3] übereinstimmten. In einigen Fällen wurden die Nebennieren ausser Sudanfärbung auch unter Polarisationsmikroskop untersucht.

Nach den histologischen Ergebnissen beschränkt sich der fetthaltige, funktionierende Teil in den ersten Tagen nach der Operation auf die Zona Glomerulosa. Andere Teile der Nebenniere sind zu diesem Zeitpunkt mit Blut und nekrotischen Gewebsresten ausgefüllt. Später tritt eine Regeneration ein, die etwa vier Wochen nach der Operation dauert.

Die Steroidsekretion der Nebennierenrinde wurde durch Analyse des in einer halben Stunde gesammelten venösen Blutes verfolgt. Es sollen hier haupt-

Tabelle I

Versuch N°	Versuchsgruppe	Zahl der Tiere	Corticosteron µg/kg/Stunde	*P*-Wert
1.	Kontrollen A	10	40,5±4,7	0,05
	1 Tag nach der Operation	8	26,6±4,05	
2.	Kontrollen A	10	40,5±4,7	
	Kontrollen B	12	47,0±3,12	
	3—6 Tage nach der Operation	18	41,2±3,2	
3.	Kontrollen A	10	40,5±4,7	<0,05
	Kontrollen B	9	53,5±3,24	
	13—25 Tage nach der Operation	14	30,2±2,8	<0,01
4.	Kontrollen A	16	26,9±1,08	<0,01
	90 Tage nach der Operation	8	17,3±2,4	

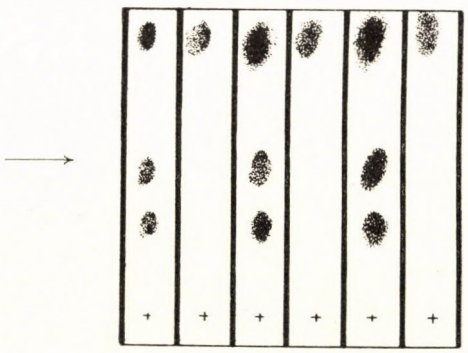
A. Kontrollen: unoperierte Tiere gleichen Gewichts wie die operierten Ratten
B. Kontrollen: rechtsseitige Nieren- und Nebennierenentfernung ohne Demedullierung, mit Kochsalz als Trinkflüssigkeit. Operation zu gleicher Zeit wie bei den demedullierten Tieren Im Versuch No. 4 schien die Untersuchung von Kontrollgruppe *B.* unnötig, da die Differenz auch zu den unoperierten Tieren signifikant war.
Die *P*-Werte wurden nur angegeben, falls die Abweichung signifikant war.
Aus der Tabelle kann entnommen werden, daß bei älteren Tieren die Corticosteronausscheidung niedriger liegt, als bei den jüngeren.

sächlich die Befunde über Corticosteron, das Haupthormon der Rattennebenniere, hervorgehoben werden.

Tabelle I zeigt eindeutig, daß die Corticosteronausscheidung nach einer überraschend leichten initialen Abnahme rasch regeneriert wird, so daß sie am 3—6 Tage schon normale Werte aufweist. Später kann eine neue Abnahme der Corticosteronsekretion beobachtet werden.

Obwohl das von uns verwendete Entwicklungsverfahren mit Tetrazoliumblau eine der empfindlichsten Methoden darstellt, kann es die quantitative Ermittlung des bei den Ratten nur in relativ kleinen Mengen sezernierten Hydrocortisons nicht ermöglichen. Doch ist es wahrscheinlich, teils auch auf Grund der später zu erwähnenden Versuche, daß eine Zunahme in der Hydrocortisonsekretion nicht auftritt.

Die Aldosteronausscheidung bleibt ebenfalls unverändert. Nach SINGER [11] liegt der dem Aldosteron entsprechende Fleck auch in dem von uns ver-



Mikrophotogramm 1. Reproduktion eines Chromatogrammes. In Richtung des Pfeiles zu lesen.

Säule 1. Hydrocortison, Cortison und Corticosteron (Standardpräparate)
Säule 2. Stark verminderte Corticosteronausscheidung in einem demedullierten Tier
Säule 3. Standardpräparate
Säule 4. Mäßig verminderte Corticosteronausscheidung in einem demedullierten Tier
Säule 5. Standardpräparate
Säule 6. Kontrolltier

wendeten System zwischen Hydrocortison und Cortison. Doch haben wir an diesem Gebiet keine meßbare Menge von Steroiden gefunden, auch wenn Eluate aus fünf Tieren vereinigt untersucht wurden, um Hormonmengen zu erfassen, die mit dem Auge vielleicht nicht entdeckt werden können. Eine fluorometrische Methode für die Mikrobestimmung von Steroiden wurde von SWEAT [9, 12] empfohlen. Wir versuchten auch mit diesem Verfahren, eine eventuelle geringgradige Aldosteronvermehrung nachzuweisen. Dazu wurde das zur Chromatographie benutzte Filterpapier (Whatman No. 1) in Soxleth-Apparat mit Ethanol extrahiert und nur dann verwendet. Das Gebiet zwischen

Hydrocortison und Cortison wurde ohne Entwickeln eluiert, das Eluat rechromatographiert, und das dem Aldosteron entsprechende Gebiet wieder eluiert. In den so erhaltenen Extrakten wurde dann die alkoholisch-schwefelsäurige fluorometrische Reaktion ausgelöst. Obwohl eine minimale Menge von Steroiden in dieser Weise nachgewiesen werden konnte, haben wir zwischen den Kontrollen und operierten Tieren keine Differenz gefunden. (In beiden Gruppen lag die Menge der Steroide unter 0,01 μg.)

Zur Klärung weiterer Möglichkeiten haben wir eine weitere Versuchsserie durchgeführt. Wir versuchten nämlich die mit Tetrazoliumblau nicht erfaßbaren $\triangle$-4, 3-Ketosteroide und 17-Ketosteroide nachzuweisen. Zu diesem Zwecke diente die von BUSH [13] eingeführte Reaktion mit NaOH und Dinitrobenzol. Die durch NaOH hervorgerufene sog. »Soda-Fluorescence« ist an die $\triangle$-4, 3-Ketogruppe, während die Dinitrobenzolreaktion an die 17-Ketosteroiden geknüpft. Zur Ausführung dieser Versuche wurden 10 Ratten nach und 9 während der beendeten Nebennierenregeneration entblutet. Das vereinigte Blut wurde extrahiert und chromatographiert. Damit wollten wir auch kleinste Hormonkonzentrationen nachweisen. Auch Kontrolltiere wurden natürlich in ausreichender Zahl parallel untersucht. Doch gelang es uns auch mit diesem Verfahren nicht, einen, in den Kontrollen nicht vorhandenen neuen Stoff nachzuweisen.

Ein mit Tetrazoliumblau entwickeltes Chromatogramm einiger demedullierten Tiere ist im Mikrophotogramm 1 wiedergegeben.

Besprechung der Ergebnisse

Unseren Befunden über das Verhalten des Blutdruckes sind keine neuen Anhaltspunkte zu entnehmen. Aus Literaturangaben ist nämlich bekannt [14, 15], daß der erhöhte systolische Druck in verschiedenen Formen der experimentellen Hypertonien mit einer Erhöhung des diastolischen Druckes einhergeht. Unsere Angaben lassen darauf schließen, daß auch die SKELTONsche Hypertonie von demselben Charakter ist.

Unsere histologischen Befunde unterstützen in jeder Hinsicht die Ergebnisse von SKELTON [3]. Da wegen des Blutergusses, der sich einige Tage nach der Operation in der Nebenniere befindet, die Messung des Drüsengewichtes irreal ist, wurden die Steroidwerte ausnahmslos auf Körpergewicht bezogen.

Die Ergebnisse unserer Steroidanalysen sind aus mehreren Gesichtspunkten neuartig und überraschend. Auffallend ist die relativ geringgradige initiale Abnahme der Corticosteronsekretion, sowie ihre nachfolgende Zunahme, die auch den normalen Kontrollwert erreicht, und sich sogar von den Werten derjenigen Tiere nicht signifikant unterscheidet, die sich — infolge einer einseitigen Nieren- und Nebennierenentfernung — im Zustand eine-

kompensatorischen Hyperfunktion befinden. Im Zusammenhang mit dieser Frage sollen noch die Beobachtungen von AYRES [16] erwähnt werden. Dieser Verfasser konnte nämlich in *in vitro* Versuchen nachweisen, daß die Zona Glomerulosa, welche durch die Demedullierung entweder nicht oder nur wenig geschädigt wird, eine wichtige Rolle in der Corticosteronsekretion spielt. Hier ist noch zu bemerken, daß die Technik der Operation außerordentlich einfach ist, und daß sich keine bedeutenden Abweichungen in der Ausführung ergeben können. Dasselbe Problem besteht auch bezüglich der Streuung der Blutdruckwerte. Was das Verhalten der Corticosteronausscheidung in späterem Zeitpunkt betrifft, stimmen unsere Ergebnisse mit den Angaben von HOLZBAUER und VOGT [17] völlig überein. Diese Verfasser untersuchten die Corticosteronsekretion nach der vollständigen Regeneration (andere Steroide wurden nicht untersucht) und fanden eine Verminderung der Hormonsekretion, auf Körpergewicht umgerechnet. Unsere Beobachtung stimmt auch mit den Ergebnissen von MASSON [18] überein, der in einer kurzen Bemerkung zu seiner neuesten Mitteilung gleichfalls über eine herabgesetzte Corticosteronsekretion berichtete. Aus MASSONS Angaben geht nicht klar hervor, in welchem Stadium der Regeneration die Untersuchungen durchgeführt wurden, doch deuten seine *in vitro* Versuche auf das Spätstadium der Regeneration. Über andere Steroide hat auch MASSON keine Angaben mitgeteilt, und auch das von ihm verwendete chromatographische System war hauptsächlich für die Analyse weniger polarer Steroide (wie z. B. Corticosteron) geeignet. Die Ergebnisse unserer Corticosteronbestimmungen widersprechen aber sowohl den alten Befunden von INGLE [19], der eine erhöhte Glykocorticoidaktivität gefunden hatte, wie auch den neuen Ergebnissen von GIRAUD [20], wonach die Corticosteronsekretion nach der Operation abnimmt, und dann — etwa parallel mit der Regeneration — am Ende der vierten Woche wieder normalisiert wird.

Das Ergebnis unserer Untersuchungen über Aldosteron bzw. eventuelle andere Steroide scheint von besonderem Interesse zu sein. Es deutet nämlich darauf hin, daß keine der bekannten Corticosteroidtypen während der Regeneration vermehrt wird, und so keine für die Hypertonie verantwortlich gemacht werden kann. Dies wurde auch durch das folgende Experiment unterstützt: Drei Wochen nach der Operation wurde fünf nach SKELTON operierten Tieren venöses Nebennierenblut entnommen und in der übliche Weise extrahiert. Der Extrakt — in 20%-igem Aethanol gelöst — wurde Normalratten intravenös gegeben. Während einstündiger Beobachtungszeit konnten wir nur einen sehr leichten Blutdruckanstieg und zwar nur in einem einzigen Fall beobachten. Dieser Versuch schließt zwar die Rolle der Steroide in der Entstehung der Hypertonie nicht völlig aus, macht aber diese sehr unwahrscheinlich, besonders wenn auch die obenerwähnten Angaben über die Steroidsekretion in Betracht gezogen werden. So sind wir der Meinung, daß in dem

Bestreben, die Ursache der SKELTONschen Hypertonie zu klären, in die Untersuchungen noch andere Faktoren einbezogen werden müssen.

*

Für die freundliche Überlassung von den Standardpräparaten Cortison, Hydrocortison und Corticosteron danken wir der Firma *Ciba*, und für die von Aldosteron der Firma *Organon*.

*

Bemerkung. Nach der Zusammenstellung dieser Mitteilung wurde uns bekannt, daß PELLEGRINO am Kongreß der Internat. Biochem. Ges. im Sept. 1958 — also nach unserem Vortrag am Kongreß der Ungarischen Physiologischen Gesellschaft — Beobachtungen über die Steroidsekretion der regenerierenden Nebenniere veröffentlicht hatte. Seine Beobachtungen stimmen mit unseren Angaben in mancher Hinsicht überein.

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COMPENSATORY FUNCTION OF THE AFFERENT SYSTEM OF THE RENAL PELVIS

By

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In interoceptive renal pelvic conditioned reflex experiments on dogs it has been demonstrated that after unilateral experimental hydronephrosis, or after unilateral nephrectomy the contralateral renal pelvis increases its afferentative activity. The interoceptive system of the unaffected kidney compensates in this way the reduction in afferent impulsation resulting from the loss of a receptor area.

*

The clinical observation that injury of one kidney is followed by the compensatory activity of the other kidney, gave rise to our experimental investigations. In these we have shown [1] that an excitatory or inhibitory process starting from the pelvis of one kidney would spread also to the symmetrical cortical area of the other kidney. We have also demonstrated that the higher nervous centres can analyse and distinguish between the afferent impulses coming from the two kidneys.

We have continued the experiments and now we wish to discuss the response given by the afferent system of one kidney to damage or removal of the other. In other words, is there a demonstrable compensatory activity in the symmetrical interoceptive systems?

Methods

Female dogs with left ureteral fistula and salivary fistula were studied, in a conditioned reflex cage, under chronic conditions.

In the first step a left renal pelvic interoceptive alimentary conditioned reflex was elaborated by our method [2]. Rhythmic dilatation of the left pelvis by a special device served as the conditioning stimulus and the alimentary response served as the conditioned reflex reinforcement. The rate of saliva excretion was expressed in Hanicke—Kupalov units.

To control the interoceptive signal function, the conditioned alimentary reflex was elaborated also to an exteroceptive stimulus sound. In each experiment we employed two interoceptive and two exteroceptive stimuli, in stereotype form. The number of experiments made on 6 dogs was 310.

After the conditioned reflex stereotype had been established, right hydronephrosis was induced experimentally in 3 animals, by ligating the right pelvis about 8 cm below the pelvo-ureteral junction. In the other 3 dogs the right kidney was removed after the reflex had been firmly established. Subsequently, the course of the extero- and interoceptive reflexes was analysed for several weeks.

Results

Left renal pelvic conditioned reflex response to hydronephrosis of the right kidney

The results are presented in Fig. 1, from which it is clear that following the ligation of the right pyelo-ureteral junction the values of the conditioned

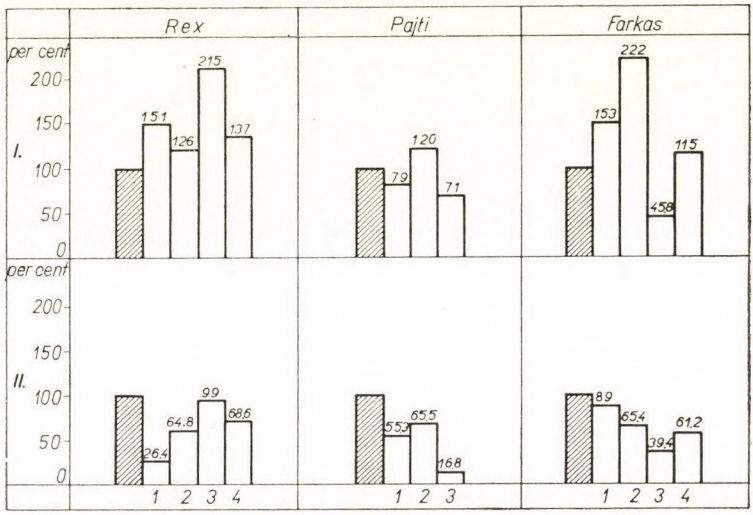

Fig. 1. Conditioned alimentary reflex responses with hydronephrosis of the right kidney
I. : Conditioned reflex responses elicited from the left renal pelvis
II. : Conditioned reflex responses elaborated to sound. Ordinata : Conditioned reflex response, expressed in per cents.
Abscissa : Number of weeks during experiment.
Shaded column : Mean of 10 experiments preceding hydronephrosis.
Empty columns : Results obtained after surgery. Each column represents the mean of the weekly results, in percentage of the reflex values obtained before operation

reflexes elicited by the stimulation of the receptors of the left renal pelvis were increased. The increase reached the maximum two to three weeks after operation ; in two dogs the values were more than double the original. Subsequently, the values decreased. At the same time, the values of the conditioned reflex elicited from the auditory receptors did not change substantially after operation, or showed a diminishing tendency. The same rule is visible in Fig. 2, showing the reflex curves for one of the dogs.

Left renal pelvic conditioned reflex response following right nephrectomy

The results are illustrated in Fig. 3. Following the removal of the right kidney, the conditioned reflex transmitted by the left renal afferent pathways were increased, reaching the maximum in 3 to 5 weeks, when the values were

nearly doubled in 2 dogs. Meanwhile, the conditioned reflex response elaborated to sound showed no substantial change. Dog *Cigány* (the third in this

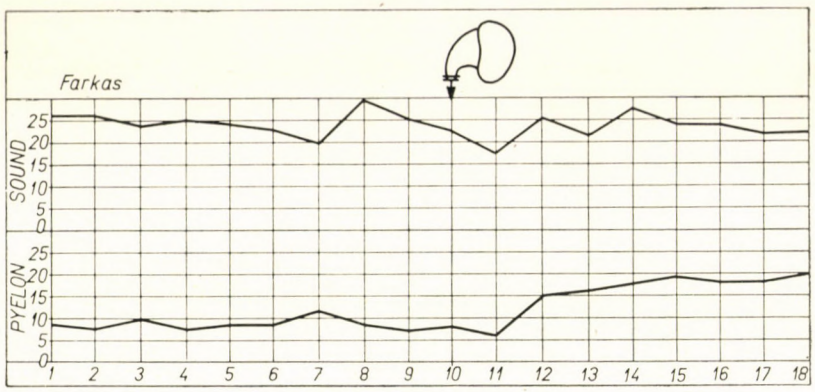

Fig. 2. Conditioned alimentary reflex responses with hydronephrosis of the right kidney, in dog "*Farkas*"

Ordinata : conditioned salivary response, in HANICKE—KUPALOV units
Abscissa : number of experiments

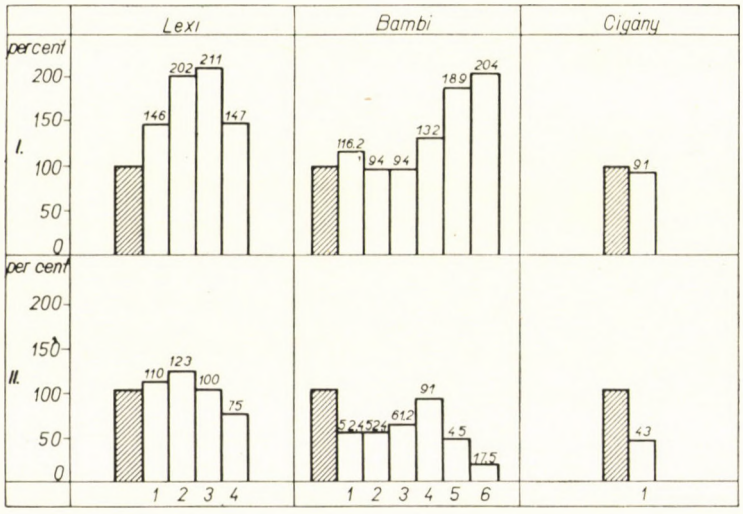

Fig. 3. Conditioned alimentary reflex responses following removal of the right kidney. Signs as in *Fig. 1*

series) was lost 3 weeks after operation (not as a result of the experimentation), and in this animal we could not follow up the increase in the values of the pelvic reflex. During the two weeks following nephrectomy the sound reflexes decreased by about 50 per cent, whereas the conditioned reflex response

elicited by the stimulation of the renal pelvis reached the pre-operative level. Thus, the results for the dog *Cigány* support the evidence obtained in the other dogs.

Discussion

The evidence found in the literature concerning the afferent innervation of the renal pelvis is chiefly morphological. By using the method of interoceptive conditioned reflex [2, 3], we have elucidated the higher nervous connections of the renal pelvis and ureters and described the laws of this renal afferentation. Also, we have demonstrated a close correlation between the symmetrical cortical projections of the afferent systems [1]. The problem then arose, how does the afferent system of one kidney respond to the injury of the other organ. Experimental hydronephrosis, studied in detail by BABICS and RÉNYI-VÁMOS [4], seemed to be eminently suitable for injuring the renal pelvis. As our results indicate, the conditioned salivary reflex elicited from the left renal pelvis was greatly increased following operation on the right kidney. The control reflex elaborated to sound having not changed, it is clear that the unconditioned alimentary reflex centre had not suffered. It is to be surmised, therefore, that after the experimental operation the afferent impulsation from the left renal pelvis did increase for some reason.

Two explanations offer themselves.

a) The affected kidney is continuously emitting pathological impulses to the higher centres, wherein they create a condition similar to VEDENSKY's hysteriosis [5]. Thereby the "threshold" of the afferent nervous centres is lowered, and this would explain the increase in interoceptive impulsation.

b) The increase in the impulsation running upward from one kidney in the presence of hydronephrosis in the contralateral organ may be explained also by some kind of a "deafferentation" in the hydronephrotic kidney, resulting from a degeneration of tissues and destruction of receptor elements caused by the compression lasting for days and weeks. The organism compensates the loss of an extensive receptor area by intensifying the activity of the still intact renal receptor system.

To decide this problem were the above nephrectomy experiments carried out. If hysteriosis caused by the pathological impulses were responsible for the increased interoceptive reflex activity, no increase in the pelvic reflex activity would result after nephrectomy. But if in the case of hydronephrosis the contralateral kidney increases its activity to compensate for the loss of the receptor area, the afferent impulses will increase even in the nephrectomy experiments.

The nephrectomy experiments confirmed the latter view. As it has been seen, after unilateral hydronephrosis and after unilateral nephrectomy alike,

the contralateral renal pelvis increased its afferent activity. It is thereby that the symmetrical interoceptive system compensates for the decrease of impulsation resulting from a loss of part of the receptor area.

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STUDY OF THE GROWTH-INHIBITING EFFECT OF CERTAIN THIOPROPIONIC ACID DERIVATIVES IN HELA CELLS

By

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Thiopropionic acid, α(S-methyl) propionic acid Na, α(S-ethyl) propionic acid Na, and α(S-propil) propionic acid were found to inhibit the growth of HeLa cells. The inhibitory effect increased with the increase of concentration. The inhibition was partially prevented with methionine and glutamic acid; cysteine, asparagine and serine were effective.

*

Experiments performed in this Institute on bacterial cultures and rats had shown α-thiopropionic acid to be an effective methionine, cysteine and glutaminic acid antagonist [10, 11]. It seemed therefore worth while examining whether certain simple thioethers derivable from α-thiopropionic acid antagonizing some amino acids.

In the presumption that the substances will antagonize S-containing amino acids, a HeLa cell culture, sensitive to the absence of these amino acids, was chosen as the test object [2]. For comparison, two methionine antagonists were used, methionine sulphoximine (MeSOI) and ethionine, both well-known in the literature [4, 16, 5, 6], but neither of them tested in this respect.

Materials

MeSOI was prepared according to Bentley *et al* [1]. Its melting point, 210—216 (decomp.) agreed well with that found in the literature (214—218, decomp.).

Thiopropionic acid (TP) and its derivatives were prepared as usual with aliphatic thiols and thioethers [14]. TP was the starting point in each case; it was brought into reaction with a calculated amount of Na-hydroxide, with an equivalent amount of alkyl halogenide and halogenized carbonic acid, respectively.

TP disulphide was prepared as follows. Oxygen was streamed through a solution of the corresponding thiol in hot water until the substance had ceased to react with alcaline nitroprussic Na.

The end products were always isolated in the form of their Na salt, and several times recrystallized from alcohol. The identity of the substances was controlled by S determination.

TP disulphide diNa salt, computed S content: 25.20%, obtained S content: 25.41%. α (S-methyl) propionic acid Na (SMe), computed S content: 23.20%, obtained S content 22.91% α (S-ethyl) propionic acid Na (SEt), computed S content: 20.51%, obtained S content: 20.78%. α (S-propyl) propionic acid Na (SPr), computed S content: 18.85%, obtained S content: 18.92%. α (S—CH_2.COONa) propionic acid Na, computed S content: 15.36%, obtained S content: 15.51%. α (S—$C_3H_4O_2Na$) propionic acid Na, computed S content: 14.41%, obtained S content: 14.33%.

Methods

The "HeLa" tissue culture (human epidermoid carcinoma cells) was maintained as described elsewhere [7]. The cultures were grown in 1 or 2 litre Roux flasks. The nutrient fluid consisted of 40 per cent human serum and 60 per cent Hanks' solution. The protein content of the human serum mixture added to the nutrient fluid was invariably the same. After the development of a suitable, one-layer cell culture in the flasks, cultures were prepared in test tubes with the usual trypsinizing procedure, so that each tube contain 60 000 cells in each ml of the nutrient fluid.

The tested substances were dissolved in 0.1—0.2 ml of sterile distilled water and added to the 3-day culture which contained 1 ml of fresh nutrient fluid. To the control tubes was added the same amount of sterile physiological saline solution. After further 3 days' incubation the nutrient fluid was poured out and the cells adhering to the wall of the tube were washed 3 times with physiological saline whereafter they were collected from the tube wall and subjected to LOWRY's [6] protein determination. This method makes it possible to discard the degenerated, necrosed cells, as they do not adhere to the tube wall and are removed together with the nutrient fluid. The effect of the test substances was estimated from the multiplication of the cells and by the number of living cells, respectively. The cellular mass is well characterized by the total protein content of the culture [15]. This method was therefore applied, instead of the time consuming and less exact method of making cell counts. Crystallized human albumin was used as the standard.

As against LOWRY's original description, the KNa-tartarate was not dissolved in a mixture of Na_2CO_3—NaOH, but a separate standard solution was prepared of it which was mixed with a $CuSO_4$ solution before use. (2% KNa-tartarate + 1% $CuSO_4$, 1 : 1). The solution prepared according to the original description was not stable and was precipitated after 1 or 2 days' standing.

Experimental

When adding the amino acids to the test substances, the methionine and cystine content of the nutrient fluid was also taken into consideration. This was $2.8 \cdot 10^{-3}$ *M* for methionine and $1.16 \cdot 10^{-2}$ *M* for cystine, as computed on the basis of EDSDALL's data [3].

In our first experiment we studied in what concentration the known methionine antagonists, namely MeSOI and ethionine would bring about a definite inhibition of growth. The control test tubes contained 0.2 ml of physiological saline and were incubated together with the experimental tubes. The average protein content of the controls was taken as 100 per cent and the results obtained for the test substances were compared with this value (Fig. 1).

The effective concentrations were usually high. This was ascribed to the supplementing effect of the (protein-bound) methionine of the nutrient fluid. There was a notable difference in effect between the two methionine antagonists.

TP inhibited also in this system. The curve of inhibition ran close to that of MeSOI.

In the next experiments, we added the examined thioethers in an increasing concentration to the cultures (Fig. 1).

In the case of SMe, SEt and Spr, a parallelism between the length of the alkylizing chain and the strength of action was observed. At a lower

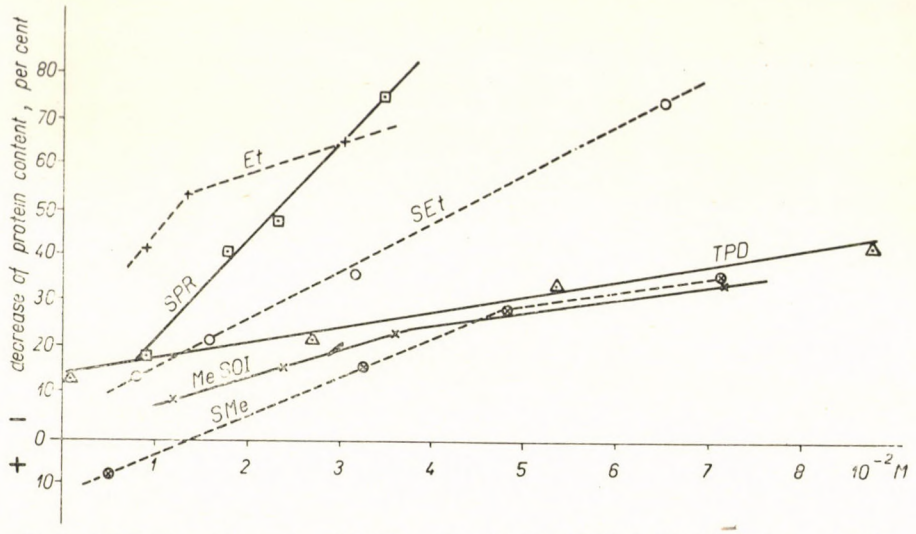

Fig. 1. Inhibition of growths of HeLa cells with Et, MeSOI, TP, SMe, SEt and SPr.

The values show the percentual decrease of protein as compared to the control tubes. At the $0.5 \cdot 10^{-2}$ *M* concentration of SMe, the value below the abscissa means increase of protein content. Abbreviations: Et: ethionine, SPr: α (*S*-propyl) propionic acid Na, SEt: α (S-ethyl) propionic acid Na, TPD: α thiopropionic acid-disulphide-Na, MeSOI: methionine-sulphoximine, SMe: α (S-methyl) propionic acid Na.

Table I

The growth-inhibitory effect of (S—CH_2COONa) propionic acid and of (S—$(CH_2)_2$COONa) propionic acid in HeLa cells

No. of experiment	Concentration of substance $n \cdot 10^{-2}$ *M*	Substance	Inhibition per cent
14	2	(S—CH_2—COONa) prop.	4
16	4		+2
15	8		7
12	2	(S—$(CH_2)_2$COONa) prop.	0
15	4		3
16	8		8

concentration, SMe promoted growth, whereas definite inhibition was manifest with SEt and SPr, even at lower concentrations.

The two substances of the dicarbonic acid type were ineffective even at high concentration (Table I).

Next, we attempted to prevent the inhibition of growth by the addition of different amino acids (Table II).

Table II

Preventability of the growth-inhibiting effect of Et, MeSOI, TP, SMe, SEt and SPr with amino acids

No. of experiment	Antimetabolite conc. $n \cdot 10^{-2} M$		Amino acid conc. $n \cdot 10^{-2} M$	Protein μg	Protein %	Preventability of inhibition*
			methionine			
14	—			127	100	—
18	TPD/2	2.67		112	88	45
11	SMe	4.8		118	90	76
12	—			142	100	—
9	SEt	0.8		137	97	21
16		1.63	3.07	91	64	0
9	—			125	100	—
11	SPr	0.3		106	85	16
14		1.8		55	44	0
8	—			120	100	—
12	MeSOI	0.6		108	90	56
14	Etio	0.92		98	82	66
			cysteine			
12	—			111	100	—
16	TPD/2	2.67		70	63	0
14	SMe	4.8	3.22	83	75	14
10	—			152	100	—
20	SEt	1.63		80	53	0
14	SPr	1.8		64	43	0
			glutamine			
20	—			132	100	—
14	TPD/2	2.67		125	95	77
12	SMe	4.8		156	118	147
14	—		6.2	145	100	—
18	SEt	1.63		137	95	76
16	SPr	1.80		85	59	0

* Computed on the basis of the data in Fig. 1.

Methionine in the concentration of $3.07 \cdot 10^{-2} M$ successfully prevented the inhibitory effect of TP and SMe, even at high concentrations of the antimetabolites, but only 3 to 4 times this amount was capable of preventing inhibition by SEt and SPr.

As regards the preventability of their inhibitory effect, TP and SMe behaved like MeSOI, whereas SEt and SPr like ethionine. Cysteine was effective only in preventing SMe by inhibition.

In our former experiments [10, 11], performed in bacterial cultures, cysteine had proved most effective in preventing inhibition by TP. This result could not be reproduced in the present tests ; moreover, in several experiments the inhibition of growth became significantly more pronounced after the addition of cysteine. This might have been due to an eventually ensuing imbalance of amino acids. The same phenomenon was shown by SEt and SPr at a higher ($11.6 \cdot 10^{-2}$ *M*) concentration, after the addition of methionine.

Glutamine significantly (147 per cent) increased growth in the presence of $4.8 \cdot 10^{-2}$ *M* SMe. It prevented the inhibitory effect of TP, while that of SEt only at 3 to 4 times that concentration and that of SPr not even at a 4 times higher concentration.

Serine, glycine and asparagine failed to prevent the inhibitory action of either of the substances.

Discussion

The 3 thioethers examined inhibited the growth of HeLa cells in a similar degree and at a similar concentration as did MeSOI and ethionine and, as regards the preventability of inhibition, they also showed the same behaviour.

These analogies, however, do not yet prove the identity of their mechanism of action with that of MeSOI and ethionine.

The mechanism of growth inhibition may at best be anticipated from the chemical structure of the substances and from the analogy — demonstrated in the experiments — with the two antimetabolites of well-known mechanism of action.

The growth-inhibitory effect of ethionine may be due to two factors. According to RABINOVITZ' experiments performed in ascites tumour cells [8], this substance is being built into proteins and some unnatural proteins are thus formed ; the inhibition exerted by ethionine were therefore of a competitive character. This does not seem to be the case with the substances under discussion, since they are not compounds of the amino acid type, possessing only a carboxyl group, so that they could be built into proteins at best as end groups.

The other way of action of ethionine [12, 13] is the forcing out of methionine from physiological methylizing processes. This leads to the formation of unnatural compounds which bring about different kinds of injury in experiments *in vivo*. This might be the mechanism of action of SEt and SPr, too.

At a low concentration SMe is stimulating growth, a fact suggesting that the HeLa cell is able to utilize the substance in its normal metabolism. There is a theoretical possibility for its being a methyl donator as, on the basis of its structure, its $-CH_3$ group may be considered as labile. This may be the case

also with SEt and SPr, so that one may assume that their inhibitory effect, similarly as that of ethionine, is based on their entrance into the physiological methylizing processes. We wish to examine this possibility in experiments *in vitro*.

The inhibitory effect of MeSOI is not competitive, but is probably based on the inhibition of glutamine synthesis [9] and is well-prevented by the administration of glutamine. Considering that glutamine was effective in preventing the growth-inhibitory action of our substances as well, such a mechanism of action is — *per analogiam* — also possible.

The action of our substances was found to be correlated with the length and nature of the alkylizing chain. The thioethers of acetic acid and propionic acid were ineffective. Thus, if the alkylizing chain was a carbonic acid, ineffective derivatives had originated from the effective basic substances.

If the alkylizing process was performed with aliphatic radicals, effective compounds were obtained, the inhibitory capacity of which increased with the length of the alkyl chain.

Thiopropionic acid, (S-methyl) propionic acid Na, α (S-ethyl) propionic acid Na, and α (S-propyl) propionic acid Na were found to inhibit the growth of HeLa cells. The inhibitory effect increased with the increase of concentration. The inhibition was partially prevented with methionine and glutamic acid ; cysteine, glycine, asparagine and serine were ineffective.

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Recensio

E. ERNST: *Die Muskeltätigkeit*

Versuch einer Biophysik des quergestreiften Muskels

Academic Press, Budapest 1958. Pp. 355, Figs. 204, Price

This monograph, summarizing the results of almost four decades of the author's research in this field, is, perhaps with the exception of physical optics and acoustics, the first attempt to give a comprehensive view on the biophysics of a whole organ or organ system. The well-known author states already in the introductory chapter (A) his views on the correlation and interaction of structure, function and energetics, which are usually treated separately in the literature. The biological phenomenon of auto-restitution, very often neglected in biophysical research, is also brought into the focus of attention. In the second chapter (B) the structural properties of muscle are treated in detail and the reader will find some important new data of the author and his associates on Z-membranes and their physical properties clearly demonstrated by micromanipulation, and a number of other observations on optical properties of living fibrils. The most important achievemnt, however, is the conformation of the muscle fibril as the smallest functional unit by demonstrating its electrical excitability and recording its rapid and reversible contraction. The third chapter (C) deals with the state of the muscle constituents. The dispute for many years about „bound potassium" and „bound water" seems to be resolved in favour of ERNST's views expressed many years ago.

The second part of the book, devoted to the mechanics of muscle, contains in the 4th chapter (D) a comprehensive discussion of the problems of muscular excitation. The author has made primary and fundamental contributions to the main three aspects of muscle excitation : *ax* by proving the intimate and direct relation of action potential and volume reduction ; *bx* by demonstrating before others the potassium-sodium exchange; and *cx* by discovering the electrical excitability of the myofibril. Some of these achievements and priorities have already been acjnowledged in the international literature, others will certainly be sooner or later. In the 5th chapter (E), dealing with muscular tension, interest centres on the crystallisation of myosin and the nature and significance of the increase in metabolism in the course of contraction. In the 6th chapter (F), on the quantitative aspects of mzuscular activity, the course of contraction, force, work and efficiency receive thorough and original consideration and mathematical analysis. Some practical aspects, as fatigue, myotonia, *etc.*, are also touched upon. The 7th chapter (G), on specific function and metabolism, is devoted to the criticism of the widely current erroneous concept connecting elementary metabolic processes (lactic acid formation, creatinine-phosphate, ATP) directly with specific function i. e. contraction. The author had opposed this conception ever since the beginning of his scientific career and it appears that recent progress will prove his views to be correct in principle. In the last chapter (H) the authors view's on the „muscular machine" are outlined. The peculiarity and biologic principle of this machine is, that if an external obstacle makes the muscle to exert force, this automatically elicits *crystallisation*, *i. e.* fortification

of structure, and *hypertrophy*, *i. e.* increased reproduction of living matter. According to the author's experiments, the work apparently lost during forced streching of muscle in tetanus is at least partly recorvered in the process of maintenance and building up of structure.

Theories, views, considerations and criticisms are supported by a wealth of experimental data, demonstrated with a great number of excellent photomicrographs, cinematic picture series' diagrams and tables. Besides the earlier work of the author these represent mainly the results obtained in the Institute of Biophysics of the Medical University, Pécs.

The material is presented in a stimulating and orignal manner, statements are sometimes rather provocative, but always interesting and logical. The author generally mistrusts theories assembling a group of facts into a well and smottohly fitting framework and claiming „final explanation" in any field of our knowledge. He relishes in aiming at the weak points of the theories „en vogue", and one must confess that he rarely misses his target. Many readers, who with the reviewer readily acknowledge the electronical excitability of the myofibril as a fundamental new fact, will perhaps not follow the author in his argumantation that the membrane theory tries only to explain the excitability, of cellular units or their equivalents — e. g. striated muscle fibres — as a whole, and that especially in striated muscle the membrane theory could not and indeed never has explained the „excitation" pf the fibril. The author's new ideas on rossible mechanisms underlying this „fiboillar excitation" — for this expression the reviewer has to bear full responsibility — are extremely interesting, though the analogies with semi-conductors may seem far-fetched and daring for the time being, but may turn out correct, or at least fruitful in the future.

Excellent printing and reproduction espicially of the photomicrographs are a credet to the Academic Press of Budapest.

J. SZENTÁGOTHAI

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Biochemia

COMPARATIVE STUDIES ON D-GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASES

VIII. A STUDY OF THE ESSENTIAL Zn IONS OF THE ENZYME

By

T. KELETI and M. TELEGDI

BIOCHEMICAL INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, BUDAPEST

(Received October 16, 1958)

The inhibitory action of 1,10-phenanthrolene on the D-glyceraldehyde-3-phosphate and D-glyceraldehyde oxidation by D-glyceraldehyde-3-phosphate dehydrogenases isolated in crystalline form from rabbit, bovine and pig muscle, from baker's and brewer's yeast, as well as from crayfish muscle, has been studied. All these enzymes were inhibited in about the same measure, irrespective of the substrate used. Inhibition was slower when the reaction mixture contained phosphate instead of arsenate. When alloxan was used instead of diphosphopyridine nucleotide, D-glyceraldehyde-3-phosphate dehydrogenase did not oxidize D-glyceraldehyde-3-phosphate and its oxidative action on D-glyceraldehyde was also questionable.

According to data in the literature, the following dehydrogenases containing essential SH groups are Zn-proteins: baker's yeast ADH* [1—7]; brewer's yeast ADH [7, 8]; horse liver ADH [9, 10, 11]; pig liver glutamic acid dehydrogenase [12]; and rabbit muscle and baker's yeast PGAD [13].

Owing to their essential Zn content, all these enzymes may be inhibited by chelate forming agents.

It is known that neither physicochemical, nor enzymological, nor immunological methods could demonstrate any difference between the PGADs isolated from different mammalian species [14—18]. On the other hand, differences have been demonstrated between mammalian, yeast and crab PGADs [19]. For this reason we have examined all these enzymes for inhibition by 1,10-phenanthrolene.

It has been reported for the ADHs isolated from yeasts that the Zn ion participates in binding the natural coenzyme (DPN, or DPNH) [20, 21, 22] and that the Zn ion binds also alloxan, which may act as coenzyme [8]. It was therefore investigated whether PGAD would oxidize the substrate when instead of DPN alloxan is present. As both the PGAD [23] and the ADH [24] use up different amounts of SH groups when oxidizing different substrates,

* The abbreviations used are as follows:

ADH = alcohol dehydrogenase
DPN = diphosphopyridine nucleotide
DPNH = reduced diphosphopyridine nucleotide
GA = D-glyceraldehyde
HDP = hexose diphosphate
PGA = D-glyceraldehyde-3-phosphate
PGAD = D-glyceraldehyde-3-phosphate dehydrogenase

we studied the inhibition of the enzyme by phenanthrolene, or its activity in the presence of alloxan in experiments involving a variety of substrates.

Methods

a) Enzyme preparations

PGAD prepared from rabbit, pig or ox muscle according to SZÖRÉNYI and DVORNIKOVA [25], as well as ELŐDI and SZÖRÉNYI [14] was used. The enzymes thus prepared were active even without the addition of cysteine.

The crystalline enzymes from baker's yeast and brewer's yeast were isolated by our method [26] and were activated with cysteine.

The enzyme from crayfish *(Potamobius astacus)* was crystallized according to SZÖRÉNYI, ELŐDI and DÉVÉNYI [19].

The enzymes were recrystallized 2 to 5 times and then dissolved in an *N*/10 glycine buffer or in an *M*/2 phosphate buffer (pH 8.5).

Aldolase was isolated from rabbit muscle according to TAYLOR, GREEN and CORI [27], and was used after 2 recrystallizations.

b) Materials and instruments

DPN: 85 per cent *(Light & Co)*.

1,10-phenanthrolene: *(Merck)* was used dissolved in distilled water.

Alloxan: *(Merck)* was dissolved in *N*/20 NaOH.

Dialuric acid: *(Polychemia)* was prepared from alloxan *(Merck)* and was dissolved in *N*/20 NaOH.

HDP: was prepared enzymatically and was kindly supplied by Professor B. TANKÓ.

GA: *(Fluka)*, was dissolved in distilled water.

The measurements were made in a *Hilger Uvispek* spectrophotometer, using quartz cuvettes, with a light path of 1 cm and 0.5 cm, at room temperature.

c) Technique of measurement

The enzymatic activity of the various PGADs was studied by WARBURG's optical method, at 340 mμ, as already described [19, 23].

The optical method [8] was employed when working with alloxan or dialuric acid.

The activity of aldolase was determined measuring after 15 minutes of hydrolysis with *N* NaOH at room temperature the rised triosephosphates. The inorganic phosphate was determined according to FISKE and SUBBAROW [28] and LOWRY and LOPEZ [29].

Phenanthrolene was added to the enzyme before making the measurement without previous incubation but before adding the coenzyme and substrate.

Experimental

a) *Oxidation of PGA*

We investigated the inhibitory action of 1,10-phenanthrolene on the PGA oxidation by PGADs of different origin. PGA was supplied by the HDP + aldolase system in the reaction mixture. The inhibition of PGADs from different mammalian muscles is shown in Fig. 1 which makes it clear that phenanthrolene inhibited them practically to the same extent.

Likewise, phenanthrolene had about the same inhibitory effect on PGAD from baker's yeast and PGAD from brewer's yeast (Fig. 2).

Fig. 3 reveals that the inhibitory action of phenanthrolene on crayfish PGAD was closely similar to that on mammalian muscle and yeast PGADs.

When in the reaction mixture phosphate was used instead of arsenate, to start the inhibition of mammalian muscle PGADs the concentration of phenan-

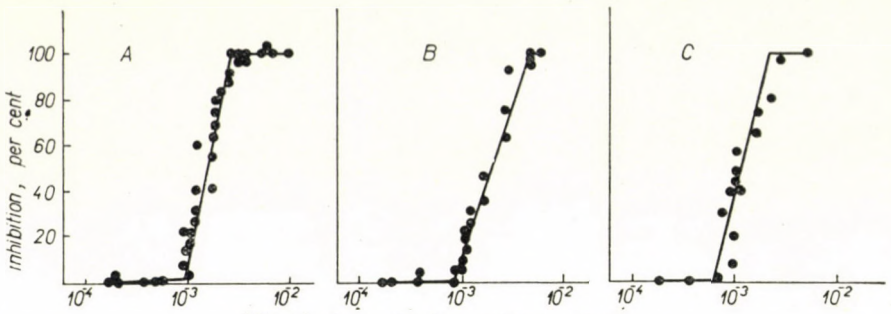


Fig. 1. PGA oxidation. Rabbit, ox and pig muscle PGAD. Inhibitory action of 1,10-phenanthrolene

A: pig PGAD B: rabbit PGAD C: ox PGAD

Curves plotted from the data of 16 different experiments. Various preparations have been used and the enzyme concentration varied from 5 to 15 μg/ml in the different experiments

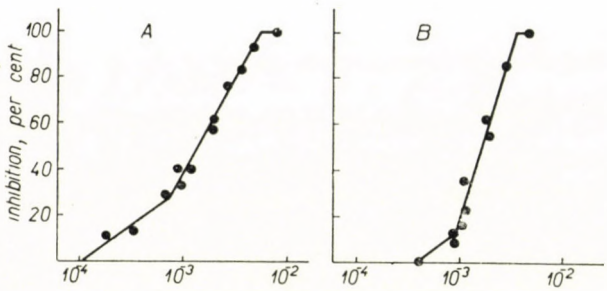


Fig. 2. PGA oxidation. PGAD from baker's yeast and brewer's yeast. Inhibitory action of 1,10-phenanthrolene

A: baker's yeast PGAD B: brewer's yeast PGAD

Curve plotted from 7 experiments. Conditions as in *Fig. 1.*

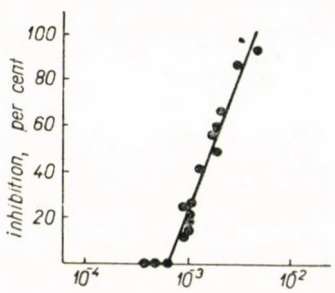


Fig. 3. PGA oxidation. PGAD from crayfish muscle. Inhibitory action of 1,10-phenanthrolene. Curve plotted from 4 experiments, carried out as specified in *Fig. 1.*

throlene had to be increased by one order of magnitude. However, the increasing of the inhibition with increasing concentration of phenanthrolene proceeded at the same rate as in the presence of arsenate ion (Fig. 4).

In the presence of phosphate ion, for starting the inhibition of PGAD from yeast a somewhat higher concentration of phenanthrolene was needed but complete inhibition took place at already a slightly lower concentration as in the presence of arsenate ion (Fig. 5).

Inhibition of crayfish PGAD in the presence of phosphate ion proceeded just as with mammalian muscle PGAD (Fig. 6).

We examined the effect of phenanthrolene on the activity of aldolase producing the substrate and found that in the concentration employed 1,10-phenanthrolene had no influence on aldolase activity.

b) *GA oxidation*

Fig. 7 makes it clear that in the case of GA oxidation the PGADs isolated from different mammalian muscles were inhibited in practically the same measure.

We did not study the GA oxidation of yeast PGADs because at the protein concentration required for GA oxidation, phenanthrolene precipitates some of the proteins and the opalescence thus resulting interferes with evaluation by the optical method.

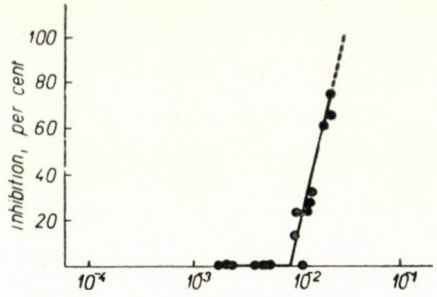
Thus far, only PGA oxidation has been demonstrated in the case of PGAD isolated from crayfish muscle [19]. We now found that not only the mammalian muscle and yeast PGADs, but also the crayfish muscle PGAD can oxidize GA (Table I).

Table I

GA oxidation of crayfish muscle PGAD, as measured by the optical method

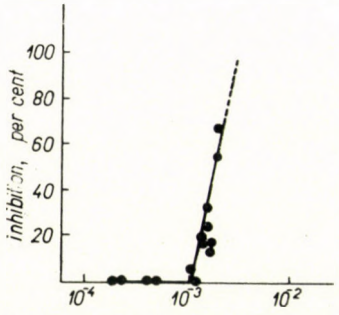
Time (sec)	Extinction at 340 mμ	
	GA oxidation in the presence of arsenate ion	GA oxidation in the presence of phosphate ion
0	0.051	0.026
30	0.068	0.058
60	0.082	0.086
120	0.108	0.132
180	0.137	0.168
240	0.161	0.196
300	0.189	0.215

The composition of the reaction mixtures used in the measurements was the same as that already described [23].



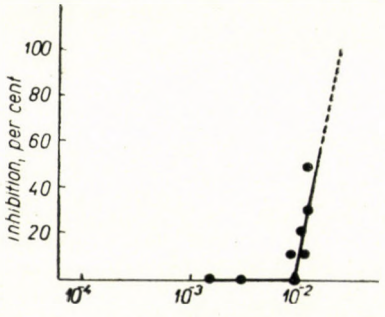
M 1,10-phenanthrolene, end concentration

Fig. 4. PGA oxidation. Pig, rabbit and ox muscle PGAD. Inhibitory action of 1,10-phenanthrolene in the presence of phosphate ion
Curve plotted from 3 experiments. The results for the different mammalian PGADs have been combined



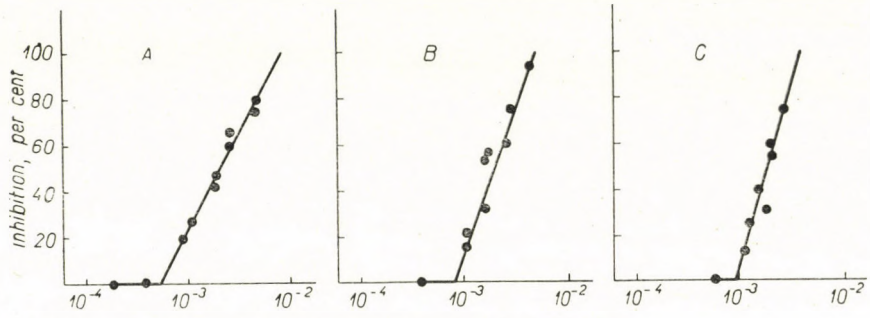
M 1,10-phenanthrolene, end concentration

Fig. 5. PGA oxidation. PGAD from baker's and brewer's yeast. Inhibitory action of 1,10-phenanthrolene in the presence of phosphate ion
Curve plotted from 4 experiments, showing the combined results for the different yeast PGADs



M 1,10-phenanthrolene, end concentration

Fig. 6. PGA oxidation. PGAD from crayfish muscle. Inhibitory action of 1,10-phenanthrolene in the presence of phosphate ion

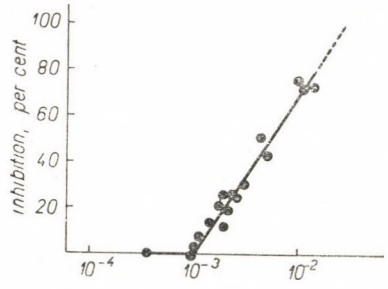


M 1,10-phenanthrolene, end concentration

Fig. 7. GA oxidation. Pig, rabbit and ox muscle PGAD. Inhibitory action of 1,10-phenanthrolene

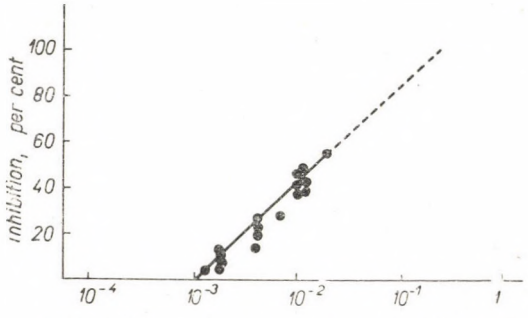
A: pig PGAD B: rabbit PGAD C: ox PGAD

Curve plotted from the results of 8 different experiments, in which several different preparations were used and the concentration of enzyme varied from 0.5 to 3.0 mg/ml



M 1,10-phenanthrolene, end concentration]

Fig. 8. GA oxidation. PGAD from crayfish muscle. Inhibitory action of 1,10-phenanthrolene? Data from 4 experiments, carried out as specified in *Fig. 7*



M 1,10-phenanthrolene, end concentration

Fig. 9. GA oxidation. Pig, rabbit and ox muscle PGAD. Inhibitory action of 1,10-phenanthrolene in the presence of phosphate ion.

The curve shows the combined results of 4 experiments with the different mammalian PGADs

The data in Table I show that GA was oxidized also by crayfish PGAD and that this reaction proceeded faster in the presence of phosphate than in the presence of arsenate ions, just as with PGADs from mammalian muscle.

The inhibition of crayfish PGAD by phenanthrolene is shown in Fig. 8. Complete inhibition of GA oxidation by crayfish PGAD required a slightly higher concentration of phenanthrolene than was necessary with the mammalian muscle PGADs.

When in tests with PGADs from mammalian muscle the reaction mixture contained phosphate ion, inhibition increased less rapidly with the increase

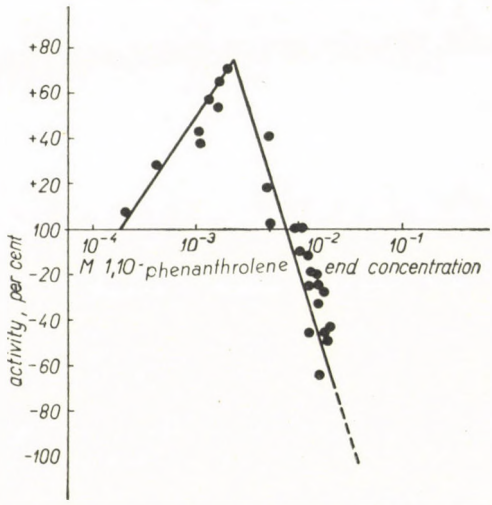


Fig. 10. GA oxidation. PGAD from crayfish muscle. Inhibitory action of 1,10-phenanthrolene in the presence of phosphate ion
Curve plotted from the results of 4 experiments

in concentration of phenanthrolene than in the presence of arsenate ion and a higher concentration was required for complete inhibition (Fig. 9).

Testing the action of phenanthrolene on the oxidation of GA in the presence of PGAD isolated from crayfish muscle it was found that in the presence of phosphate ion at low phenanthrolene concentrations the enzyme was activated and inhibition took place only at higher concentrations of phenanthrolene (Fig. 10).

It is apparent from Fig. 10 that in the presence of about $4 \cdot 10^{-3}$ *M* of 1,10-phenanthrolene the enzyme was about 75 per cent more active than the original preparation. As compared to the mammalian muscle PGADs, inhibition of crayfish PGAD begins at a higher phenanthrolene concentration, but increased more steeply and complete inhibition took place at a lower concentration.

The protective effect of the phosphate ion on the inhibition of the enzyme with phenanthrolene may be studied well by following the course of the reaction at the phenanthrolene concentration which in the presence of arsenate causes practically complete inhibition. In the presence of coenzyme and substrate the enzyme-phenanthrolene complex is dissociable, but its dissocia-

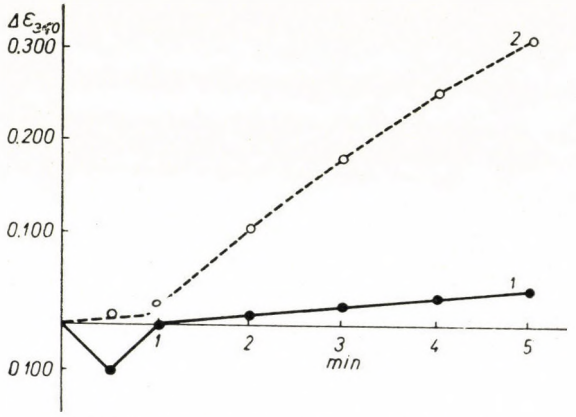


Fig. 11. Dissociation of the PGAD-phenanthrolene complex in the presence of coenzyme and substrate, with phosphate or with arsenate ion

1 : arsenate ion 2 : phosphate ion

The curve shows an experiment made with PGAD isolated from ox muscle. The experiments with enzymes from the other mammalian muscles yielded similar results

tion takes place much faster in the presence of phosphate ion than in the presence of arsenate ions (Fig. 11).

c) *Experiments with alloxan*

It was found that in the presence of alloxan the various PGADs cannot oxidize PGA. At the same time, higher concentrations of alloxan inhibit the PGA oxidation observable in the presence of excess DPN. The results for GA oxidation, when alloxan was used instead of DPN, were not unequivocal. In the presence of both phosphate ions and alloxan, GA was never oxidized, whereas in the presence of arsenate a very slight reaction resulted in a few instances. In tests of the faster opposite process by the use of dialuric acid, a slight reaction was observed.

Discussion

It has been demonstrated that the proteins of crystalline PGAD isolated from rabbit muscle and baker's yeast contain Zn ions. As the enzyme activity is inhibited if these Zn ions are made to form a complex with chelate forming

agents, the Zn ions in the protein may have some role to play in the activity of the enzyme [13]. We have found the crystalline PGADs isolated from the bovine and pig muscle, brewer's yeast and crayfish muscle to be inhibited by phenanthrolene to about the same extent as the PGADs isolated from rabbit muscle and from baker's yeast. This suggests that all these enzymes are apparently Zn proteins and the metal ion in the proteins is essential for enzymatic activity.

Evidence has been obtained to show that the protein-bound Zn ion plays a role in the oxidation of both phosphorylated and non-phosphorylated substrates. There was no difference in this respect between the various kinds of PGAD tested (isolated from muscles of various mammals, yeasts and from the muscle of crayfish).

The phosphate ion afforded a certain protection against the inhibitory action of phenanthrolene. In the case of PGADs isolated from mammalian muscle this protection manifested itself with the fact that the inhibition of PGA oxidation began at a phenanthrolene concentration one order of magnitude higher than in the presence of arsenate. However, the increase in the extent of inhibition with the increase in the concentration of phenanthrolene was just as fast as in the presence of arsenate ions. In the case of GA oxidation, inhibition started at about the same concentration of phenanthrolene as in the presence of arsenate, but increasing the concentration of phenanthrolene caused a much slower increase in the extent of inhibition. The phosphate ion had the same effect on the PGA oxidation of crayfish PGAD. On the other hand, in GA oxidation the activity was first extremely enhanced and only subsequently did the inhibition take place. All these findings demonstrate that differences exist between the PGADs from mammalian muscle, yeasts and crayfish muscle, as it had been suggested [19].

The protective action of phosphate ions may in part be due to a promotion of the dissociation of the enzyme-phenanthrolene complex. This suggests that phenanthrolene and the phosphate ion link up with the enzyme molecule through the same site, the Zn ion. We shall discuss this problem in detail in report on studies on the oxidative phosphorylation catalysed by PGAD [30].

The strong activation of GA oxidation of crayfish PGAD by low concentrations of phenanthrolene in the presence of phosphate is not believed to have been due to a formation of complex between phenanthrolene and the heavy metal contamination in the phosphate, which might have inhibited the enzyme. Perfectly identical results are namely achieved with the use of phosphate of analitical purity. There were probably no traces of heavy metal in the enzyme preparation or in some component of the reaction mixture ; had there been such traces, activation would have occurred in the presence of arsenate as well. However, such activation did not occur in any of the experiments with arsenate.

It has been established that in the presence of alloxan, PGAD cannot oxidize PGA. The GA oxidation detected in the presence of alloxan in a few instances (and its failure in others) suggests that under the given circumstances the reaction proceeds at such a slow rate that the minute quantity of risen dialuric acid cannot be measured because its reoxidation by air.

Acknowledgement

We are indebted to MRS. L. GASPARIK for the valuable technical assistance.

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Physiologia

ELECTROPHYSIOLOGICAL ANALYSIS OF REPETITIVE RESPONSES ON THE SAPHENOUS NERVE OF THE RAT

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Veratrine applied to an injured area of the skin has been found to facilitate the effect of touching the area under the lesion. At a concentration of 1 : 1000, dinitrophenol inhibited the facilitation by veratrine. K^+, Na^+ and Li^+ ions when applied to the injured skin area, elicited repetitive responses by acting on the axons and not on the receptors. Ethylenediamine tetraacetate, which forms a complex with Ca^{++}, caused no repetitive discharges, in contrast with sodium citrate. For this reason the autorhythmic activity elicited by sodium citrate appears to be due to a direct action of the citrate anion. Dinitrophenol was found to inhibit the repetitive responses to ions. The results obtained have been discussed in the light of recent evidence concerning the processes of nervous impulse.

It has been known for long that the isolated muscles and axons of cold-blooded animals respond to treatment with various inorganic ion by repetitive discharges. Studies of this phenomenon, especially in warm-blooded animals merit attention, because it has been suggested that the mechanism of impulse formation is the same in the receptors and axons [1, 2].

There is evidence to show that in the receptors [3, 4, 5], axons [6, 7, 8, 9] and even in the myoneural junctions [10, 11] a local response (generator potential) precedes the repetitive discharges. Nothing certain is known about the genesis of the local response and it is also possible that certain receptors initiate a series of impulses even without generator potential [12]. At any rate, when the action potential starts, the impedance of the nerve membrane decreases and its permeability for sodium increases [13, 14, 15]. The process requires energy only for restoring the original conditions. This is maintained by the so-called Na-pump. According to TEORELL [16], Na^+ may be substituted by the Li^+ ion in the maintenance of membrane potential. Both cause rhythmic changes in the frog skin potential. As K^+, Rb^+ and Cs^+ have no such property, but when injected intradermally such ions cause intense pain [17], it is surmised that the nerve uses a metabolic mechanism against the changes in the ionic milieu and the repetitive response is a result of this.

Starting from the above data we have investigated

a) how does the blocking of the Na-pump mechanism required for maintaining the action potential, or

b) an enhancement of its activity, influence nervous excitatory processes,

c) do ions elicit the repetitive response in warm-blooded animals through an excitation of receptors or through direct axonal effects?

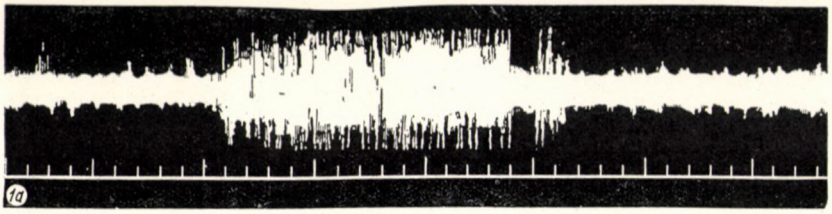
Methods

In rats the injured skin areas supplied by the saphenous nerve were studied under Intranarcon (sodium cyclohexenylallyl thiobarbiturate) anaesthesia induced by 50 mg/kg, intraperitoneally. The lesion involved an ellipsoid area 3 to 4 mm in diameter. The prepared nerve fibre was about 0.1 mm thick. It was fastened to a thin recording electrode and was coated with a 6 per cent ion-free saccharose solution to avoid short-circuit. In other experiments the action potentials were led off by means of platinum electrodes, by the wet-chamber technique. The two methods yielded comparable results. An ultrathermostat was used to maintain the temperature of the rat and of the nerve at 38° C. Repeated touch control tests were made to ascertain that the preparation was intact. Nervous function persisted for 3 to 4 hours. The ions and compounds tested were applied to the injured skin area by means of cotton swabs soaked in Tyrode's solution (composed of 0.8 per cent NaCl; 0.02 per cent KCl; 0.02 per cent $CaCl_2$; 0.01 per cent $MgCl_2$; 0.1 per cent glucose; 0.005 per cent NaH_2PO_4; 0.1 per cent $NaHCO_3$). The action potentials were transmitted through a conventional R. C. amplifier to a magnetic tape recorder and to two cathode-ray oscilloscopes. This method allowed simultaneous visual and auditory observation. The findings were evaluated after playing back several times the magnetic tapes, taking photographs from the important parts. The method has been described in detail in a previous paper [18]. About 100 animals were involved in the experiments.

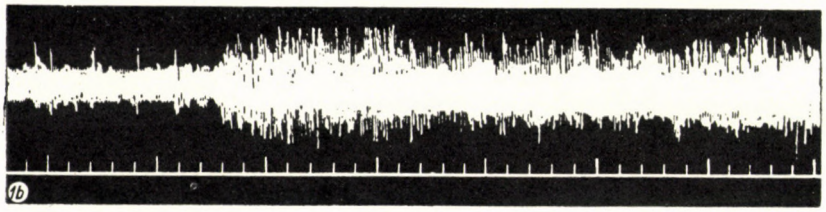
Results

I. The action of veratrine causing repetitive responses and its antagonism with dinitrophenol

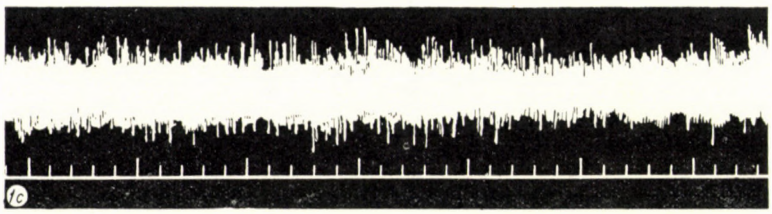
It is known that in response to veratrine the permeability of the nerve membrane increases, sodium inflow and the O_2 uptake of the axon are enhanced [19, 20, 21]. The increase in O_2 consumption is ascribed to an increased activity of the Na-pump mechanism. For these reasons veratrine seemed suitable for use in studies concerned with the problems outlined in the introduction; it was applied to the lesioned skin area at a concentration of 1 in 10 000, one which was found to cause no spontaneous nervous excitation, does not elicit repetitive responses and does not bring about depolarisation, *i.e.* blocking of conduction. However, 5 to 8 minutes after the application of veratrine a very long series of impulses was elicited if the veratrinized area under the lesion was touched. Such an experiment is illustrated in Fig. 1, which shows that without veratrine treatment touching the area under the injured part of the skin produced in the electroneurogram of the saphenous nerve spikes that lasted only as long as the touch (0.3 sec) (part *a.* of Fig. 1). On the other hand, after treatment with veratrine, touch of the same intensity elicited nervous activity which sometimes lasted as long as 40 sec. (parts *b.*, *c.*, and *d.*). The frequency of the action potentials increased steeply and declined thereafter gradually so that siren-like sounds were heard from the loudspeaker. The amplitude of the single discharges was also variable; and so was the size of the spikes in the electroneurogram. Thus, the effect of touch was "facilitated" by veratrine. This veratrine facilitation persisted long after the cotton swab containing the drug had been removed from the skin and the effect began to



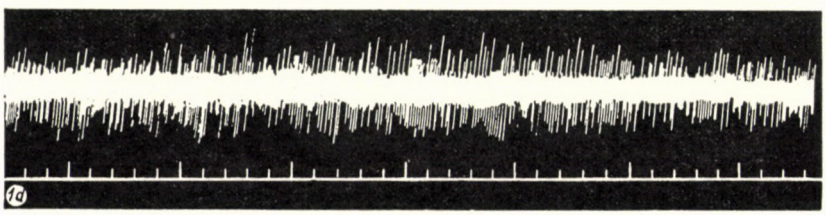
a. The spikes elicited by a single touch last only as long as touching lasts



b. After 8 minutes' treatment with veratrine, a single touch induces long-lasting repetitive discharges

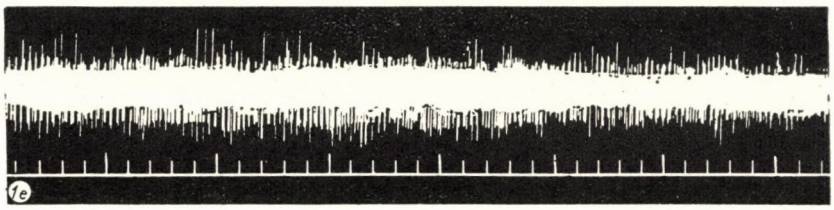


c. b. continued



d. c. continued

abate in about 30 minutes. It was noteworthy that such lasting, synchronized series of discharges were not elicited by every touch. If the area was repeatedly touched in rapid succession, there occurred no effect, but 2 or 3 minutes later even the gentlest touch sufficed to induce a strong activity. If the veratrine applied to an uninjured area of the skin, even 25 minutes' exposure to a tenfold concentration (1 in 1 000) failed to elicit a similar response.



e. d. continued.

Fig. 1. Experiment No. 75. Rat weighing 220 g. Effect of veratrine on touching potentials. Action currents from saphenous nerve. Time signal: 0.1 sec. *a.;* Spikes elicited by a single touch. *b. c., d., e.;* Response to a single touch after 8 minutes' treatment with veratrine

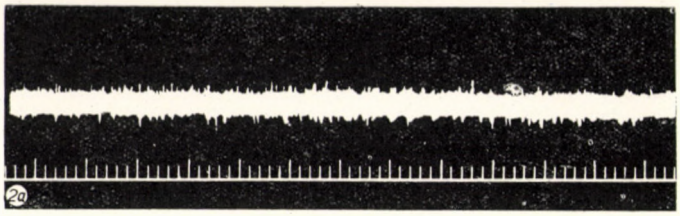
These results indicate the increased activity of the Na-pump mechanism to be responsible for the increased excitability. To corroborate this view, attempts were made to lessen the response by the use of dinitrophenol (DNP), on grounds of the considerations outlined below.

The formation of impulses is in close connexion with a sudden increase in the permeability of the nerve membrane. It is known, that first Na enters, then during the next few msec. K leaves the axoplasm [13, 15, 22]. The Na-pump mechanism is responsible for the return to the resting phase and its function requires high-energy phosphate esters. This view is supported by the observation made by HODGKIN [23]: DNP inhibits the resting Na^{24} outflow and relieves even the so-called posttetanic hyperpolarisation[24]. The important role of metabolism in nerve inpulse has been emphasized also by VOGEL [25] who found that in the isolated nerve of the frog DNP decreased the amplitude of action potential. For these reasons we thought that DNP would be able to inhibit repetitive responses.

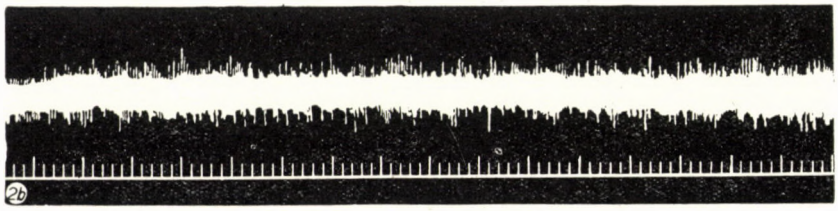
We applied DNP at a concentration of 1 in 1 000 to the veratrinized area, with the result that the so-called touch-facilitation was immediately abolished. DNP did not depolarize the nerve membrane, because in the control experiments involving 11 animals the same concentration caused no block of conduction even after 5 to 8 minutes of treatment. Moreover, DETTBARN and STÄMPFLI [26] reported that at pH 7.8 DNP reduces the membrane potential of the frog's nerve only by 3 mV.

II. Studies with NaCl, KCl, LiCl, Sodium Citrate and EDTA (ethylenediamine tetraacetate, Komplexon III)

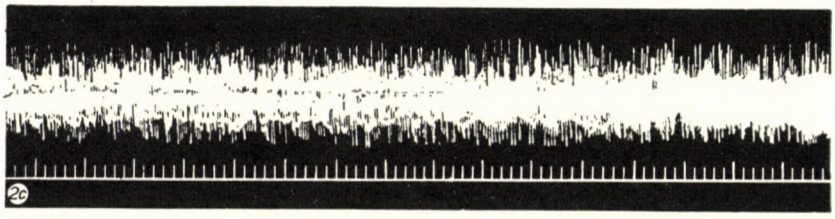
These compounds have been reported to elicit long-lasting repetitive responses [27, 28, 29]. We found in the rat the lowest effective concentrations of these ions to be much higher than those reported in the literature as effective for cold blooded animals. For example, of NaCl, KCl, and LiCl 5 per cent solutions were active. Sodium citrate was usually effective even in its iso-



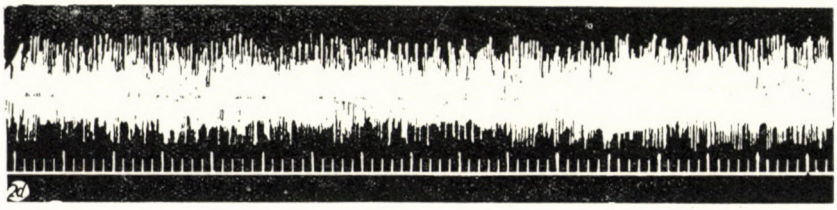
a. Spontaneous activity of the saphenous nerve



b. 40″ after KCl



c. 120″ after KCl



d. 121″ after KCl

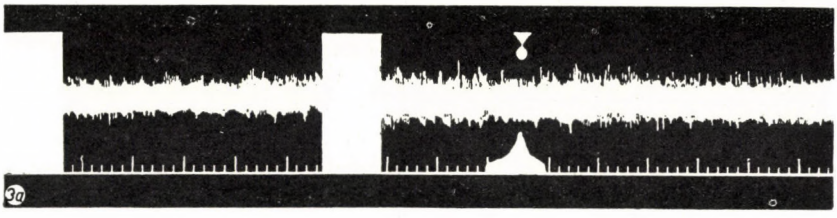
Fig. 2. Exepriment No. 52. Rat weighing 160 g, desensitized with capsaicine. Effect of KCl on the electroneurogram of the saphenous nerve
Time signal: 0.1 sec. Biphasic lead. Note the significant increase in both the amplitude and the frequency of resting activity in response to KCl

tonic solution. Concentrations lower than those mentioned were inactive. The non-synchronized repetitive responses of musical nature began usually after a latency of 20 to 40 sec, to reach the maximum level in about 2 minutes, with frequencies varying from 100 to 600 Hz, then the effect decreased gradually

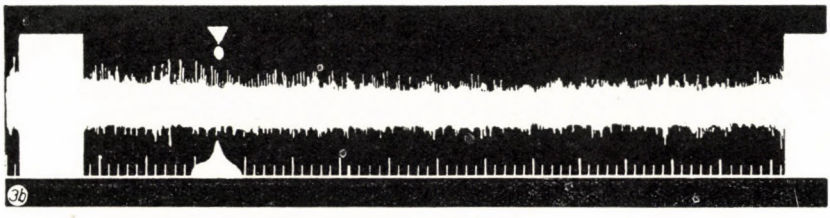
and ceased in 4 minutes (Fig. 2). In such cases touching the area under the lesion caused no change in the electroneurogram : depolarisation, conduction block developed. The repetitive responses caused by the ions showed a very slight tendency to fluctuation, the frequency rose steeply. Siren-like activity was heard from the loudspeaker, but, the discharges having been synchronized corresponding to the activity of different fibres, the sounds changed evenly.

The size (impulse/sec) and the duration of repetitive responses caused by the ions varied greatly with the skin areas. For instance, KCl had hardly any effect at the toes, whereas it elicited considerable repetitive responenses when applied to the back of the foot. The strongest response was elicited from the area above the internal ankle. This might have been due not so much to differences in the number of receptors in the different skin areas as to the fact that in the ankle area there are more afferent nerve fibres than at the toes.

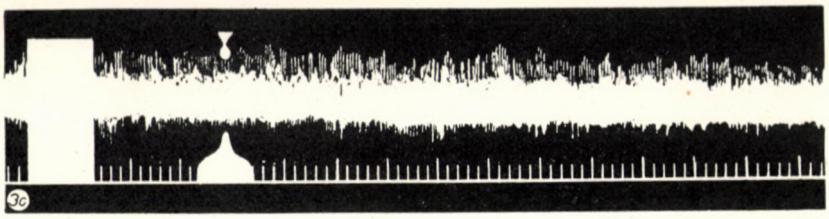
Even isotonic solutions of sodium citrate usually gave rise to long-lasting, non-synchronized, non-fluctuating discharges of musical nature. MONNIER [29] ascribed the citrate effect to a binding of calcium, but LORENTE DE NO [30] attributed this action to the citrate itself. Thus, the role of Ca is not clear. To decide the issue, we used EDTA which binds Ca in the form of a complex [31], but at a concentration of 3.8 per cent (identical with that of sodium citrate) EDTA was found absolutely ineffective. It is believed therefore



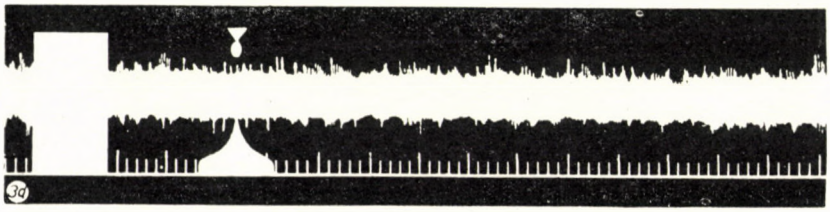
a. Spontaneous activity of the saphenous nerve. The wide white area marks the onset of the KCl effect



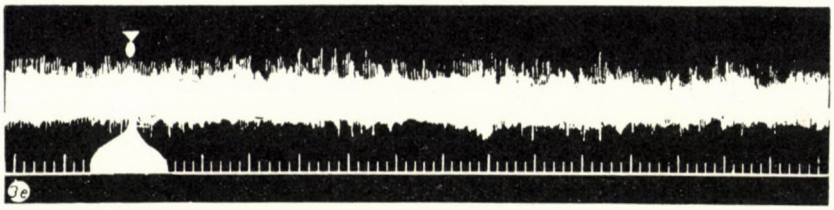
b. Increasing KCl effect



c. Maximum of KCl effect

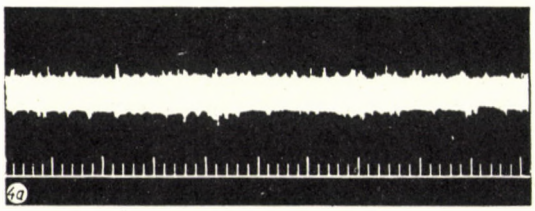


d. KCl effect about to cease

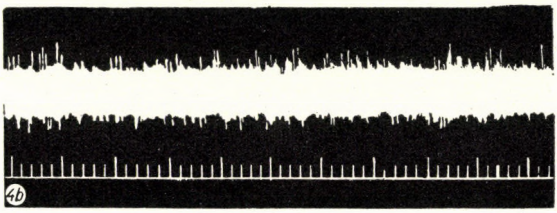


e. Substitution of KCl swab with one soaked in Tyrode's solution results in reappearance of repetitive responses

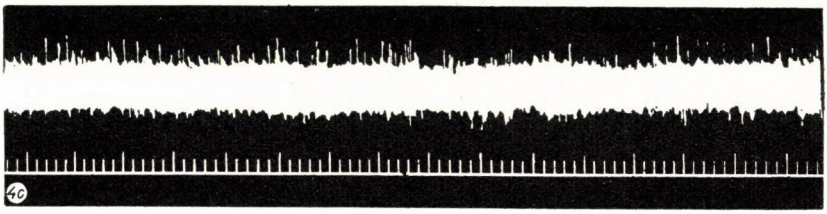
Fig. 3. Experiment No. 85. Rat weighing 200 g. Action currents from saphenous nerve. Time signal: 0.1 sec. Biphasic lead. After the gradual cessation of the repetitive responses to KCl, treatment of the injured skin area with Tyrode's solution gives once again rise to significant repetitive discharges



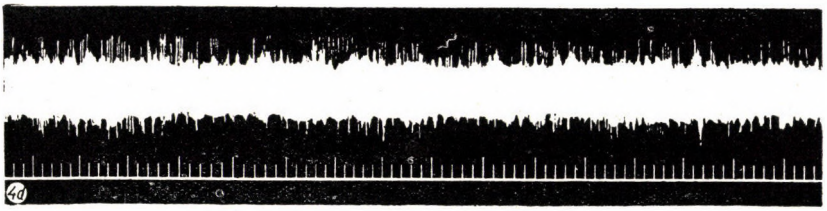
a. Basal activity



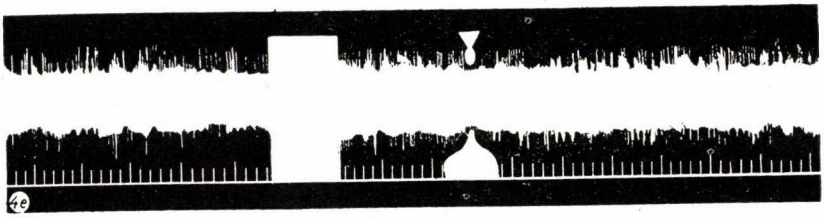
b. Onset of sodium citrate effect



c. Sodium citrate effect increasing



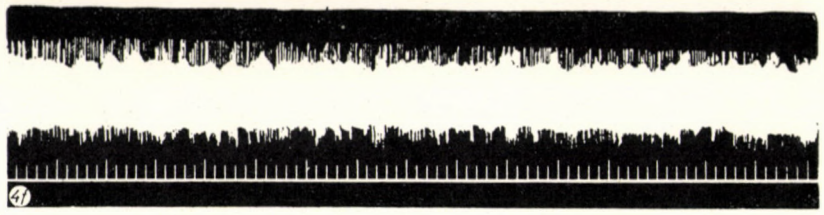
d. Peak of sodium citrate effect



e. The thick white line indicates that the swab soaked in sodium citrate has been substituted by one soaked in Tyrode's solution

that the repetitive responses elicited by treatment with sodium citrate are due to a direct action of the citrate anion.

If the KCl or Na citrate solution was substituted by Tyrode's solution after the repetitive responses had considerably decreased or ceased, they reappeared again (Fig. 3, Fig. 4). The explanation for this will be dealt with in the discussion.



f. Repetitive sodium citrate effect after washing

Fig. 4. Experiment No. 89. Rat weighing 180 g. Action currents from saphenous nerve. Time signal: 0.1 sec. The cotton swab soaked in 3.8 per cent sodium citrate, when applied to the injured skin area, gives rise to marked repetitive responses (parts *b.*, *c.* and *d.*). Substituting the sodium citrate swab with one soaked in Tyrode's solution further enhances the repetitive responses

III. The role of receptors in the repetitive responses

It is generally believed that ions cause pain or elicit repetitive responses by stimulating the receptors. This conclusion has been drawn by GESSLER [27] from experiments with hypertonic NaCl. However, a direct axonal effect appears just as probable. The chemical desensitization described by JANCSÓ [32, 33] seemed to be suitable for deciding this problem. JANCSÓ found namely that in rats treated for 2 or 3 days with increasing subcutaneous doses of capsaicine the skin and mucous membranes become insensitive to the widest variety of chemical irritants, including hypertonic (30 per cent) NaCl applied to the eye. In our experiments, 30 per cent NaCl continued to act as an irritant when in such desensitized animals it was applied to the injured skin area, though it was ineffective in the eye ; the threshold concentration did not change. It appears therefore that ions elicit repetitive responses not by exciting the receptors, but by acting on the axons and it is in this way that they cause pain in the lesioned skin area.

IV. The antagonistic action of dinitrophenol on the effect of ions

In analogy to the veratrine experiments, we investigated whether the repetitive responses elicited by ions could be antagonized by DNP. In these experiments we used mainly KCl (5 per cent solution). First, we examined the effect of KCl at the injured skin area, then after 8 to 10 minutes' treatment with DNP we repeated the experiment. Control experiments proved that from the same lesioned area KCl can elicit repetitive responses several times in succession. We then made experiments at various sites on both legs of 6 animals and found that DNP blocked the repetitive responses caused by KCl. Likewise, it inhibited the repetitive responses induced by sodium citrate and by hypertonic NaCl. The inhibitory action was slightly reversible.

It remained to be examined whether DNP blocked the so-called direct receptor actions. In 10 rats we determined the closing time of the palpebra in response to the instillation of 20 per cent NaCl before and after treatment with 1 : 1000 DNP, and found that it did not change significantly after 10 minutes of treatment with DNP. It seems therefore justified to surmise also on this basis that it is by different mechanisms that the ions elicit series of impulses in the receptors and the axons.

Discussion

Repetitive excitation of nerve elements has been induced in two ways, *viz.* by treatment with veratrine and monovalent cations (K^+, Na^+, Li^+) and by eliminating Ca^{++}. On the basis of our own findings and those in the literature, touch facilitation caused by veratrine may be explained in the following way. At the site of the injury veratrine increases the permeability of axons for Na^+ and lessens at the same time accommodation [34]. The lessening of accommodation usually increases the tendency to repetitive responses and thus excitability increases [35]. For this reason the action potentials induced by touching the injured area elicit a new process of impulses, as a result of the decreased accommodation of nerve elements in the lesioned area. DNP, which blocks the Na-pump mechanism required in the repolarisation of nerves, abolishes the repetitive responses. Accommodation presumably increases in response to DNP, as in the 1 : 1000 concentration it causes no conduction block. Depolarisation alone thus cannot explain the antagonism between DNP and veratrine. Ether has been reported to enhance the accommodation of the frog's nerve [36], suggesting that a correlation may exist between the metabolism and accommodation of nerves.

Likewise, DNP, a metabolic inhibitor, blocks the repetitive responses to K^+, Na^+, Li^+ and sodium citrate (Ca^{++} deprivation), indicating that the intactness of nerve metabolism is essential in the elicitation of repetitive responses. The mechanism by which ions elicit repetitive responses is still unclear. In this paper we do not wish to discuss all data pertaining to the process of stimulation and impulse formation, (the interested reader should consult references [37, 38, 39 and 40]) let it suffice that the classical membrane theory had failed to explain why the action potential is higher than the resting one and had to be revised. The membrane permeability theory of HODGKIN and HUXLEY [41, 42] overcame this problem and showed that excitation reverses the polarity of the membrane and increases the Na^+ permeability constant. The Na^+ entering the cell (axon) is driven out by Na-pump [43]. However, even the revised membrane theory could not explain all pertaining problems, for instance why electric phenomena are detectable in certain nerves even in the absence of Na^+ [44]. More recently, by some

authors the membrane concept has been abandoned and it has been claimed that it is the physico-chemical properties that determine the uneaqual distribution of ions. A denaturation of protein, a process undoubtedly taking place on excitation, may alter the fixation of ions and this may explain how without a membrane the ional shifts and electric phenomena are brought about in the functioning nerve [45, 38, 39]. Although there still remains considerable disagreement as to certain aspects of the problem, it is clear that a significant advance has been made toward the explanation of the process of nervous excitation and the electric phenomena connected with it.

If we want to explain the repetitive responses elicited by ions exclusively by the "spontaneous indirect reversible reaction" of proteins (SEGAL, [38]) (*i. e.* with a reversible denaturation of proteins) caused by electrolyte solutions, we shall encounter difficulties because 1./ the autorhythmic activity (repetitive response) continues under a constant ionic effect until depolarisation has taken place and 2./ the autorhythmic activity continues to increase for a while even after the normalisation of ional preponderance, and only then does it gradually cease and is the functional integrity restored. Our observations significantly emphasize the role of metabolic processes, especially that of high-energy phosphate esters, in the process of nervous excitation.

It would be tempting to light upon a common denominator in regard to the Na-pump mechanism with the repetitive responses caused by different ions. There is much in support of this view. Ca^{++} deprivation is known to increase the permeability of the membrane to Na^+ (activating its Na^+ metabolism [46]). Moreover, the preponderance of Na^+ in the extracellular medium overburdens the Na-pump by means of the diffusion gradient, in regard to nervous metabolism Li^+ may physicochemically substitute Na^+. The outflow of Na^+ from the cell is increased by 30 per cent by a preponderance of K^+ [47]. However, the fact that EDTA does not cause spontaneous nervous excitation even when present in much higher amounts than Ca^{++} is in contradiction to this attractive hypothesis. Anyway, the role of Ca^{++} in nerve impulse is rather unclear, because for example ROEDER [48], GORDON and WELSH [49], HEILBRUNN [39] claim that Ca^{++} ions are released in the moment of excitation, whereas according to KEYNESS and HODGKIN [50] excitation during Ca^{++} would enter the axon.

Our results for the animals desensitized against chemical irritants by capsaicine treatment indicate that it is by different mechanisms that the ions give rise to impulses in the axons and the receptors. Our experiments on the rat's eye also support this view.

Summing up, it may be stated that high concentrations of K^+, Na^+ and Li^+ ions outside the nervous membrane activate the Na-pump mechanism maintaining the membrane potential. The repetitive response, *i.e.* the rapid de- and repolarisation, ceases when the Na-pump mechanism is inhibited by

DNP, which blocks oxidative rephosphorylation. The reduced accommodation is probably bound to an increased activity of the Na-pump mechanism. Any intervention inhibiting metabolism results in an increase of accommodation. This, however, should be proved by further studies.

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EFFECT OF HYPOTHALAMIC LESION ON PHOSPHATIDE METABOLISM IN THE LIVER

By

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The quantity of arsenocholine built into phosphatides is significantly increased during 24 to 48 hours' fasting. In adrenalectomized rats the markedly enhanced phosphatide metabolism setting in after fasting remains absent. Three weeks after electrolytic injury to the antero-medial area of the hypothalamus, the increase of arsenocholine turnover released in normal animals by fasting does not occur. In lesions to other areas of the hypothalamus and in extra-hypothalamic lesions, such effects were not observed.

Under conditions where glyconeogenesis and fat consumption are increased (as in hunger or diabetes mellitus) there is also an increase in the phosphatide metabolism in the liver [1, 2, 3]. The hormones which accelerate glyconeogenesis and fat consumption (cortisone, ACTH, adrenaline) or diminish them (insulin) influence the phosphatide metabolism of the liver in the same way [3, 4, 5, 6]. An explanation for this may be that the phospholipids of the liver are components of enzyme systems which are directly or indirectly involved in the oxidation of fatty acids [7]. In the present work we investigated what neuro-humoral factors regulate phosphatide metabolism in the liver.

Methods

The experiments were carried out on male Wistar rats. Phospholipid synthesis was determined by the quantity of arsenocholine built up into phosphatides. The arsenocholine was administered intraperitoneally in doses of 4 mg/100 g body weight, and six hours thereafter the animals were decapitated. Removing the livers, total As was determined and the fat extracted according to FOLCH *et al.* [8]. After digestion of the liver and the extracted fat, the As content was determined by the method of J. E. EASTOE and B. EASTOE [9], the inorganic phosphate by FISKE and SUBBAROW's [10] method.

The adrenals were removed retroperitoneally. From then on the rats were given 1 per cent NaCl to drink *ad libitum*. The experiments were made 8 to 12 days later.

Lesion of the central nervous system was inflicted under Evipan anaesthesia by means of the stereotaxic apparatus as modified by SZENTÁGOTHAI [11], with 5 mA for 3 seconds. The second week after the lesions were made, we determined the animals' fluid consumption the data on which will be given in 100 g/24 hours, and carried out the Thorn test by injecting 50 μg adrenaline subcutaneously. The changes in eosinophil count will be presented in per cent of the initial value. Liver metabolism was controlled on the third week after inflicting the lesions. The exact site of the lesion was confirmed histologically in all cases.

Results

In conformity with our previous investigations, the quantity of arsenocholine built into phosphatides was found considerably to increase on 24 or

48 hours' fasting ($P < 0.01$) (Fig. 1). Fig. 2 shows the absolute quantitative changes in liver phosphatides. Fasting induced a decided rise in the quantity of liver phosphatides only after 24 hours, in spite of the fact that the turnover greatly increased both after 24 and 48 hours' fasting.

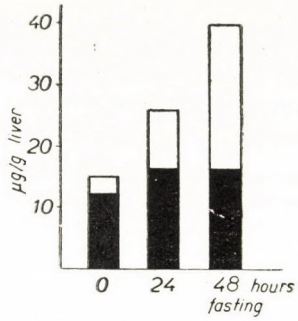

Fig. 1. Arsenocholine built into phosphatides on fasting. Ordinate: Amount of arsenocholine in As µg/g liver wet weight. Abscissa: Duration of fasting, in hours. The complete columns show the normal values, the black ones those for previously adrenalectomized animals

During fasting, glyconeogenesis and fat consumption are maintained by the activity of the adrenal cortex. We therefore investigated liver phosphatide metabolism in adrenalectomized animals both before and after fasting.

It is to be seen from Figs. 1 and 2 that in the adrenalectomized animals, phosphatide metabolism was not enhanced by fasting.

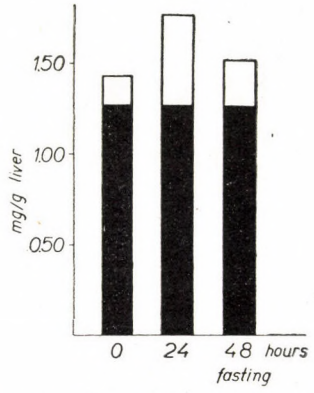

Fig. 2. Changes in the absolute quantity of phosphatides on fasting. Ordinate: Amounts of phospholipids in inorganic P mg/g liver wet weight. Abscissa: Duration of fasting, in hours. Complete columns show normal values, the black ones those for previously adrenalectomized animals

It is known from the literature that the glucocorticoid secretion of the adrenal cortex is regulated by ACTH release by the hypothalamus-pituitary system. Therefore, in the next series of experiments, we studied the effect of fasting on the phosphatide metabolism of the liver in animals in which

hypothalamic or extra-hypothalamic lesions had been brought about. From 156 such animals two groups were selected by means of the Thorn test and on the basis of their daily fluid consumption. One group was composed of animals which drank a great deal and whose eosinophil count did not diminish on

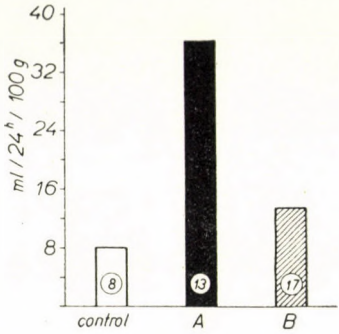

Fig. 3. Fluid consumption of normal animals and of those with hypothalamic injury. Ordinate: Fluid consumed in 24 hours, calculated per 100 g body weight. Abcissa: White columns show the values for control rats, the black ones for those with hypothalamic lesion in the antero-medial region, the shaded columns the values for rats injured in other areas of the hypothalamus. The numbers inside the columns indicate the number of animals studied

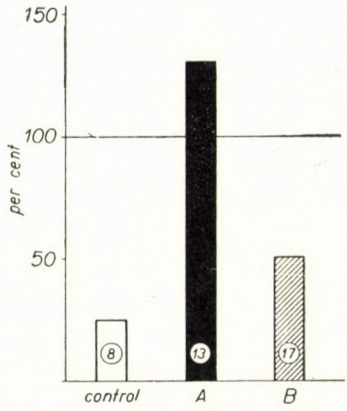

Fig. 4. Eosinophil count (Thorn test) in normal rats and in rats with hypothalamic lesion. Ordinate: Percentual change in eosinophil count following the subcutaneous injection of 50 μg adrenaline. 100 per cent means the initial value. Abscissa: White columns show the control, black columns the animals with antero-medial lesion of the hypothalamus, the shaded columns the rats injured before or in other areas of the hypothalamus

subcutaneously administered adrenaline. 13 of the 156 animals fell to this group. The other group contained those animals the daily fluid consumption of which did not markedly differ from that of the normal controls, and in which the most pronounced decrease in eosinophil count was caused by adrenaline. Of the 156 animals 17 fell to this group.

Fig. 3 shows the daily fluid consumption of the control animals and of the two groups mentioned above. It may be seen that the mean value for

24 hours was 8.5 ml in the control animals, whereas in the first injured group, 37 ml ; in the second 13.5 ml.

The Thorn test values are given in Fig. 4. In the control animals adrenaline caused the eosinophil count to drop to 25 per cent of the initial value.

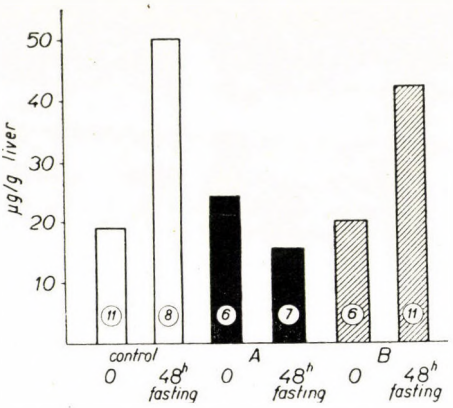

Fig. 5. Arsenocholine built into liver phosphatides in control rats and those with hypothalamic lesions. Ordinate : Amount of arsenocholine in As µg/g liver wet weight. Abscissa : White columns show the control, black columns the animals with antero-medial hypothalamic lesion, the shaded columns the rats injured in other areas

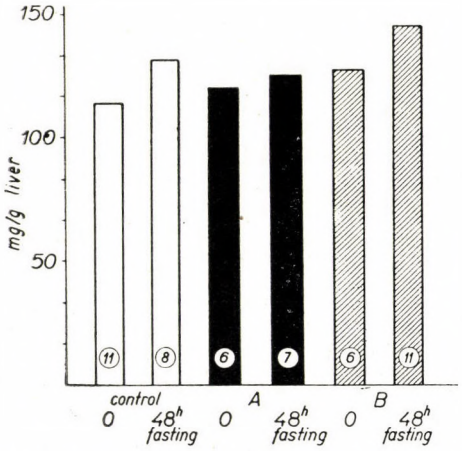

Fig. 6. Absolute quantity of phosphatides in normal rats and those with hypothalamic lesion. Ordinate : Amount of phospholipid inorganic P mg/g liver wet weight. Abcissa : White columns show the control rats, black columns the animals with antero-medial lesion of the hypothalamus, shaded columns the rats injured before or in other areas of the hypothalamus. The encircled numbers inside the columns show the number of animals studied

In the first group of injured animals, instead of a decrease, a mean 30 per cent increase occurred in the eosinophil count ; in the other group a mean 51 per cent decrease was registered.

Histological control of the exact site of the brain lesion revealed that in every animal where the daily fluid consumption was high and the Thorn test negative, the lesion had been inflicted in the antero-medial part of the hypothalamus. In the rats drinking little fluid and reacting normally to the Thorn test, the lesion was found either before or behind of the antero-medial part of the hypothalamus.

Comparing Figs. 5 and 6, as well as Tables I, II and III for greater detail, it may be seen how, as effect of fasting, the phosphatide metabolism of the liver changed in the control animals, in the rats injured in the antero-medial area of the hypothalamus, and in those lesioned in other areas.

Table I

Normal control animals

Non-fasted

Series No.	Weight of animal in g	Total As μg/g	Lipid As μg/g	Lipid P mg/g
1	126	91	16.1	1.07
2	104	105	—	1.16
3	108	80.5	14.3	—
4	125	118	16.5	1.08
5	125	105	30.3	1.05
6	110	107	25.3	1.23
7	138	101.5	18.9	1.28
8	112	83	17.8	1.22
9	125	92	13.8	1.17
10	120	178	9.2	1.05
11	116	131	29.5	1.16
		108.3 $\pm$8.2	19.1 $\pm$2.2	1.15 $\pm$0.02

As may be seen in Table I and in conformity with our previous results, in the control animals fasting caused the lipid As content to rise from 19.1$\pm$2.2 μg/g to more than twice as much, 49.2 $\pm$ 4.2 μg/g. This increase is statistically highly significant : $t = 6.510$; $P < 0.01$. In other words, in the non-fasting animals 42.0 μg/g arsenocholine was built into phospholipids, and in the fasting animals 108.2 μg/g. The lipid P content also increased on fasting, though the difference between the fasting and non-fasting animals was less pronounced : $t = 2.226$; $P < 0.02$. It follows that, under the given experimental conditions, in the non-fasting animals 0.7 per cent of the hepatic phosphatides contained arsenocholine, whereas in the fasting animals 1.6 per cent.

Fasted

Series No.	Weight of animal in g	Total As μg/g	Lipid As μg/g	Lipid P mg/g
1	100	128	44.4	1.22
2	105	138	29.0	1.55
3	106	177	67.0	1.61
4	110	101	42.8	1.43
5	96	167	62.5	1.56
6	111	138	51.6	1.06
7	128	116	54.0	1.07
8	102	149	43.6	1.10
		139.2 ±8.8	49.2 ±4.2	1.32 ±0.08

Fasted, non-fasted, lipid As. $t = 6.510$; $P < 0.01$
Fasted, non-fasted, lipid P. $t = 2.226$; $0.05 > P > 0.02$

Table II

Animals with lesion to the medial part of the supraoptic region of the hypothalamus

Non-fasted

Series No.	Weight of animal in g	Total As μg/g	Lipid As μg/g	Lipid P mg/g
1	138	129	31.4	1.23
2	175	135	25.4	1.26
3	148	106	7.3	1.16
4	103	124	18.6	1.07
5	128	153	26.2	1.25
6	125	117	29.2	1.26
		127.3 ± 6.5	23.0 ± 3.6	1.20 ± 0.03

In Table II are presented the fasting and non-fasting values for animals injured in the antero-medial area of the hypothalamus. The lipid As value for the non-fasting animals was 23 ± 3.6 μg/g, hence the amount of arsenocholine built in was 50.6 μg/g. In the fasting animals the quantity of lipid As was 15.9 ± 1.8 μg/g, *i.e.* 34.9 μg/g arsenocholine was built into liver phosphatides. There was no statistical difference between the values for fasting and non-fasting animals, for $t = 1.34$, $0.20 > P > 0.10$. The lipid P value for non-fasting rats was 1.20 ± 0.04 mg/g, and for the fasting ones 1.24 ± 0.04 mg/g.

Fasted

Series No.	Weight of animal in g	Total As μg/g	Lipid As μg/g	Lipid P mg/g
1	98	104	7.5	1.09
2	135	190	18.6	1.26
3	148	—	21.1	1.22
4	162	139	17.3	1.26
5	104	115	14.6	1.20
6	110	170	19.9	1.26
7	98	109	12.2	1.42
		137.8	15.9	1.24
		± 14.4	± 1.8	± 0.04

Non-fasted, fasted, lipid As. $t = 1.734$ $0.20 > P > 0.10$
Non-fasted, fasted, lipid P. $t = 0.850$ $0.50 > P > 0.20$

Evaluating the two figures statistically, $t = 0.850$, $0.50, > P > 0.20$. Of the total phospholipid content of the liver, arsenocholine containing phosphatides amounted to 0.8 per cent in the non-fasted animals, and to 0.6 per cent in the fasted ones. In this group of animals, then, phosphatide metabolism in the liver was not augmented on fasting as in the adrenalectomized animals.

Table III

Animals with hypothalamic and extra-hypothalamic injury at sites other than to the antero-medial cell group

Non-fasted

Series No.	Weight of animal in g	Total As μg/g	Lipid As μg/g	Lipid P mg/g
1	185	138	10.0	1.18
2	180	119	24.7	1.16
3	160	126	34.0	1.20
4	140	125	21.5	1.40
5	150	130	20.0	1.21
6	180	118	10.2	1.24
		126.0	20.0	1.23
		± 3.0	± 3.7	± 0.03

In Table III may be seen the values for those animals in which the lesion did not fall to the antero-medial area of the hypothalamus. The effect of fasting on this group of animals was to increase the incorporation of arseno-

Fasted

Series No.	Weight of animal in g	Total As μg/g	Lipid As μg/g	Lipid P mg/g
1	150	175	34.8	1.11
2	90	151	39.6	1.36
3	180	115	37.0	1.37
4	90	100	47.0	1.67
5	100	140	25.3	1.31
6	160	130	60.0	1.53
7	180	138	—	—
8	180	—	48.6	1.42
9	180	128	36.6	0.89
10	150	125	35.3	1.65
11	150	—	52.2	1.74
		133.5 ±	41.6 ± 3.2	1.40 ± 0.08

Non-fasted, fasted, lipid As. $t = 4.053$ $P < 0.01$
Non-fasted, fasted, lipid P. $t = 1.409$ $P < 0.10$

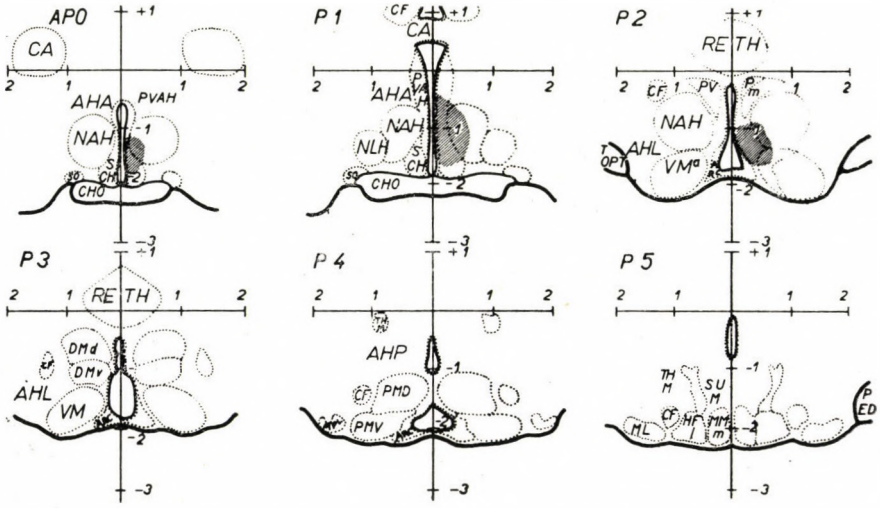

Fig. 7. Localization of hypothalamic lesion inhibiting the fasting-induced increase of phospholipid synthesis. The six schematic figures show the localization of cells in the different levels of the hypothalamus. The slices follow one another at a distance of 1 mm according to SZENTÁGOTHAI [11]. The cell boundaries are shown by lightly broken lines, the extent of the lesion in the different slices by continuous black lines. The area including all effective lesions has been shaded. The scale shown respresents millimetres. All lesions are bilateral in every case.

choline into liver phosphatides, just as in the control animals. The difference between the lipid As values for non-fasting rats (20.0 $\pm$ 3.7 μg/g) and those fasting (41.6 $\pm$ 3.2 μg/g) was statistically significant, $t = 4.053$, $P < 0.01$. As in the control animals, the quantity of phospholipids was not significantly elevated, $t = 1.409$, $P < 0.10$ since the lipid P amounted to 1.23 $\pm$ 0.03 mg/g and 1.40 $\pm$ 0.08 mg/g, respectively. In the non-fasting animals, 0.7 per cent of the phosphatides contained As ; in the fasting animals, 1.3 per cent.

Fig. 7 shows the localization of the hypothalamic lesions which inhibited the increase in phospholipid synthesis during fasting. The sites of all the lesions inflicted in the present experiments are shown in Fig. 7. The area, the injury of which in every case decidedly inhibited the enhancement of phospholipid turnover by fasting, is shown by shadowed lines. It may be seen that the area in question lies in the antero-medial part of the hypothalamus and touches the suprachiasmatic, filiform, anterior, arcuate, paraventricular, and medial nuclei.

Discussion

Our earlier investigations have shown that the sharp increase in phosphatide synthesis occurring after fasting is deterred by adrenalectomy. According to the literature and our own studies, certain cell groups of the hypothalamus influence adrenocortical function. After hypothalamic lesion, the adrenal cortex fails to respond to different influences. As to the question, which of the hypothalamic cell groups is the most influential in this respect, opinions in the literature are not uniform. Some authors found the increase in adrenocortical activity inhibited after lesioning the anterior hypothalamus, whereas others after injuring the middle or posterior areas [12, 13, 14].

In our previous investigations into the regulation of adrenocortical activity carried out in more than 500 rats it was shown that lesion of the antero-medial cell group of the hypothalamus affords the most effective protection against the eosinopenic reaction induced by adrenaline or the decrease in the ascorbic acid content of the adrenal cortex [15]. In animals with injured hypothalamus the sensitivity to ACTH decreases, but at the same time their sensitivity to cortisone does not differ from that of uninjured control animals. Lesions of other central, posterior or lateral areas also inhibit activation, but in this case the inhibitory effect wears off gradually in four to six weeks [16].

It is known that after lesions of the antero-lateral cell group of the hypothalamus the animals develop diabetes insipidus. The antero-lateral lesion — as has been mentioned — is not a specific inhibitor of adrenocortical activity. We have shown in numerous experiments that in some of the

animals with diabetes insipidus both the eosinopenic reaction to adrenaline and the ascorbic acid depletion occur just as in uninjured animals [17].

In view of the two above mentioned facts the animals in the present arsenocholine experiments were selected on the basis of their daily fluid consumption as well as according to the extent of their eosinopenic reaction.

The investigations on liver phosphatide metabolism made with arsenocholine were carried out in the third week after the hypothalamic lesion had been inflicted, as previously it was found that for one or two weeks after the lesion the aspecific stimulus of the injury had a disturbing effect [15].

From the present results it has been concluded that if the lesion is inflicted so as to affect the antero-medial cell group of the hypothalamus, phospholipid metabolism in the liver is the same as that of adrenalectomized animals, *i.e.* the increase of phospholipid metabolism released in normal animals by fasting remains entirely absent. In addition to a high daily fluid intake, it is typical of these animals that the eosinophil count is not decreased by subcutaneously administered adrenaline. Had some hypothalamic area other than the antero-medial been injured, phosphatide metabolism in the liver, the eosinophil response to adrenaline and fluid consumption were the same as in uninjured animals.

Our investigations, therefore, show that the changes brought about by fasting in liver phospholipid metabolism are under a neuro-hormonal regulation, via the hypothalamus, pituitary, adrenocortical system.

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Pathophysiologia

UNTERSUCHUNGEN ZUR TRENNUNG VON EIWEISS-MANGELHUNGERZUSTÄNDEN

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Im Verlauf isokalorischer Hungerzustände ist die Qualität der verbrauchten Eiweiße für Albinoratten von großer Bedeutung.

Die Folgen des Hungerns werden durch Verfütterung von Weizenkleber am wenigsten verschlechtert. Durch Verfütterung von Leim, Hefeeiweiß und Gelatine + Kaseinhydrolysat wird der Zustand der Tiere bedeutend verschlimmert.

Unter gleichen Verhältnissen wird die schädliche Wirkung der Kleberverfütterung durch Verabreichung einer größeren Klebermenge gemildert, während der Prozeß nach Darreichung der drei anderen mangelhaften Stickstoffquellen progrediert.

Durch Komplettierung konnte von den vier Eiweißmangeldiäten lediglich die schädigende Wirkung der Hefeeiweißnahrung nicht behoben werden.

Hunger und Eiweißmangel kommen beim Menschen gewöhnlich gleichzeitig zustande. Die menschlichen Hungerzustände gehen im allgemeinen mit Eiweißmangel und insbesondere mit erhöhten Mangel an den sog. qualitativ wertvollen Eiweißen einher. Als beispielsweise die Kalorienversorgung in Ungarn in den Jahren 1944—1945 infolge der kriegsbedingten Schwierigkeiten im Vergleich zum Normalen auf 80% gesunken war, betrug nach den Untersuchungen von Sós der Gesamteiweißverbrauch nur noch 50—60% und der Verbrauch tierischer Eiweiße bloß 20—30%. Die Frage, welche Bedeutung den mit Hungern verknüpften Eiweißversorgungsstörungen vom pathologischen Gesichtspunkt zukommt bzw. welche Wirkung die Anwendung verschiedener Eiweiße, die Veränderung und Komplettierung ihrer Menge auf die hungerbedingten Krankheitsprozesse ausüben, ist sowohl theoretisch wie in volksgesundheitlicher Hinsicht sehr wesentlich.

Methodik

Die Untersuchungen wurden an 74 ± 2 g schweren 200 Albinoratten in Angriff genommen, die wir in 20 Versuchsgruppen zu je 10 Tieren einteilten. Die Ergebnisse analysierten wir an vier allgemeinen pathologischen Erscheinungen: an der Veränderung des Körpergewichts, an der Gestaltung des relativen Gewichtes der Organe, an der Zusammensetzung der Blutserumeiweiße und an der Veränderung des Sauerstoffverbrauchs der Leberzellen. Mit Ausnahme einer normalen Kontrollgruppe (N) waren die anderen sog. halbhungernde Gruppen, die täglich eine der Hälfte ihres Kalorienbedarfes entsprechende Nahrungsmenge erhielten. Einer Gruppe, der hungernden Kontrollgruppe (NE), gaben wir normale Nahrung, den anderen die experimentelle. Die normalen Tiere verzehrten täglich durchschnittlich 10 g Nahrung, die anderen 5 g. Die Zusammensetzung der einzelnen Diäten zeigt Tabelle I.

Bei der Normalnahrung handelte es sich um das 18% Kasein enthaltende bekannte halbsynthetische Gemisch. Von den einseitigen N-Quellen verwendeten wir Leim, Hefe, Weizenkleber (Gliadin), Gelatine und ein säurehydrolysiertes, neutralisiertes und getrocknetes Kasein-Aminosäurengemisch.

Tabelle I

Prozentuale Zusammensetzung der einzelnen Nahrmengen

Jedes Gemisch enthielt 4% komplexe Salzmischung und 10% Fett, wovon 2% Lebertran waren

Bezeichnung der Diät	Diätart	Kasein	Leim	Kleber	Gelatine	Kasein-Hydr.	Getr. Hefe	Stärke
N	normal	18	—	—	—	—	4	64
F	eiweißreich	50	—	—	—	—	4	32
M	Leim	—	18	—	—	—	4	64
L	Kleber	—	—	18	—	—	4	64
S	Hefe	—	—	—	—	—	26	60
T	Gel.-Hydr.	—	—	—	9	9	4	64
MD	leimreich	—	50	—	—	—	4	32
LD	kleberreich	—	—	50	—	—	4	32
TD	gel.-hydr.-reich	—	—	—	25	25	4	32
KM	kompl. Leim	8	18	—	—	—	4	56
KL	„ Kleber	8	—	18	—	—	4	56
KT	„ Gel.-Hydr.	8	—	—	9	9	4	56
KS	„ Hefe	8	—	—	—	—	26	52
KMD	„ leimreich	8	50	—	—	—	4	24
KLD	„ kleberreich	8	—	50	—	—	4	24
KSD	„ hefereich	8	—	—	—	—	64	14
KTD	„ gel.-hydr.-reich	8	—	—	25	25	4	24
E	eiweißfrei	—	—	—	—	—	4	82

Der Leim war ein hauptsächlich methioninfreies Eiweiß und wies daneben in geringerem Maße auch Tryptophanmangel auf [12, 14]. Das Eiweiß des Hefemehles war von nekrogener Leberwirkung [6, 7, 14] und vor allem arm an S-Aminosäuren [14]. Der Weizenkleber war lysinarm [8, 9]. Die Gelatine- und Kaseinhydrolysate waren hauptsächlich tryptophanfrei [2, 3, 4, 14]; ihre anderen Mängel rückten auch dadurch in den Hintergrund, daß wir in den Diäten ihre Mischung im gleichen Verhältnis als N-Quelle benutzten.

Sämtliche N-Quellen wurden in normaler Menge (18% Eiweiß entsprechend), weiterhin in größerer Menge (50%) und außerdem auch mit 8% Kasein komplettiert verwendet. Die erhöhten Mengen der N-haltigen Substanzen wandten wir in den Nahrungen zu Lasten der Stärke an, d. h. die N-haltige Substanz war zu Lasten der Kohlenhydrate vermehrt.

Ergebnisse

Das Gewicht der normal ernährten Kontrolltiere stieg in der üblichen Weise (Abb. 1, Kurve N). Das Gewicht der mit Normalnahrung gefütterten, halbhungernden Tiere sank in 4 Wochen um 15% (Kurve NE). Das Gewicht der mit eiweißfreier Nahrung gefütterten, halbhungernden Ratten zeigte

36%ige Senkung, und die Hälfte der Tiere ging in der 4. Woche zugrunde (Kurve E). Das Gewicht der mit Lysinmangeleiweißdiät gefütterten (L) und zugleich halbhungernden Ratten liegt etwas unter dem der an Normalnahrung gehaltenen halbhungerden Tiere. Vom Verbrauch dieses Eiweißes wird jedoch der Hungerzustand nicht wesentlich verschlechtert. Der Verbrauch der Methioninmangel und Tryptophanmangeleiweiße führt ungefähr dieselbe

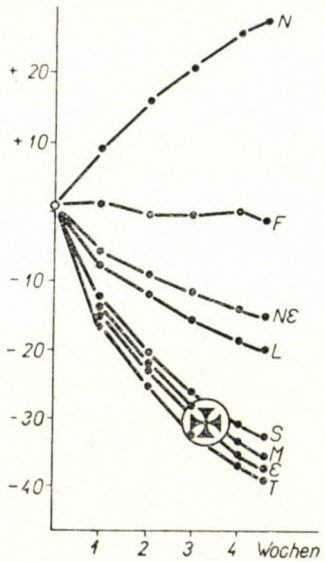

Abb. 1. Gewichtskurven der Ratten. Ordinate: Gewichtsveränderung in %. *N* = normale Kontrolltiere; *NE* = mit Normalnahrung gefütterte halbhungernde Tiere. Die anderen Bezeichnungen s. Tabele I. Anfangsgewicht 74 ± 2 g. Die Kurven entsprechen den Mittelwerten von je 10 Tieren. Die senkrechte Linie am Anfang der *N*-Kurve zeigt die Größe der Streuung (σ) an

Situation herbei wie die Anwendung der Eiweißmangelnahrung. Wie die Gewichtskurven S, M, E und T der Abb. 1 zeigen, kommt es in diesen Gruppen zu starker und nahezu gleicher Gewichtssenkung.

Es ist deutlich zu sehen, daß die Hungerzustände in drei Gruppen geteilt werden können. Unter den isokalorischen Hungerzuständen ist der jenige am wenigsten schädlich bei dem kein Eiweißmangel vorliegt (Gruppe F). Mittelschwer ist der durch Normalnahrung (NE) bedingte Hungerzustand, in dem das Kalorien- und Eiweißdefizit von gleichem Ausmaß ist, und ähnlich verhält es sich bei dem durch einseitigen Klebereiweißverbrauch bedingten Hungerzustand (L). Schwerere Wirkungen zeigen die partiellen Hungerzustände, die durch eiweißfreie, ferner infolge Tryptophan- und Methioninmangel einseitige Stickstoffversorgung ergebende Ernährungsformen verursacht sind.

Weitere Analysemöglichkeiten bieten die mittels Vermehrung und Komplettierung der Menge der einzelnen Eiweißarten durchgeführten Versuche (Abb. 2).

Die Folgen des Eiweißmangels des durch Kleberdiät bewirkten Hungerzustandes lassen sich durch Erhöhung des Klebergehaltes zum Teil beheben (Abb. 2, A, Kurve LD), noch besser jedoch durch Komplettierung mit Kasein

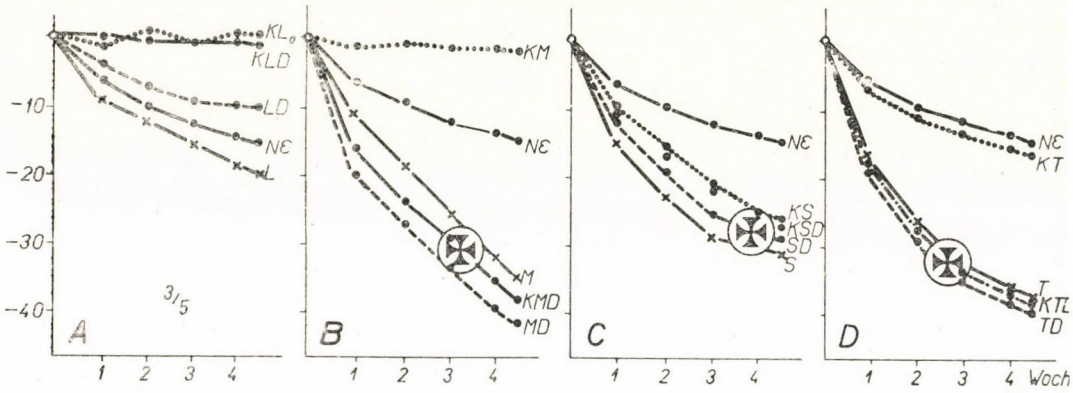

Abb. 2. Senkung der Rattengewichte im Falle 50%iger Kalorienversorgung mit verschiedenen eiweißhaltigen Diäten. Das + an den Gewichtskurven zeigt den Beginn der spontanen Verendungen an. *A* = Gewichtskurven der Klebernahrung (Lysinmangel); *B* = Gewichtskurven der Leimeiweißnahrung (Methioninmangel); *C* = Gewichtskurven der Hefeeiweißdiät (S-Aminosäurenmangel); *D* = als Stickstoffquelle diente ein Gemisch von Gelatine und säurehydrolysiertem Kasein (Tryptophanmangel). Die Bezeichnungen der einzelnen Kurven s.-Tabelle I

(Abb. 2, A, Kurven KL und KLD). Offensichtlich handelte es sich um einen einfachen, reinen Mangelzustand (Deficientia pura, vera). Kleber ist für seinen Lysinmangel bekannt, die Substitution ist in jeder Form erfolgreich. Die erzielte Gewichtskurve übersteigt die der bei Normalnahrung gehaltenen halbhungernden Tiere (Kurve NE) und erreicht den Wert, der dem Hungerzustand bei eiweißreicher Nahrung (Abb. 1, Kurve F) entspricht.

Die pathogene Leimeiweißwirkung wird unter gleichen Bedingungen nicht abgewehrt, wenn man mehr davon verabreicht (Abb. 2, B, Kurve MD), doch übt die Komplettierung mit Kasein normalisierenden Effekt aus (Kurve KM). Interessanterweise hat jedoch die Komplettierung der leimreichen Nahrung (KMD) die Lage nicht verbessert, was wahrscheinlich darauf beruhen dürfte, daß die größere Leimmenge das Aminosäurengleichgewicht der Nahrung derart verändert (imbalance), daß es von Kasein nicht mehr wiederhergestellt werden kann. Es ist aber auch möglich, daß dieses Eiweiß einen schwach toxischen Faktor enthält, der bei Anwesenheit von 18% Leim noch latent bleibt, aber bei Anwesenheit von 50% bereits schädlich wirkt.

Die Anwendung von Hefeeiweiß als N-Quelle ruft beim Hungern ebenfalls einen schweren Mangelzustand hervor, der sich weder durch Vermehrung

der Hefeeiweißmenge (von 18% auf 50%) noch durch Komplettierung (Beimischung von 8% Kasein) noch durch die Kombination der beiden Verfahren zufriedenstellend verbessern läßt (Abb. 2, Kurven des Teils C). Das Körpergewicht bleibt in allen Fällen beträchtlich unter dem der mit Normalnahrung gefütterten halbhungernden Ratten. Dieser Umstand läßt den Schluß zu, daß nicht nur einfacher Aminosäurenmangel, sondern auch toxischer Mangel (Deficientia toxica) vorkommen kann. Für diese Möglichkeit zeugt auch, daß mehrere Autoren nach Verfütterung größerer Hefeeiweißmengen schwere Lebernekrosen beobachtet haben. Bei unseren Versuchen war massive Nekrose nur bei 2 der 10 mit hefereicher Nahrung gefütterten Ratten eingetreten. Mikrohepatonekrosen waren indessen histologisch bei sämtlichen Tieren der Hefeversuche anzutreffen.

Das Verenden der mit Leimdiät und Hefediät gefütterten Ratten begann in der 3. Woche, woraus die Schwere dieser beiden Eiweißmangelhungerzustände gegenüber der klebergefütterten Gruppe noch stärker hervorgeht. Die Verlustgefahr beginnt bei etwa 30% Köpergewichtsabnahme.

Bei Anwendung von Gelatine und dem mit säurehydrolysiertem Kaseingemisch hergestellter Nahrung erleiden die Ratten schwere Gewichtsverluste und beginnen gleichfalls in der 3. Woche zugrunde zu gehen. Die Vermehrung der mangelhaften N-Quelle (TD) führt keine Besserung herbei, wohl aber die Komplettierung mit Kasein (Abb. 2, D, Kurve KT). Dadurch erreichen die Ratten die Gewichtskurve der Tiere, welche die normal zusammengesetzte Nahrung verbrauchen. Wird das komplettierende Kasein der mit einer großen Gelatine- und Hydrolysatmenge (25 + 25%) hergestellten Nahrung zugegeben, so bleibt seine günstige Wirkung ebenso wie bei den vorangegangenen 2 Gruppen auch hier aus (KTD).

Die Analyse der Gewichtskurven zeigt, daß die Fütterung mit den vier verschiedenen Eiweißen während der isokalorischen Hungerzustände jeweils eine andere Situation zustande bringt.

Eine allgemeine organpathologische Auswertung ermöglicht die auf das Körpergewicht bezogene Veränderung der Organgewichte.

Das relative Gewicht der Nebennieren war bei allen hungernden Gruppen wesentlich erhöht, was auf eine allgemeine, unspezifische Schädigung hinweist. Dieser Umstand entspricht dem SELYEschen Stressprinzip und bildet seit den Untersuchungen von VERZÁR und BEZNÁK [1, 16, 17] ein bekanntes Symptom einzelner Hungerformen. In dieser Hinsicht wurde die Lage weder durch vermehrte Zugabe der Eiweißart noch durch Komplettierung mit Kasein gebessert oder verschlechtert. Das Nebennierengewicht der normalen Kontrollen betrug 25 $\pm$ 3 mg/100 g, während das der Tiere in sämtlichen halbhungernden Gruppen zwischen 34—45 mg/100 g schwankte.

Ganz ähnlich war das relative Schilddrüsengewicht in sämtlichen Gruppen pathologisch erhöht. Gegenüber dem Wert von 12 $\pm$ 2 mg/100 g bei den norma-

len Kontrolltieren war das Schilddrüsengewicht der Tiere aller Versuchsgruppen relativ höher (18—22 mg/100 g) und konnte weder durch eine größere Menge der Eiweißmangeldiät noch durch Komplettierung normalisiert werden.

Bewertbare Differenzen zeigten sich indessen in der Entwicklung der Leber- und Nierengewichte. Das relative Lebergewicht der Kontrolltiere war 4,8 ± 0,3 g/100 g, das der bei Normalnahrung hungernden 4,0 ± 0,3 g/100g, d. h. verringert. Das relative Lebergewicht der bei eiweißreicher Nahrung halbhungernden Ratten fällt zwischen diese beiden Werte. Die Kleberdiät führte zu Werten, die gegenüber denen der hungerden Kontrolltiere erhöht und im wesentlichen normal waren. Das Lebergewicht der mit Leim und Gelatinehydrolysat gefütterten halbhungernden Tiere war beträchtlich erhöht (5,7 ± 0,4 g), konnte aber durch Komplettierung normalisiert werden. Bei den mit Hefeeiweiß ernährten Gruppen zeigte das Lebergewicht keine bewertbare Veränderung.

Das relative Nierengewicht hat bei Anwendung der Kleber- und Hefeeiweißdiät etwas, aber im Vergleich zu den normalen Kontrollen (1,00 ± 0,08 g/100 g) und halbhungernden Kontrollen (1,12 ± 0,10 g/100 g) nicht signifikant zugenommen. Methionin und Tryptophanmangeldiät bewirkten wesentliche Erhöhung (1,44 ± 0,12 g/100 g). Eine noch stärkere Organhypertrophie trat ein (1,52 ± 0,12 g/100 g), wenn wir größere Mengen dieser Stickstoffquellen gaben. Durch Komplettierung mit Kasein wurde das relative Gewicht der Nieren fast völlig normalisiert.

Aus der Gestaltung der Organgewichte kann demnach geschlossen werden, daß von den mangelhaft zusammengesetzten N-Donator-Nahrungen die Kleber- und Hefediät die schwächste schädigende Wirkung ausüben, während die Leim und Gelatinehydrolysat enthaltende unter gleichen Bedingungen schwer schädigenden Effekt zeigen.

Die Gesamtmenge der Blutserumeiweiße ist bei allen Hungerzuständen, wenn auch Eiweißmangel eingetreten ist, erniedrigt. Bei den an eiweißreicher Diät gehaltenen halbhungernden Tieren, die also an Kaloriendefizit ohne Eiweißmangel leiden, ist die Senkung der Serumeiweiße unbeträchtlich.

Sämtliche Hungerformen bewirkten die Senkung der Albuminfraktion und die Erhöhung der Globulin-, vor allem die relative Steigerung der γ-Globulinfraktion. Daraus ergibt sich auch die Senkung des A/G-Quotienten. Unter den von den vier verschiedenen einseitigen Eiweißdiäten bewirkten Hungerzuständen ruft die Fütterung mit Kleber die geringste Abweichung hervor (Tabelle II). N-reiche Diät und Komplettierung mit Leim bessern die Situation. In den anderen Fällen sind die Abweichungen nicht von solchem Ausmaß, daß die Differenz signifikant wäre. Deshalb haben wir die weiteren Angaben in der Tabelle nicht angeführt.

Die Atmung der Leberzellen haben wir im Warburg-Apparat auf übliche Weise am O_2-Verbrauch des Leberbreis gemessen. Zwecks Veranschaulichung

Tabelle II

Blutserumeiweiße bei verschiedenen Hungerzuständen

Die Gesamteiweißwerte entsprechen g/100 g, die Fraktionen der prozentualen Aufteilung dieser Werte

Fütterungsform	Gesamteiweiß	Globulinfraktionen					
		Alb.	α_1	α_2	β	γ	A/G
Normale Kontrolltiere	6,6 ± 0,4	59,1	8,5	7,1	14,6	9,9	1,48
Halbhungernde bei Normaldiät ...	5,8 ± 0,5	50,0	9,0	9,7	17,0	14,8	1,00
Halbhungernde bei eiweißreicher Diät	6,5 ± 0,4	55,8	7,0	7,3	14,4	16,2	1,24
Halbhungernde bei Kleberdiät	6,2 ± 0,4	56,5	5,2	6,8	14,4	16,8	1,30
Leimdiät	5,5 ± 0,5	51,7	7,4	11,4	15,8	13,5	1,07
Hefediät	5,6 ± 0,5	57,7	5,6	8,1	13,9	14,5	1,37
Gel.-Hydr.-Diät	5,8 ± 0,5	56,6	4,4	7,7	13,2	18,0	1,31

der bewertbaren Abweichungen zeigt Abb. 3 die 6 charakteristischsten der vielen Angaben. Der O_2-Verbrauch der normalen Kontrollen (N) weist in der 20.—70. Minute nach Exstirpation der Leber sinkende Tendenz auf, und die Senkung erstreckt sich bis zu 40%. Bei den halbhungernden (NE) und mit Leimmangeleiweiß halbhungernden Ratten (MD) ist der O_2-Initialverbrauch viel höher, sinkt aber in 70 Minuten auf denselben Wert wie bei den normalen Kontrolltieren. Die Lebern der mit Gelatine + hydrolysatreicher Nahrung

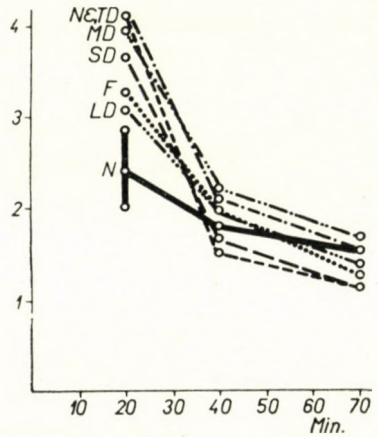

Abb. 3. Sauerstoffverbrauch des Leberbreis in vitro. Ordinate: O_2-Verbrauch ml/g Trockensubstanz/Stunde. *N* = normale Kontrollratten. Die senkrechte Linie am Anfang der Kurve zeigt den Streuungswert (σ) an. *NE* = bei Normaldiät halbhungernde Tiere; *TD* = mit stickstoffreicher Tryptophanmangeldiät gefütterte Tiere; *SD* = mit Hefeeiweiß gefütterte Tiere; *FD* = mit kaseinreichem Nährmittel gefütterte Tiere; *LD* = mit kleberreichem Nährmittel gefütterte Tiere; *MD* = mit leimreichem Nährmittel gefütterte Tiere

gefütterten Tiere (TD) verhalten sich bezüglich des O_2-Verbrauchs auf ähnliche Weise. Ganz ähnlich ist auch die Situation der mit hefereicher Diät ernährten Tiere. Die Werte der mit eiweißreicher und mit kleberreicher Diät gefütterten Tiere fallen anfangs zwischen die der vorigen und der normalen Kontrollen und zeigen später allmählich die gleiche Größenordnung. Der Effekt der Komplettierungen ließ sich nicht feststellen. Die Werte waren um die entsprechende eiweißreiche Kurve verstreut und von dieser nicht signifikant verschieden. Auch nach diesen Untersuchungen erscheint die Verfütterung des Gelatinehydrolysates am meisten pathogen und der Kleberverbrauch am wenigsten schädlich. Die Differenz ist bereits zwischen der N- und F-Gruppe signifikant, $P < 0{,}01$.

Obwohl der Grundumsatz im Hungerzustand bekanntlich sinkt, hat der O_2-Verbrauch bei den halbhungernden und Eiweißmangelgruppen im Vergleich zu den Kontrollen zugenommen. Die erwähnte Grundumsatzsenkung bezieht sich jedoch auf den Gesamtorganismus. GRANDE *et al.* beobachteten die Senkung des O_2-Verbrauchs auch an halbhungernden Menschen (5). Der Brei des isolierten Organs verhielt sich unter unseren experimentellen Bedingungen gegensätzlich. Zwischen den einzelnen Hungerformen entwickelten sich indessen Unterschiede.

Im Verlauf isokalorischer Hungerzustände führt demnach die Eiweißqualität sehr unterschiedliche Situationen herbei. Der Kleber- bzw. Cerealiaeiweißmangel verursacht die geringste pathologische Abweichung, und die Folgen des Mangelzustandes lassen sich durch Komplettierung und Verabreichung einer größeren Klebermenge beheben.

Die Verfütterung der als S-Aminosäuremangeldiät bekannten Leim- und Hefeeiweißnahrung löst bei nicht hungernden Tieren ungefähr denselben pathologischen Prozeß aus (VÉGHELYI *et al.* [15]). Die charakteristischsten Züge dieses Krankheitsbildes waren Pankreas- und Leberdegeneration [13]. Die vorliegenden, mit Hungern kombinierten Versuche ergaben in der Wirkung der beiden Eiweiße wesentliche Unterschiede. Die pathogene Wirkung des Leimeiweißverbrauchs kann man durch Komplettierung gut abwehren, den pathogenen Effekt des Hefeeiweißes jedoch nicht. Am stärksten pathogen wirkt die Gelatinehydrolysat enthaltende Diät. Vom Gesichtspunkt der Körpergewichtsabnahme führte ihre Komplettierung zum Erfolg, aber das relative Nieren-, Nebennieren-, Schilddrüsengewicht und der erhöhte Sauerstoffverbrauch der Leber blieben pathologisch. Schon allein aus diesem Befund geht hervor, daß neben dem Verhalten des Körpergewichtes auch andere allgemeine pathologische Anzeichen beobachtet werden müssen.

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ON THE HUMORAL TRANSFER OF RENAL HYPERTENSION

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Renal hypertension has been transferred humorally in rats, by means of parabiosis. This is another proof of the humoral mechanism of renal hypertension. Renal hypertension and renoprive hypertension induced in parabiotic animals show differences indicating that the two kinds of hypertension are based on different mechanisms and that the kidney has an active pressor function.

The humoral mechanism of renal hypertension has been proved by more than fifty years'research into the renal pressor system, as well as by the successful induction of hypertension after sympathectomy [1] or by producing ischaemia in the transplanted kidney [2].

On the other hand, contradictory evidence has been published as to the humoral transfer of renal hypertension. According to Braun-Menendez and Fasciolo [3], blood from animals with ischaemized kidney elevates the blood pressure of normal ones, but Govaerts [4] could not confirm this view. According to Fasciolo, Houssay and Taquini [2], blood of animals with renal hypertension exerts a vasoconstrictor action, but Mason [5] and Heymans [6] deny this.

Humoral transfer is rendered difficult by the fact that in the normal kidney renal pressor agents are inactivated. In all animal species but the rat elevation of blood pressure induced by the ischaemia of one kidney is prevented if the other kidney is left intact. The fact that one functioning kidney can prevent the development of renal hypertension, makes it clear that the humoral transfer of hypertension cannot be brought about without bilateral nephrectomy. However, blood pressure increases spontaneously in the nephrectomized animal, which is therefore unsuitable for such experiments [7].

According to Wilson and Byrom, in the rat unilateral renal ischaemia still gives rise to hypertension. This means that in this animal species the presence of one intact kidney does not prevent the development of hypertension [8].

On this basis we surmised that if renal hypertension is humoral in origin, it will be transferable to unilaterally nephrectomized rats.

Uniting such animals in parabiosis with animals suffering from renal hypertension, we succeeded in transferring high blood pressure humorally.

According to GROLLMANN and RULE [9], in rats renal hypertension cannot be transferred by parabiosis. The difference between their data and ours is due to the fact that we had induced more malignant hypertension and carried out unilateral nephrectomy in the normotensive rats.

Methods

1. *Parabiosis.* A total of 102 couples of albino rats of the same sex, weighing 150 to 250 g each and maintained on a standard diet, was used. Under ether anaesthesia skin and scapula sutures were placed according to BUNSTER and MEYER [10], followed by coelioanastomosis according to SAUERBRUCH and HEYDE [11]. Mortality rate varied from 20 to 30 per cent in the different groups. Since data in the literature indicate that capillary connections are established from the third day after uniting, the hypertension operation was performed on the fourth day, invariably on the animals on the left side, designated by the letter "A", as opposed to the animals on the right side, designated "B". Parabiotic connection was controlled at the last measurement of blood pressure, when Nembutal injected into animal "B" caused narcosis also in animal "A".

2. *Hypertension operation.* Renal hypertension was induced by the operation described by LŐRINC and GORÁCZ [12]. In brief, this operation consists of placing both kidneys into a slightly tight rubber ball, which causes ischaemia by increasing the intrarenal pressure. Of the known techniques, this method causes the most rapid elevation of blood pressure. As determined in 30 animals, the blood pressure response was comparable to that claimed by LŐRINCZ and GORÁCZ. The mortality rate was 60 per cent. Using 60 parabiotic couples, the operation was performed on the "A" animals, removing simultaneously the left kidney of the "B" animals. In 5 couples we controlled the effect of parabiosis on blood pressure, in 5 others a sham operation was performed ; this consisted in placing a loose rubber envelop around the spleen through an incision similar to the above, and left nephrectomy in the "B" animals.

In 7 couples bilateral nephrectomy was performed in the "A" animals and unilateral nephrectomy in the "B" animals. All these interventions were carried out under ether anaesthesia.

3. *Measurement of blood pressure.* Blood pressure was measured by the photoelectronic device of RÓZSA, GÁTI and WEISZ [17], under 4 mg/100 g of Nembutal injected intraperitoneally.

Results

One or two days after the LŐRINC—GORÁCZ operation 18 of the 30 animals died ; 2 developed uraemia and hypotension ; in 10 blood pressure rose in 2 days from 100 to 130 mm Hg to 160 to 238 mm Hg. Average blood pressure in these animals was 190 mm Hg. Extensive renal infarction and excessive hypertrophy of the adrenals were detectable in every animal.

In 5 couples the effect of parabiosis on blood pressure was studied for a period comparable to that of the experiment. Before uniting, blood pressure varied between 104 and 138 mm Hg, averaging 117 mm Hg. As determined at 2 days intervals over one week, the maximum deviation was 18 mm Hg, indicating that no significant change in blood pressure had taken place in any of these rats.

Two days after the sham operation the 5 couples involved showed a maximum increase of 11 mm Hg ; thus, the sham operation had no significant influence on blood pressure.

In 7 couples, bilateral nephrectomy was performed in animal "A" and unilateral nephrectomy was carried out in animal "B". In 2 days 3 o

the "A" animals showed an increase of blood pressure by 24, 34 and 39 mm Hg, respectively ; 2 showed no increase exceeding 10 mm Hg ; in 1 blood pressure dropped by 20 mm Hg. One couple was lost. In agreement with data in the literature [16], the elevation of blood pressure in animals "B" did not follow that in animals "A" in any of the cases.

In 60 couples the L RINC—GORÁCZ operation was performed in animals "A" and unilateral nephrectomy in animals "B". Eleven of these couples succumbed one day, 35 two days after operation. The 75 per cent mortality rate has been attributed to the extremely rapid rise in blood pressure, which the animals were unable to tolerate after the parabiosis operation.

In 10 of the 14 surviving couples blood pressure of "A" rose parallel with that of "B", in "A" from the 104—136 mm Hg level (average 120 mm Hg) to 160—250 mm Hg (average 195 mm Hg), and in "B" from 100—140 mm Hg (average 116 mm Hg) to 155—220 mm Hg (average 178 mm Hg). The maximum rise in the animals "A" was 138 mm Hg, the minimum 40 mm Hg, and the average rise was 75 mm Hg. In the animals "B" the maximum rise was 96 mm Hg, the minimum 45 and the average 62 mm Hg.

The detailed data are presented in Table I.

Table I

Changes in blood pressure in parabiotic animals

Animals "A" underwent hypertension operation ; animals "B", unilateral nephrectomy

Couple N°	Blood pressure of animal "A"		Blood pressure of animal "B"	
	before operation	2 days after op.	before operation	2 days after op.
	mm Hg		mm Hg	
1.	128	200	140	220
2.	112	250	126	200
3.	128	190	114	160
4.	104	220	124	220
5.	136	190	118	185
6.	120	160	112	164
7.	120	185	100	160
8.	120	178	100	160
9.	135	190	120	165
10.	100	190	110	155

It is clear from the above that the renal ischaemia in 2 days gives rise to severe hypertension which can be transferred from one parabiotic animal to the other. During the same period, in 2 days, bilateral nephrectomy raises blood pressure much less and this hypertension does not influence the blood

pressure of the other animal of the parabiotic couple. As LEDINGHAM [16] has failed to transfer grave renoprive hypertension in parabiotic animals, it is obvious that in such models the two kinds of hypertension behave differently. The difference between renal and renoprive hypertension is shown in Fig. 1.

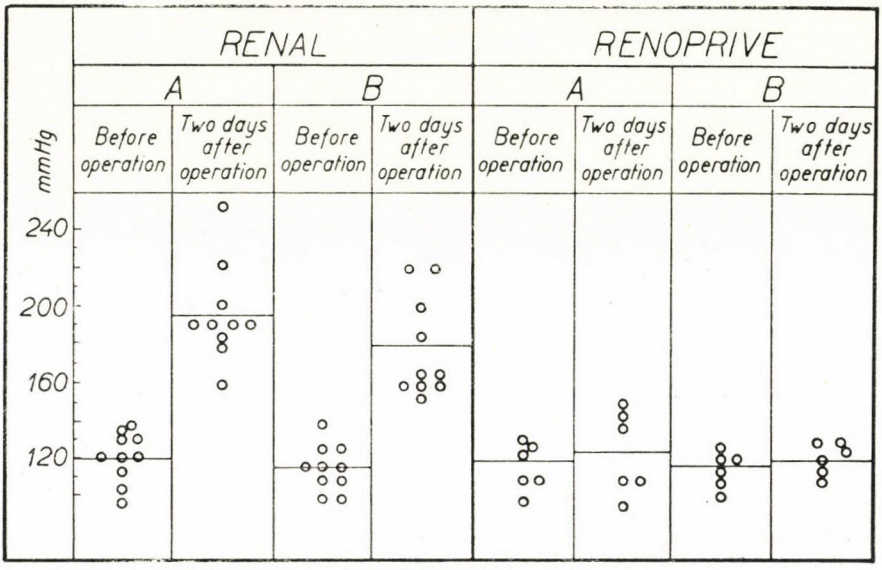

Fig. 1. Comparison of renal hypertension and renoprive hypertension in parabiotic animals Animals "A": bilateral renal ischaemia or bilateral nephrectomy. Animals "B": unilatera nephrectomy

Two days after operation the animals were killed. Histologic study revealed in animals "A" extensive renal infarction and a thickening of the fasciculate zone of the adrenals. The results of these studies have been described in detail elsewhere.

The left kidneys removed from animals "B" averaged in weight 392.3 mg/100 g. Two days after operation the average weight of the right kidneys was found to be 423.1 mg/100 g. This average increase of 30.8 mg/100 g means an unusual hypertrophy within the short time of two days.

Two days after operation the average adrenal weight for animals "A" was 21.7 mg/100 g, and for animals "B", 15.7 mg/100 g. The average for the controls from the same stock having been 13.2 mg/100 g, hypertrophy was very marked in animals "A" and slight in animals "B" (Table II).

In 3 couples the hypertension in animals "A" was not followed by an elevation of blood pressure in their "B" counterparts. Adrenal hypertrophy in the "A" animals and the renal hypertrophy in the "B" ones were comparable to what was found in the rest.

Table II

Changes in renal and adrenal weight (in mg/100 g of body weight)

Couple N°	Animal "A"	Animal "B"		
	adrenal weight	left kidney	right kidney	adrenal weight
1.	19.0	340	380	14.2
2.	18.8	380	450	13.1
3.	22.0	330	360	14.0
4.	23.5	380	450	19.3
5.	21.0	370	401	14.5
6.	28.5	400	450	15.7
7.	31.2	455	466	25.3
8.	14.0	330	390	12.6
9.	16.5	344	420	13.1
10.	23.0	413	470	15.0

Finally, in one case blood pressure was 135 mm Hg in animal "A" and 190 mm Hg in animal "B". Animal "A" died a few minutes after blood pressure had been recorded ; the paradoxical difference between the two partners was apparently due to a terminal drop in blood pressure.

Discussion

From the experiments it has been concluded that the blood of animals suffering from renal hypertension contains pressor agents capable of eliciting severe hypertension in normotensive animals. The rise in blood pressure in response to the humoral effect was so marked that it may be claimed that renal hypertension develops on the basis of a humoral mechanism. According to BLACKET *et al.* [13], renin does not raise blood pressure by more than 40 mm Hg. In our experiments the maximum rise was 96 mm Hg ; this suggested the contribution of some extrarenal factors. In our experiments on the role of the adrenal cortex, carried out in co-operation with GLÁZ and WEISZ, it has been found that adrenal hypertrophy accompanying hypertension is not associated with an increased corticosterone output.

The renal effects cannot be differentiated from the extrarenal ones and the problem requires a more detailed study. It remains to be investigated whether the behaviour of animal "B" is completely passive, or pressor agents are being produced during the experiment in that animal, too.

GROLLMANN [14] makes no distinction between renal and renoprive hypertension, suggesting that an active pressor function of the kidney has no significant role to play, either in ischaemia, or in nephrectomy experiments. Hypertension is invariably due to a failure of the kidney's antihypertensive

function. In 2 days time only half of the nephrectomized animals in parabiosis showed a rise in blood pressure and this was never followed by hypertension in the parabiotic counterpart. Renal ischaemia induced graver hypertension in every "A" animal, followed by an elevation of blood pressure in the "B" animal. The difference between the two experiments is, obviously, due to an active pressor function of the kidney.

BRAUN-MENENDEZ [15] united normal rats with animals in which hypertension had been induced by perinephritis ; after parabiosis the hypertension ceased. (We, too, obtained similar results with GROLLMANN's operation). In these experiments both kidneys of the "B" animals remained in place and the hypertension was also slight. Thus, the depressor function of the intact kidneys gained preponderance. In view of the marked hypertrophy of the kidneys in the normal animals, BRAUN-MENENDEZ suggested that "renotropic" substances would be formed in the ischaemized or nephrectomized animals and these would be responsible for the hypertension. The excessive renal hypertrophy observed in our present experiments corroborates the existence of renotropic substances. On the other hand, it does not seem probable that these should induce hypertension, because in our experiments renal hypertrophy developed also in those animals to which hypertension could not be transferred.

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Pharmacologia

RELATION BETWEEN THE STRUCTURE AND ACTION OF MORPHINE AND ITS DERIVATIVES

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In morphine derivatives, quaternarization of the tertiary N enhances the analgesic activity, although EDDY has claimed that the quaternary C atom linked to the tertiary N by a CH_2—CH_2 chain is an essential element of analgesic action. The potency of analgesic action does not change parallel with toxicity, respiratory depressant action or tolerance. The tested effects of the nor-compounds were weak. N-allyl-normorphine antagonizes better than diacetyl-N-allyl-normorphine the analgesic action of morphine derivatives possessing a free OH group, as compared to those substituted at this site. N-allyl-normorphine enhances, diacetyl-N-allyl-normorphine reduces respiration. The former compound is 1.63 times more potent in analgesic action than the latter.

SERTÜRNER, a young German chemist, published a most significant paper in 1806, in TROMMDORFFS Journal der Pharmazie, under the title: "Darstellung der reinen Mohnsäure nebst einer chemischen Untersuchung des Opiums mit vorzüglicher Hinsicht auf einen darin neu entdeckten Stoff und dahin gehörigen Bemerkungen" [1]. Ever since then, the pharmacology of morphine and its derivatives has been the subject of continuous research. In their work "The Pharmacology of the Alkaloids" KRUEGER, EDDY and SUMWALT [2] listed 9069 papers published before 1938. In this report entitled "Sites and Mechanisms of Action of Morphine and Related Drugs in the Central Nervous System", ABRAHAM WIKER [3] mentions 279 papers on morphine published between 1938 and 1950. The correlation between the structure and effect of morphine derivatives has been extensively investigated, for example by SCHAUMANN [4, 5], BRAENDEN, EDDY and HALBACH [6] in foreign countries, and KELENTEY *et al.* [7, 8] in this country.

We have analysed the actions of the morphine and codeine derivatives containing identical radicals prepared by Professor BOGNÁR [9], of the Institute of Organic Chemistry, Debrecen. The actions of these compounds on pain and respiratory volume, tolerance, toxicity, as well as the antagonizability of the analgesic action by N-allyl-normorphine and diacetyl-N-allyl-normorphine were examined. From the results conclusions have been drawn as to the relation between structure and effect and the nature of antagonism.

Methods

a) Analgesic action was measured by the method of WOLFE and MACDONALD [11] as modified by HERR and PÓRSZÁSZ [10]. Mice were placed on a tin cylinder filled with water of 54° C and by means of a stopper watch the appearance of the painful response (licking, shaking of a leg, or jumping) was recorded. This normal pain reaction time is designated by K_n. Thirty minutes after the subcutaneous injection of the analgesic drug, the prolongation of the pain reaction time was determined. Stock solutions were added diluted with physiological NaCl to contain the test compound in a concentration of 0.01 ml/g. Accordingly, the percentage of analgesic effect was calculated by the formula $(2.5\ K_n - K_n) : 100 = (K_{30} - K_n) : x$ where K_n is the normal duration of the response to pain and K_{30} is the prolonged reaction time, as measured 30 minutes after the injection of the test drug. A 100 per cent analgesic effect was accepted to be that causing a 2.5-fold prolongation of the normal reaction time. Each drug to be tested was examined in at least four doses, using for each dose 25 mice maintained on the same diet.

b) The effect on respiratory volume was measured by the method of DRESER [12], as modified by HERR and PÓRSZÁSZ [13]. The animal inspires through a Müller valve and the expired air drives out an equal volume of water from a vessel. After determining the normal respiratory volume, we measured the changes resulting $^1/_4$, $^1/_2$, 1 and 2 hours after the injection of the test drug by the subcutaneous route. Each dose was tested in 5 rabbits, making 5 measurements in every case.

c) In the tolerance experiments, groups of 25 mice were given daily near-100 per cent analgesic doses (see Table I) over a period of 3 weeks. From the reduction in the analgesic action conclusions were drawn as to the degree of tolerance. Administration was discontinued if these doses no longer had an analgesic effet, or if the response was greatly reduced.

d) In the studies on antagonism, the near-100 per cent analgesic dose of the test drug and a dose of N-allyl-normorphine or diacetyl-N-allyl-normorphine corresponding to $^1/_4$ of the near-100 per cent analgesic dose were injected together, subcutaneously. The analgesic action was measured as specified under *a)*.

e) In the toxicity tests, groups of 10 mice each were treated with subcutaneous injections of the different doses. Evaluation was made by the method of LITCHFIELD—WILCOXON[14].

f) In statistical analysis [15] the standard error of the mean was computed from the formula

$$s_{\bar{x}} = \sqrt{\frac{\Sigma(x-\bar{x})^2}{n\,(n-1)}}$$

where $(x - \bar{x})$ is the deviation of the single values from the mean and n is the number of experiments. The doses and the percentage activities belonging to them were plotted on log-probit paper, from which the ED_{50} could be read. The equation for the curve is given as

$$Y = b\,(x - \bar{x}) + y$$

where b is the regression coefficient, computed, from the formula

$$\frac{\Sigma\{(x-\bar{x})\,(y-\bar{y})\}}{\Sigma(x-\bar{x})^2}$$

and $(x - \bar{x})$, $(y - \bar{y})$ are the deviations from the mean of the single doses and effects, respectively.

Results

The drugs tested were morphine, dihydromorphine, 6-acetyl-morphine-metobromide, normorphine, codeine, dihydrocodeine, 6-acetyl-codeine, 6-acetyl-codeine-metobromide, norcodeine, heroine, N-allyl-normorphine and diacetyl-N-allyl-normorphine. The structures of these derivatives are shown in Fig. 1. R_1 means substitution at C of the 3rd position, R_2 at C of the 6th position, and R_3 at N. There is no double bond between C_7 and C_8 in dihydro-

codeine and dihydromorphine. 6-acetyl-morphine-metobromide and 6-acetyl-codeine-metobromide are quaternary derivatives.

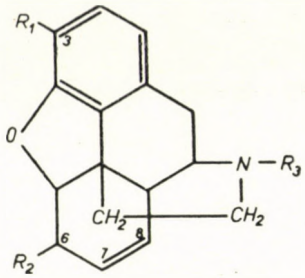

Fig. 1. Structure of morphine derivatives

N°	Compound	Radical		
		R_1	R_2	R_3
1.	Morphine	OH	OH	CH_3
2.	Dihydromorphine	OH	OH	CH_3
3.	6-acetyl-morphine-metobromide	OH	CH_3COO	$-\overset{+}{N}(CH_3)_2\,Br^-$
4.	Normorphine	OH	OH	H
5.	Codeine	CH_3O	OH	CH_3
6.	Dihydrocodeine	CH_3O	OH	CH_3
7.	6-acetyl-codeine	CH_3O	CH_3COO	CH_3
8.	6-acetyl-codeine-metobromide	CH_3O	CH_3COO	$-\overset{+}{N}(CH_3)_2\,Br^-$
9.	Norcodeine	CH_3O	OH	H
10.	Heroine	CH_3COO	CH_3COO	CH_3
11.	N-allyl-normorphine	OH	OH	$CH_2—CH=CH_2$
12.	Diacetyl-N-allyl-normorphine	CH_3COO	CH_3COO	$—CH_2—CH=CH_2$

The results for analgesic action and toxicity are shown in Table I. As regards their analgesic action, 6-acetyl-morphine-metobromide was twice, 6-acetyl-codeine 1.10 times, heroine 3.64 times as potent as morphine. On the other hand, normorphine was 11.88 times, codeine 7.31 times, dihydro-codeine 2.73 times, 6-acetyl-codeine-metobromide 3.79 times, norcodeine 24.46 times and N-allyl-normorphine 7.68 times less potent in analgesic action, as compared to morphine.

It is remarkable that 6-acetyl-morphine-metobromide was found twice as potent as morphine, and the same quaternary derivative of codeine 1.87 times as active as codeine. Quaternarization had no influence on toxicity.

Table I

No.	Compound	Analgesic						Equation of curve
		dose mg/kg	effect %	effect stand. error	AD_{50} mg/kg	Index Mo = 1	LD_{50} sc. mg/kg	
1.	Morphine	2	23.4	5.1				
		4	34.1	2.3	5.21	1	225	$y = 8.41 \times + 6.14$
		7	75.0	4.2				
		10	85.5	3.1				
		1	26.8	5.1				
2.	Dihydromorphine	5	44.3	7.5	5.75	1.10	140	$y = 4.34 \times + 25.03$
		10	80.3	5.8				
		15	83.3	6.8				
		0.25	26.9	5.5				
3.	6-acetyl-morphine-metobromide	0.5	51.2	4.9	0.31	0.06		
		1.0	71.9	6.6	2.62	0.50	228	$y = 5.52 \times + 48.26$
		5.0	87.5	7.2				
		10.0	96.2	5.4				
		20	38.5	5.2				
4.	Normorphine	40	43.9	8.1	61.9	11.88	140	$y = 0.27 \times + 33.27$
		60	76.5	5.2				
		10	33.1	5.5				
5.	Codeine	20	33.7	6.5	38.09	7.31	290	$y = 0.63 \times + 26$
		40	60.3	7.1				
		80	73.3	2.4				
		5	45.2	7.8				
6.	Dihydrocodeine	40	71.0	6.4	14.23	2.73	215	$y = 0.78 \times + 38.9$
		60	85.3	5.2				
		1	20.8	6.2				
7.	6-acetyl-codeine	2.5	42.5	7.7	4.71	0.90	120	$y = 6.40 \times + 19.8$
		10.0	83.3	5.3				
		5	32.3	6.6				
8.	6-acetyl-codeine-metobromide	10	47.8	5.7				
		40	73.5	6.9	19.74	3.79	282	$y = 0.55 \times + 39.14$
		80	77.3	5.8				
		60	16.5	5.5				
9.	Norcodeine	80	26.0	6.6	126.27	24.46	150	$y = 0.51 \times - 14.4$
		160	67.4	7.3				
		0.5	42	7.7				
10.	Heroine	1	42.6	6.9	1.43	0.27	50	$y = 10.25 \times + 35.3$
		3	68.3	7.5				
		5	85.6	3.9				
11.	N-allyl-normorphine	5	10.4	2.5				
		20	17.5	4.1	40.40	7.68	500	$y = 1.23 \times + 0.30$
		40	52.7	7.0				
12.	Diacetyl-N-allyl-normorphine	2.5	22.8	5.4				
		20	60.2	6.8	24.79	4.76	185	$y = 0.73 \times + 31.9$
		80	87.5	4.1				

Table II

N°	Compound	Action on respiratory volume					Equation of curve	Note
		dose mg/kg	effect %	stand. error	DRD_{50} mg/kg	Index Mo = 1		
1.	Morphine	1 2 4 6	38.5 57.5 53.7 62.0	1.7 2.9 2.2 2.8	3.29	1	$y = 7.11 \times + 26.6$	—
2.	Dihydro-morphine	1 2 4	12 44 65	1.4 2.6 2.4	2.97	0.9	$y = 16.8 \times + 0.07$	—
3.	6-acetyl-morphine-metobromide	0.5 1 4 8	26 38 48 58	2 1.6 1.9 1.4	5.46	1.66	$y = 3.73 \times + 29.6$	—
4.	Normorphine	16 32	23.8 31.6	3.6 4.6	70.45	21.41	$y = 0.48 \times + 16.18$	—
5.	Codeine	—	—	—	—	—	—	—
6.	Dihydrocodeine	4 8 16 24	16 30 46 68	6.0 3.4 2.6 1.0	17	5.16	$y = 2.5 \times + 7.5$	—
7.	6-acetyl-codeine	4 8 30	17 27 48	1.9 2.5 1.9	31.42	9.55	$y = 1.11 \times + 15.12$	—
8.	6-acetyl-codeine-metobromide	2 4 12 16	27.6 25 30 35	1.4 1.3 2.8 2.8	43.96	5.14	$y = 0.58 \times + 24.5$	—
9.	Norcodeine	—	—	—	—	—	—	—
10.	Heroine	0.25 0.50 1	16 42 60	7.7 4.0 1.4	0.77	0.23	$y = 55.82 \times + 6.98$	—
11.	N-allyl-normorphine	2 4 8 16	10.2 12 19 23	1.2 1.2 0.9 3.0	37.2		$y = 1.14 \times + 7.5$	increases! respira-tory volume
12.	Diacetyl-N-allyl-normorphine	1 2 8 16	18 0 25 39	1.9 2.1 5.0	17.57	5.34	$y = 2.72 \times + 2.2$	—

The LD_{50} of morphine is 225 mg/kg, that of its quaternary derivative was 228 mg/kg. The LD_{50} of codeine is 290 mg/kg, that of its quaternary derivative was 282 mg/kg. N-allyl-normorphine was 2.22 times less toxic, and diacetyl-

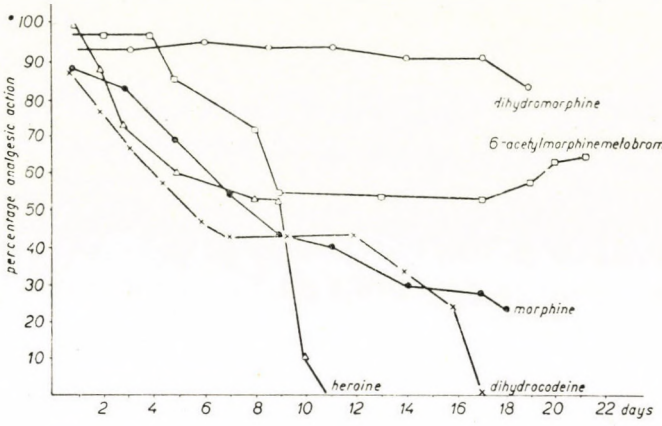

Fig. 2

N-allyl-normorphine 1.27 times more toxic, than morphine in intravenous mouse tests. Diacetyl-morphine was 3.7 times more toxic than diacetyl-N-allyl-normorphine.

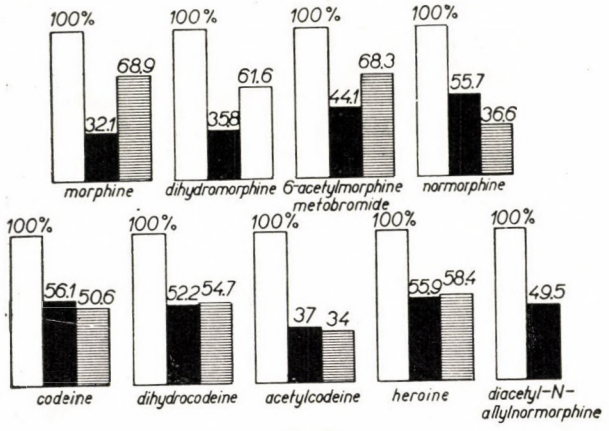

Fig. 3

The effect on the respiration of rabbits is shown in Table II. The percentage of action means the percentage increase or reduction in respiratory volume, as determined 2 hours after the subcutaneous injection of the test drugs. This point of time was chosen because, according to the evidence obtained by us and by others, for instance by BARGETON and KRUMM-HELLER

[16], the maximum respiratory response to subcutaneous doses of morphine and its derivatives may be expected to set in after 2 hours.

The dose of morphine reducing the initial respiratory volume by 60 per cent was 3.29 mg/kg. A similar effect was produced by 4.26 times less heroine and by 1.10 times less dihydromorphine. The other derivatives were less potent than morphine as regards its action on respiratory volume. N-allyl-normorphine augmented the respiratory volume. In the doses tested, codeine and nor-codeine had no influence on respiratory volume.

The results of the tolerance studies are shown in Fig. 2, with the days of administration on the x, and the percentage analgesic action on the y axis. To facilitate evaluation, we present the curves for heroine, dihydrocodeine, 6-acetyl-morphine-metobromide and dihydromorphine only. The tolerance curves for the other derivatives tested ran between those of morphine and 6-acetyl-morphine-metobromide.

Fig. 2 makes it clear that mice developed tolerance most easily to heroine and dihydrocodeine. The near 100 per cent effective analgesic dose of the former became ineffective after 11, that of the latter after 17 days. Tolerance to dihydromorphine developed the latest ; the analgesic effect was still 80 per cent after 20 days. The analgesic potency of morphine, 6 acetyl-morphine-metobromide and the other derivatives tested, but not represented in Fig. 2, varied between 30 and 50 per cent on the 23rd day.

The antagonistic effect of N-allyl-normorphine or diacetyl-N-allyl-normorphine on the analgesic action is shown in Fig. 3. The empty columns indicate the effect of the near 100 per cent analgesic doses of morphine and its derivatives. The solid and shaded columns show the reduction caused in that effect by N-allyl-normorphine and diacetyl-N-allyl-normorphine, respectively, administered in corresponding to $\frac{1}{4}$ of the near 100 per cent analgesic quantities doses.

Thus, with the exception of normorphine the analgesic effect of the morphine derivatives was inhibited more effectively by N-allyl-normorphine than by diacetyl-N-allyl-normorphine (lower solid columns and higher shaded columns, respectively). As indicated by the comparable heights of the solid and shaded columns, the two antagonists were about equally active on the analgesic action of codeine derivatives.

Discussion

Some of the results of these experiments agree with the generally accepted knowledge on the relation between the structure and action of morphine derivatives, whereas others are in disagreement with earlier claims.

According to BRAENDEN *et al.* [6], hydration of the double bond between C_7 and C_8 enhances the effect, though not in every case. We found dihydromor-

phine to be 1.10 times less potent than morphine in analgesic action, 1.6 times more toxic, and 1.17 times less active as a respiratory depressant. The ¼ dose of N-allyl-normorphine antagonized the analgesic action of the two compounds in an approximately equal measure. The same was the situation with diacetyl-N-allyl-normorphine. It is remarkable that mice developed tolerance to morphine in 18 days, a period during which the decrease in the effect of dihydromorphine was still slight.

Dihydrocodeine exerted an analgesic action 2.73 times weaker than that of morphine, but 2.67 times stronger than that of codeine. Thus, in this respect there was no analogy between codeine-dihydrocodeine and morphine-dihydromorphine. On the other hand, there was a similarity between the two couples of drugs in regard to the antagonizability of toxicity, and to the respiratory depressant and analgesic actions. Tolerance to dihydromorphine was slight, whereas tolerance to dihydrocodeine was developed sooner than that to morphine.

Likewise, the above-cited authors claim that the morphine derivatives containing quaternary nitrogen were ineffective. In contrast with this, we found 6-acetyl-morphine-metobromide to be an analgesic twice as potent as morphine, and 6-acetyl-codeine-metobromide an analgesic 1.87 times more potent than codeine. The toxicity of two quaternary derivatives did not differ from the corresponding tertiary ones. As a respiratory depressant, 6-acetyl-morphine-metobromide was about half as potent as morphine ; there was no parallelism between the analgesic and respiratory depressor actions. Lower tolerance, greater analgesic and lower respiratory depressor actions make 6-acetyl-morphine-metobromide therapeutically superior to morphine.

There was no parallelism between the analgesic action and toxicity of normorphine and norcodeine, either. These compounds were 11.88 times resp. 3.34 times less potent analgesics than morphine and codeine, respectively, whereas they were more toxic than the corresponding basic compounds.

It should be emphasized that N-allyl-normorphine was a more potent antagonist of the analgesic effect of morphine derivatives than diacetyl-N-allyl-normorphine. Both N-allyl compounds decreased the analgesic actions of codeine derivatives in about the same measure. The analgesic action of the morphine derivatives containing an esterified phenolic hydroxyl group was reduced less effectively by N-allyl-normorphine. It seems that morphine and its derivatives are connected by their groups on the C atoms 3 and 6 to the pain-sensory receptors of the body and axon of nerve cells. Phenolic hydroxyl and acetyl groups have the greatest affinity to these receptors. The derivatives possessing these groups are the most potent analgesics. The analgesic action of the derivatives containing a phenolic OH is antagonized most effectively by N-allyl-normorphine, which possesses a similar group. N-allyl-normorphine and diacetyl-N-allyl-normorphine compete about equally in the separation

from the receptors of such derivatives as contain other groups at C_3 and C_6. This theory will be proved only after morphine and N-allyl-normorphine become detectable on the body or the axon of the nerve cell by histochemical methods.

N-allyl-normorphine was 7.86 times less potent in its analgesic action than morphine. This finding is in agreement with the evidence reported by other workers [17]. Diacetyl-N-allyl-normorphine was only 4.76 times less potent as an analgesic than morphine. The former N-allyl derivative by itself enhanced, the latter reduced the respiratory volume in rabbits.

On the basis of the above results attempts have been made to assess the therapeutic value of the derivatives tested by comparing the AD_{50} (analgesic dose) with the LD_{50}. The AD_{50} of 6-acetyl-morphine-metobromide was found 87 times, that of morphine 43 times lower than the LD_{50}. The two drugs being comparable in regard to toxicity, 6-acetyl-morphine-metobromide may be claimed to be a better analgesic than morphine.

Heroine was found to have the most potent analgesic action in mice, yet we accept 6-acetyl-morphine-metobromide as the better analgesic agent, the latter being 4.5 times less toxic than the former. Likewise, among the compounds of satisfactory analgesic action, 6-acetyl-morphine-metobromide exerted the less depression on the respiration of rabbits. The doses causing a 50 per cent reduction of the respiratory volume were : 0.77 mg/kg of heroine ; 3.29 mg/kg of morphine : and 5.46 mg/kg of 6-acetyl-morphine-metobromide. To this may be added that tolerance to 6-acetyl-morphine-metobromide is only slight. These favourable properties of the compound call for clinical trials.

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STUDIES ON PROMETHAZINE

II. ANTIHISTAMINIC ACTIVITY OF ITS OPTICAL ISOMERS

By

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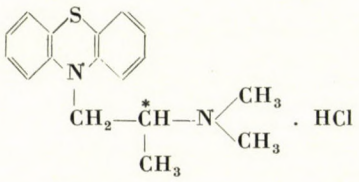
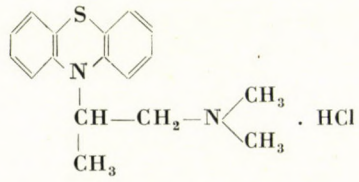
The optical isomers of promethazine have been investigated as to toxicity, antihistaminic activity and anaesthesia potentiating effect. The toxicity of promethazine was found to be identical with that of the (+) and (—) isomers. The same was true for the antihistaminic action (guinea pig intestine, blood pressure of the cat and histamine-induced asthma of the guinea pig) as well as for the anaesthesia potentiating effect. Accordingly, a difference must exist between the antihistaminic activity of the structural isomers of promethazine (iso-promethazine) described by other authors and that of the optical isomers investigated in the present study.

Promethazine (I) is well-known as a potent antihistaminic agent [1, 2]. CHARPENTIER and DUCROT [3], investigating the antihistaminic activity of the chain isomer (II), stated that the iso-form was 3 to 10 times less active than promethazine. Also N. D. EDGE [4] found promethazine to be a much more potent antihistaminic agent than the iso-form, even though not so specific.



I. Promethazine



II. Iso-promethazine

On account of the asymmetric carbon atom (C*) promethazine has two optical isomers (+ and — forms). Unlike the structural isomers, the optical isomers have not been isolated and their biological activity is still unknown.

To study this question, racemic promethazine base was resolved by means of dibenzoylic-D-tartaric acid [5]. Both the (+) and (−) forms obtained in this manner decompose at 220—221°C. The optical activity, $[\alpha]_{20}^{D}$ of (+) promethazine is +7.6° ; that of (−) form −7.6°.

In the present study the toxicity and antihistaminic activity as well as the anaesthesia potentiating effect of the optical isomers of promethazine have been investigated, as compared to the racemic form.

Methods

1. Toxicity

Toxicity was investigated in mice weighing 16 to 20 g. The preparations dissolved in physiological saline were administered intravenously at five dose levels. Evaluation of the results after 24 hours was made by the probit method of LITCHFIELD and WILCOXON [6]

2. Antihistaminic activity

a) Antihistaminic activity was investigated on guinea pig ileum, according to the method of MILLER, BECKER and TAINTER [7]. The pD of the preparations was determined with the modification that the basis of the dilution quotient was expressed in mg/l. The ED_{50} values were then computed from the data obtained in the above manner.

b) In cats under intraperitoneal chloralose-urethane anaesthesia (50 mg/kg and 40 mg/kg, resp.) it was investigated to what extent the individual compounds inhibit the hypotensive effect of histamine. Following two identical histamine reactions, the animals were treated with different doses of the inhibitors. The amounts causing 50 and 100 per cent inhibition, resp., were determined.

c) Inhibition of histamine-induced asthma in the guinea pig: untreated guinea pigs exposed to a 0.4 per cent histamine aerosol in a one-liter vessel developed within one minute grave dispnoe and cough, and lay on this side for a short time. When after careful observation the animals were taken out of the vessel at this stage, most of them survived and could be easily investigated at 30 minutes intervals. Guinea pigs pretreated with 0.5 to 1.0 mg/kg promethazine isomer or the racemic drug were examined at 30 minutes intervals through 3 hours and the incidence of asthma was compared to that simultaneously observed in animals pretreated with physiological saline. The effect of the drug was considered protective when, during an exposure time of 3 minutes, no sign preliminary to shock had developed.

3. Anaesthesia potentiating effect

In these experiments mice ranging in weight from 16 to 20 g were used.

First, the Hexobarbital dose was determined, the intravenous administration of which reliably made all animals to sleep but to awake within three minutes (controls). This dose was 37.5 mg/kg. The mice were pretreated with logarithmically increasing amounts of racemic, (+)-and (—)-promethazine, respectively, and one hour later, the standard Hexobarbital dose was administered. The number of animals not awaking within three minutes was determined. All drugs were administered at five dose levels, to about 40 mice each. From these data the ED_{50} value was computed by the method of LITCHFIELD and WILCOXON [6].

Results

1. Toxicity

The results are shown in Table I.

Table I

Intravenous LD_{50} values of racemic, (+)- and (—)- promethazine on mice

Compound	Number of animals	DL_{50} mg/kg intravenously	95% confidence limit		S*	*P*
			lower	upper		
Racemic promethazine	80	67	62.6	71.6	1.14	—
(+)-promethazine	80	70	66.2	72.4	1.34	>0.2
(—)-promethazine	80	64	60.1	67.8	1.22	>0.2

S* = Slope

The LD_{50} of racemic promethazine was found to be 67 mg/kg (95 per cent confidence limit : 62.6 to 71.6) ; that of the (+) isomer, 64 mg/kg (60.1 to 67.8) ; that of the (—) isomer, 70 mg/kg (66.2 to 72.4). The differences between the individual values proved statistically insignificant ($P > 0.2$).

Table II

Antihistaminic activity of racemic, (+)- and (—)- promethazine on guinea pig ileum

Racemic promethazine				(+)-promethazine				(—)-promethazine			
pD*	Positive / Investigated	%	P**	pD*	Positive / Investigated	%	P**	pD*	Positive / Investigated	%	P**
7.46	4/12	33.3	4.56	7.46	4/16	25	4.33	7.46	4/12	33.3	4.56
7.28	10/16	62.5	5.32	7.28	12/16	75	5.67	7.28	9/12	75	5.67

ED_{50} Racemic promethazine HCl = pD 7.4 $\pm$ 0.45 = $4 \cdot 10^{-8}$ mg/l
ED_{50} (+)-promethazine HCl = pD 7.35 $\pm$ 0.39 = $4.5 \cdot 10^{-8}$ mg/l
ED_{50} (—)-promethazine HCl = pD 7.35 $\pm$ 0.42 = $4.5 \cdot 10^{-8}$ mg/l

*pD = the dilution value expressed in mg/l
**P = probit

2. *Antihistaminic activity*

a) *Guinea pig ileum.* The antihistaminic activity of the three compounds is shown in Table II. As it may be seen, the ED_{50} of the isomers as well as their diffusion values (pD) were essentially of the identical order of magnitude.

Table III

Effect of racemic, (+)- and (—)-promethazine on the histamine-induced asthma

Compound	mg/kg s. c.	Number of animals	Protected animals, per cent					
			30′	60′	90′	120′	150′	180′
0.9% NaCl	1 ml	12	0	0	0	0	0	0
Racemic promethazine	0.5	12	33.3	33.3	33.3	33.3	25.0	16.7
	1.0	9	100	100	100	66.6	66.6	66.6
(+)-promethazine	0.5	12	50	33.3	33.3	33.3	33.3	16.7
	1.0	9	100	100	100	100	66.6	66.6
(—)-promethazine	0.5	12	33.3	50.0	33.3	33.3	33.3	16.7
	1.0	9	100	100	100	100	55	55

b) *Antagonistic effect towards histamine hypotension.* In an amount of 50 μg/kg, both racemic promethazine and the isomers abolished the depressor effect of the standard histamine dose. The approximate ED_{50} was 20 μg/kg with either of the compounds. A partial antagonistic action is shown in Fig. 1.

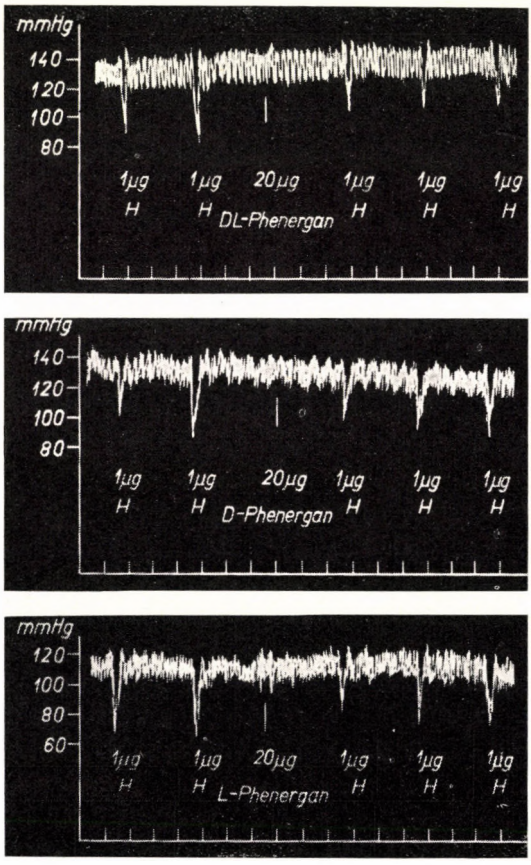


Fig. 1. Antihistaminic effect of promethazine isomers. Blood pressure. Cat weighing 3 kg. Chloralose-urethane anaesthesia. Time : 1 minute. H = Histamine dichlorhydrate

c) *Inhibition of histamine-induced asthma.* The results of these experiments are presented in Table III. Both the protective dose and the duration of action were practically identical with either of the three preparations ; 0.5 mg/kg protected for 2 to 2.5 hours one-third of the animals while 1 mg/kg afforded 100 per cent protection for 1.5 to 2 hours.

Table IV

Prolongation of Hexobarbital anaesthesia by racemic, (+)-and (—)-promethazine on mice

Compound	Number of animals	ED_{50} mg/kg i. p.	95 per cent confidence limit		S*	*P*
			lower	upper		
Racemic promethazine	206	5.0	3.85	6.5	3.35	—
(+)-promethazine	228	4.8	3.62	6.35	3.08	>0.2
(—)-promethazine	200	6.2	4.75	7.2	3.58	>0.2

S* = Slope

3. *Anaesthesia-potentiating effect*

Antihistaminic drugs are known to prolong barbiturate sleep [8]. The ED_{50} of the isomers investigated are shown in Table IV. The optical isomers did not differ in this test either ; the value was 5 mg/kg in the case of racemic promethazine ; 4.8 mg/kg with (+)-promethazine ; 6.2 mg/kg with (—)-promethazine.

Discussion

The toxicity, antihistaminic activity and central nervous action of the optical isomers of promethazine have been investigated. Unlike the iso-promethazine described by CHARPENTIER and DUCROT as well as by N. D. EDGE [3, 4], the optical isomers failed to show any difference in either of the above tests. Accordingly, the optical antipods of promethazine do not reveal such differences in activity as is usual with other kinds of drugs (*e. g.* the analgetics). The observation that the optical isomers of promethazine differ neither in antihistaminic effect nor in central nervous action indicates that the sensitivity to the individual optical isomers of the peripheral histamine receptors is the same as that of the central nervous system. Another conclusion from the above findings is that, as far as the pharmacological effectiveness is concerned, the separation of promethazine into optical isomers is superfluous.

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PHARMACOLOGICAL ACTIONS OF PANCREATIC ELASTASE

By

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The pharmacological properties of pancreatic elastase have been studied with the results as follows:

In mice and rats the intravenous toxicity of preparations with varying elastolytic activity proved to be the function of the elastase content. The more elastolytic a preparation is the higher its toxicity.

The toxic and subtoxic effects of the enzyme administered intravenously manifested themselves mainly in the lungs and spleen, organs rich in elastic fibres. The enzyme presumably plays a role in the maintenance of the state of collagenic tissue and it increases capillary permeability.

In cats under chloralose-urethane anaesthesia, elastase decreased blood pressure. The hypotensive effect of elastase was analysed. The findings suggest the hypotensive action of elastase to be due mainly to dilatation of the peripheral blood vessels and capillaries. However, the influence on the vasomotor centre should not be overlooked, either.

Studies performed on isolated organs suggested the biological behaviour of elastase to resemble that of crystalline trypsin.

Baló and Banga [1, 2] in 1949 isolated from ox pancreas a new enzyme which *in vitro* dissolved the elastic fibres of blood vessels. This proteolytic enzyme dissolving elastin was called "elastase". The biochemical properties made it possible to separate elastase from the other proteolytic enzymes (trypsin, chymotrypsin) present in the pancreas [3]. From that time on numerous authors have dealt with the purification and biochemical properties of elastase [4—10]. These investigations revealed the enzyme to consist of different components [9, 11], a fact very probably responsible for the manifold enzymatic action. Banga and Schuler [12], as well as Hall *et al.* [4], demonstrated the mucolytic activity of elastase, while a lipolytic activity was reported by Lansing *et al.* [5]. According to Saxl [13], the enzyme is involved in the formation of the "clearing factor".

The physiological and pharmacological properties of elastase have hardly been studied. Baló and Banga [14] demonstrated that the elastase content of the pancreas was considerably lower at those who had died with grave arteriosclerosis than in other individuals. Lansing *et al.* [15], investigating the systemic effects of the enzyme, stated that in cholesterol-fed rabbits the development of fatty liver and atheromatosis was inhibited by elastase treatment. On the contrary, Tennent *et al.* [16] found that elastase failed to prevent in chicken the development of arteriosclerosis.

The antiatherogenic action of the elastolytic enzyme has been pointed out recently by COHEN *et al.* [17]. BALÓ *et al.* [18] demonstrated that in the rat, on intraperitoneal administration of toxic doses, the enzyme produced extreme dilatation of the peritoneal capillaries and diapedetic bleedings in the abdominal cavity, with subsequent collapse and death. On the other hand, chronic administration of elastase induced hypertrophy and hyperplasia in the glandular tissue of the pancreas, with a comitant marked increase of the elastase content. KOVÁCS and BAGDY [30] administered high doses of elastase intrabronchially to rabbits, without any untoward consequences. LANSING *et al.* [19] demonstrated that elastase was produced in fish by pancreatic-α-cells. CARTER [20] found the elastase content of the dog pancreas to diminish on treatment with cobalt. In the dog the presence of elastase in the pancreatic juice was demonstrated by KOKAS *et al.* [21] and, in the rat, by COHEN *et al.* [17]. Accordingly, elastase may be ranged among the digestive enzymes.

As it seemed probable from the literary data that the new enzyme may play a part in arteriosclerosis [1, 2, 14, 15, 17], it seemed important to investigate its pharmacological properties.

The present report deals with experiments concerning the acute toxicity of elastase, as well as its action on blood vessels and on various smooth muscles.

Methods

The elastase used in the experiments was prepared according to the adsorption technique of BAGDY and BANGA [10]. The preparations, varying in elastolytic activity from 24 to 120 units/mg, were dissolved in 1/15 *M* phosphate buffer at pH 7.2 and diluted with physiological saline.

Toxicity. The investigations concerning the acute toxicity of elastase were performed on white mice weighing 16 to 20 g, and on Wistar rats ranging in weight from 120 to 160 g. The preparations were administered by the intravenous route. From the mortality rate observed after 24 hours, the LD_{50} values were calculated according to the probit method of LITCHFIELD and WILCOXON [22]. The wet weight of the organs was measured on a torsion balance.

Effect on the circulation. The effect of elastase on blood pressure was investigated in 45 cats under chloralose-urethane (50 mg/kg and 40 mg/kg, resp.) anaesthesia. Blood pressure was recorded from the carotid artery. The preparations were injected into the femoral vein. The adrenolytic and noradrenolytic actions of the preparations as well as their influence on both the carotid sinus reflex and the sympathetic ganglia were similarly studied in cats under the above-described anaesthesia. Investigation of the carotid reflex was made by compressing both carotid arteries for 30 seconds. In these cases blood pressure was recorded from the femoral artery. To investigate the adrenolytic and noradrenolytic actions, 5 to 10 μg/kg adrenaline, resp. noradrenaline was used. The ganglionic blocking action was measured by registering the contractions of the nictitating membrane after electric stimulation of the preganglionic fibres of the superior cervical ganglion of the cat.

Direct vasodilator action. These experiments were performed on the isolated hind limb of the cat under the above anaesthesia. The left hind limb of the animal was isolated from the body except for the femoral artery and vein. Both vessels were cannulated. The blood flowing through the isolated limb was conducted from the femoral vein into a drop-counter and then, after rewarming, reinfused into the right femoral vein. The vasodilator or vasoconstrictor effect of the preparations was judged by the changes in the number of blood drops flowing through the limb. The preparation investigated was introduced into the cannula of the femoral artery in a maximum volume of 0.1 ml. These experiments involved ten cats.

Capillary permeability. These experiments were carried out on 8 rabbits ranging in weight from 2 to 2.5 kg. Under urethane anaesthesia the abdominal wall of the animal was shaved ; 1 per cent trypane blue was administered intravenously and then the elastase dissolved in 0.2 ml physiological saline was administered by the intradermal route. The extent of dye-binding was registered half-hourly by measuring the diameters of the spot for 3 hours after the intradermal injection of the elastase.

Effect on the heart. The effect of the elastase on heart function was studied on the frog heart isolated according to STRAUB. The influence of the preparation on coronary flow was investigated on rabbit hearts isolated according to LANGENDORFF and by the method of NIESCHULZ, POPENDIKER and HOFFMANN [23] on alive rats. The animals weighing 120 to 160 g were anaesthetized with ether and lead II was recorded by means of "Orion EMG" type electrocardiograph. When 1 U/kg posterior pituitary total-extract was administered by rapid intravenous injection, this resulted in the elevation of the T-wave, on account of the constriction of the coronaries. The elastase was impeted intravenously 5 minutes prior to the pituitary extract and the per cent of animals protected from the elevation of the T-wave was determined.

Effect on smooth muscles. These experiments were made on excised rabbit intestine and uterus, on guinea-pig intestine, and on the uterus of rats pretreated with 100 μg/kg diethyl-stilboestrol for 1 to 2 days. Rabbit and guinea-pig intestines were suspended in 10 ml oxygenized Tyrode solution of 38° C. With the rabbit and rat uteri Tyrode solution poor in calcium was used and the experiments were performed at 30° C. The contractions evoked at 8 minutes intervals were registered by means of a frontal recorder (transmission 1 : 5).

The preparations used in the experiments *in vitro* were histamine dihydrochloride *(Chinoin);* Parenzyme aqueous (lyophilized trypsin, *National Drug Co.) ;* serotonine-creatinine sulphate *(Light and Co., Ltd.)* and kallikrein (Padutin, *Bayer).*

Results

Toxicity

In the mouse, the intravenous LD_{50} of different elastase preparations varied from 2960 to 4240 units/kg, with a mean of 3455 $\pm$ 207 U/kg. In the rat, one of the preparations showed an intravenous LD_{50} of 4400 U/kg. This same preparation gave, in the mouse, the identical value of 4240 U/kg. The results of these experiments are shown in Table I.

Table I

LD_{50} values of enzyme preparations with varying elastolytic activity

Species	Number of animals	Elastolytic activity in unit/mg	LD_{50} i. v. in mg/kg	LD_{50} i. v. in elastolytic unit/kg	s. e.*
mouse	60	100	35	3500	$\pm$ 150
,,	50	72	41	2960	$\pm$ 275
,,	60	40	106	4240	$\pm$ 182
,,	100	24	130	3120	$\pm$ 220
			Mean	3455 U/kg	$\pm$ 207
rat	45	40	110	4400 U/kg	$\pm$ 262

* s. e. = standard error

Some minutes following the intravenous administration of elastase, decreased motility, ataxia and flaccid paralysis were observed in both the mouse and the rat. Side position was, however, not tolerated. These effects developed after the injection of about 800 to 1000 U/kg enzyme. After higher doses some animals exhibited within a short time an unusual bloody-frothy discharge from the snares and the mouth. These animals soon died under convulsions. Post mortem the lungs showed unusual hyperaemia, oedema and markedly increased weight. The alveoli were filled mainly with blood or in some places with plasma.

Table II

Effect of elastase on the weight of the lungs, liver and spleen in the mouse

Standard dose = 180 mg/kg intravenously, of a preparation containing 24 elastolytic units/mg [= 4320 units/kg]

Group	Number of animals	Weight of animals, g	Weight of lungs, mg	Weight of liver, mg	Weight of spleen, mg	Weight change, per cent			Index			Spleen mg
						lungs	liver	spleen	lungs*	liver**	spleen ***	Lungs mg
Treated animals died within 30 minutes	79	17.5	309.2	—	—	+ 54	—	—	17.6	—	—	—
Controls.....	52	17.4	202.2	—	—	—	—	—	11.9	—	—	—
Treated animals died within 30 minutes	29	17.7	311.5	941.5	198.8	+ 56.0	— 7	∅	17.6	53.1	11.3	0.64
Treated animals *survived* 30 minutes	12	17.1	236	1281	485.5	+18	+30	+144	13.8	75.5	28.4	2.05
Controls.....	22	17.8	200	986.2	199.3	—	—	—	11.2	55.4	11.1	1.0

$$\text{* Lung index} = \frac{\text{weight of lungs, mg}}{\text{body weight, g}}$$

$$\text{** Liver index} = \frac{\text{liver weight, mg}}{\text{body weight, g}}$$

$$\text{*** Spleen index} = \frac{\text{spleen weight, mg}}{\text{body weight, g}}$$

Table II shows the increase evoked by toxic doses of elastase in the weight of the lungs. After the intravenous administration of 4320 U/kg enzyme, 80 to 90 per cent of the animals died under the signs of pulmonary oedema and pulmonary haemorrhage. The weight of the lungs increased on the average

by 54 per cent. The pulmonary index rose from 11.9 to 17.6. In animals dying within the shortest time following the above dose of the elastase, the weight of the liver and spleen as well as the index liver : spleen were hardly altered. On the other hand, in mice surviving the enzyme administered more than 30 minutes it was the spleen the weight of which augmented most (by 114 per cent) with a concomitant rise in the spleen index from 11.1 to 28.4. In these animals the liver became 30 per cent heavier and the liver index increased from 55.4 to 75.5, changes considerably less than those of the spleen. At the sam etime, of the thre eorgans they were the lungs which exhibited the slightest increase in weight and the lung index was augmented to a considerably smaller degree (from 11.2 to 13.8) than in the animals which died immediately after the administration of elastase.

Effects on the circulation

In the cat, the intravenous administration of elastase evoked a fall in blood pressure, the extent of which depended on the dose employed. 5 mg/kg (120 U/kg) of a preparation with an enzyme content of 24 U/mg caused a decrease of 15 to 50 mm Hg ; 10 mg/kg (240 U/kg) led to a fall of 20 to 60 mm Hg.

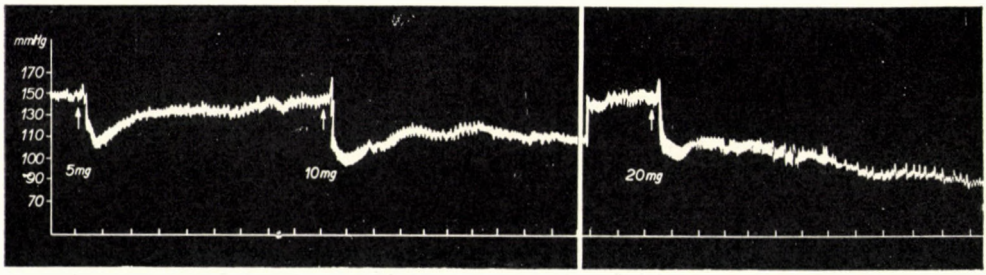

Fig. 1. Blood pressure decreasing effect of different doses of elastase. Cat: 3.2 kg. Chloralose Urethane anaesthesia. T : 130 mm Hg. E = Elastase 24 U/mg. Time 1 minute

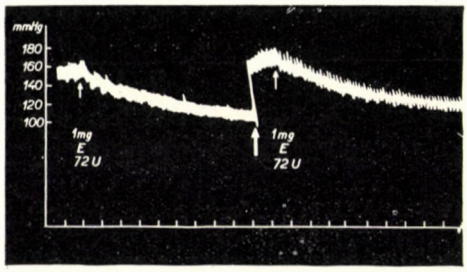

Fig. 2. Effect of vagotomy on elastase hypotension. Cat : 2.8 kg. Chloralose-Urethane anaesthesia. T : 160 mm Hg. Elastase 72 U/mg. Time 1 minute

Increasing the dose to 20 mg/kg (480 U/kg), the depressor response was hardly greater than that observed after 10 mg/kg, even though the action was prolonged.

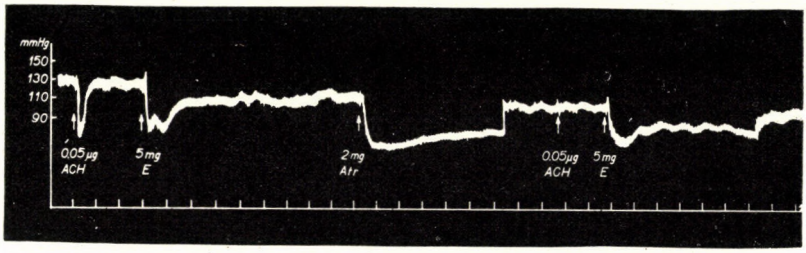

Fig. 3. Blood pressure decreasing effect of elastase before and after atropine treatment. Cat: 2.6 kg. Chloralose-Urethane anaesthesia. T: 134 mm Hg. E = Elastase 24 U/kg, ACh = Acetylcholine, Atr = Atropine sulphate

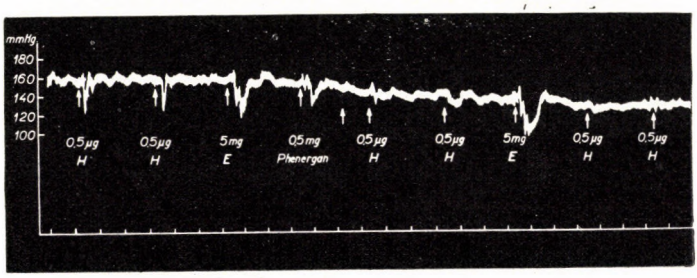

Fig. 4. Effect of promethazine on elastase hypotension. Cat: 2.9 kg. Chloralose-Urethane anaesthesia. T: 160 mm Hg. Time 1 minute

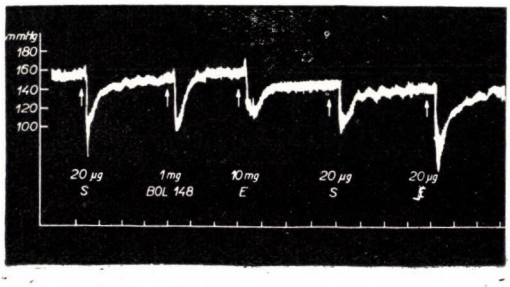

Fig. 5. Effect of BOL-148 on the hypotensive effect of elastase and serotonine. Cat: 2.8 kg Chloralose-Urethane anaesthesia. T: 160 mm Hg. Time 1 minute. E = Elastase 24 U/mg. BOL-148 = Brome lysergic acid diethylamide, S = Serotonine creatinine sulphate

In most cases small doses (2.5 to 10 mg/kg) caused only a transient fall in blood pressure, lasting 2 to 18 minutes, while the effect of greater amounts (20 mg/kg ± 480 U/kg) was sometimes irreversible. The extent and duration of the vasodepressor action markedly varied with the sensitivity of the animals.

In the greater part of the experiments a biphasic reaction, too, could be observed with a rise of 10 to 20 mm Hg some seconds prior to the fall of blood pressure. These effects are demonstrated in Fig. 1.

In the following experiments, the mechanism of this hypotensive action had to be studied.

Fig. 2 shows that, at a dose level of 1 mg/kg, the hypotensive action of a preparation containing 72 U/mg elastase was not influenced by vagotomy, while Fig. 3 demonstrates that 2 mg/kg atropine, too, failed to affect the hypotensive action of elastase. At the same time, the vasodepressor effect of the standard acetylcholine dose was abolished. Correspondingly, the vasodepressor action

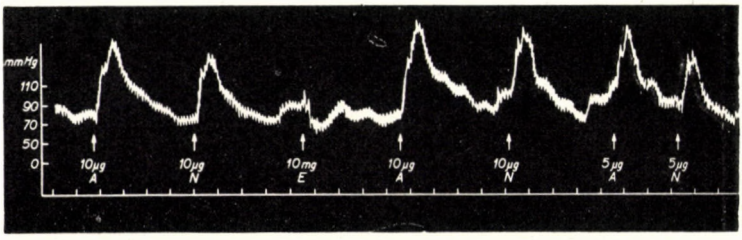

Fig. 6. Effect of Adrenaline on the blood pressure increasing effect of adrenaline and noradrenaline. Cat: 2.7 kg. Chloralose-Urethane anaesthesia. Atropine 1 mg/kg. Vagotomy. T:-90 mm Hg. Time 1 minute. A = Adrenaline, N = Noradrenaline, E = Elastase 24 U/mg

of the elastase was not due to a stimulation of the parasympathetic nerve endings. Neither was this action of reflex origin (BEZOLD-reflex) since vagotomy hardly influenced the extent of the depressor response.

The hypotensive action of the enzyme differs from that of histamine in that antihistaminic drugs failed to counteract the elastase action (Fig. 4).

Fig. 4 shows that the hypotensive effect of elastase manifested itself also after the administration of 0.5 mg promethazine by which the depressor response to the standard histamine dose was completely abolished.

Serotonine is known to induce a fall in the blood pressure of the cat. The question arose whether it was a contamination with serotonine that caused the preparation to lower blood pressure. This possibility was excluded by the finding that the action of elastase, unlike that of serotonine, was not inhibited by BOL—148 (bromo lysergic acid diethylamide). This is shown in Fig. 5.

It shows that 1 mg/kg BOL—148, while decreasing the hypotensive effect of serotonine, failed to inhibit the vasodepression induced by 10 mg/kg (240 U/kg) elastase.

From the investigations of ABELONS and BARDIER [24], as well as FREY, KRAUT and WERLE [25, 26, 27], it has been known for long that the pancreas contains a hormone with hypotensive action. This hormone was

called kallikrein (Padutin). It had to be decided whether or not the hypotensive effect of elastase and that of kallikrein could be distinguished from each other. For this purpose we relied on the known fact that kallikrein is adrenolytic in action.

As it can be seen from Fig. 6, elastase had neither adrenolytic nor noradrenolytic properties. In fact, it transiently somewhat potentiated the

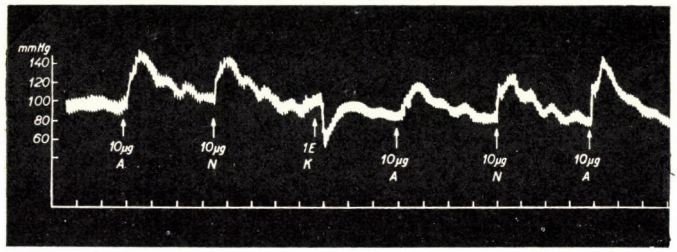

Fig. 7. Effect of kallikrein on the blood pressure increasing effect of adrenaline and noradrenaline. Cat: 2.7 kg. Chloralose-Urethane anaesthesia. Atropine 1 mg/kg. Vagotomy. T = 100 mm Hg

effect of both pressor amines, on the other hand, Fig. 7 shows that kallikrein decreased the effect of adrenaline and left unchanged the effect of noradrenaline. After the administration of 1 U/kg kallikrein the vasopressor effect of 10 μg/kg adrenaline decreased, while the effect of 10 μg/kg noradrenaline remained unaltered.

Summing up the above results, the hypotensive action of elastase was not counteracted either by vagotomy or by previous treatment with atropine. These facts indicate that the vasodepressor effect is not connected to an excitation of the parasympathetic nerve endings, or to that of the sensory fibres of the vagal nerve. The action of elastase differed from that of histamine not only in the character of the blood pressure change but also in the fact that antihistaminic drugs did not abolish its effect. Neither was the hypotensive action due to an eventual contamination with serotonine, since BOL—148, which inhibited the effect of serotonine left unchanged that of elastase. Elastase differs also from kallikrein, the hypotensive hormone known to be present in the pancreas. Elastase potentiated namely the effect of adrenaline and of noradrenaline, while kallikrein exerted an adrenolytic action.

Effect on the carotid sinus reflex

Considering that intravenously or intraperitoneally administered elastase was found to cause sedation and a decrease of spontaneous activity, the role played by central mechanisms in the hypotensive action of elastase was studied.

For this purpose we examined the influence of elastase on blood pressure rise following the compression of both carotid arteries. It was found that the intravenous administration of 120 to 240 U/kg elastase inhibited the

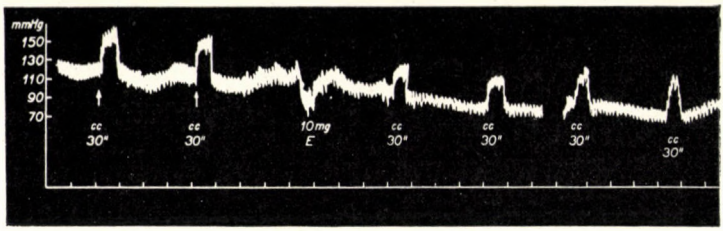

Fig. 8. Effect of elastase on blood pressure increase caused by the compression of bilateral carotis. Cat : 3 kg. Chloralose-Urethane anaesthesia. T : 130 mm Hg. E = Elastase 24 U/mg cc = carotis compression 30 sec

carotid reflex by 20 to 50 per cent. This is shown in Fig. 8. This effect was observed especially in the case of a prolonged hypotensive action (8 to 20 minutes).

Ganglionic blocking action

Hypotensive doses of elastase (120 to 240 U/kg) failed to exert any ganglionic blocking effect, as judged from tests on the nictitating membrane of the cat.

Table III

Effect of elastase on the isolated hind limb of the cat

Number of experiment	Substance investigated	Dose dissolved in 0.1 ml saline	Changes in the drop number after the injection, per cent					
			30 sec	60 sec	90 sec	180 sec	210 sec	240 sec
1.	Elastase	50 E. U.	+148	+72	0	—24	+19	+10
2.	Elastase	50 E. U.	+ 68	+12	—20	—12	+12	+ 5
3.	Elastase	50 E. U.	+ 38	+10	— 6	0	+ 6	+12
4.	Elastase	50 E. U.	+ 25	—15	—10	—15	—10	— 5
5.	Elastase	50 E. U.	+114	+76	+24	—27	—20	—20
6.	Elastase	50 E. U.	+ 56	+24	—20	—12	—10	+10
7.	0.9% NaCl	0.1 ml	0	0	0	+ 2	— 2	+ 5
8.	0.9% NaCl	0.1 ml	0	+ 5	— 2	+ 5	+ 2	+ 1
9.	0.9% NaCl	0.1 ml	+ 4	+ 2	+ 2	+ 2	+ 5	+ 3

Effect on peripheral blood vessels

Table III shows the results of the experiments performed on the isolated hind limb of the cat. Throughout the experiments the intraarterial injection of 50 U/kg elastase evoked vasodilatation lasting 0.5 to 1 minute, manifesting itself with a 25 to 148 per cent increase of the number of drops. This vasodilatation was followed by a slight reactive vasoconstriction.

Effect on capillary permeability

Elastase administered intracutaneously increased the capillary permeability in the shaven abdominal wall of the rabbit, since trypane blue introduced by the intravenous route was soon bound at the site of the elastase injection. The colouring of tissues was already observable after a dose as small as 2 U and was very marked after the administration of 10 to 20 U.

These findings indicate that capillary permeability was increased by the enzyme and that the binding of the circulating dye to tissues was also promoted.

Effect on the frog heart

On the frog heart isolated according to STRAUB, 20 to 50 U of elastase evoked a transient rise in the amplitude (Fig. 9). Higher doses (200 to 500 U) led to a diastolic standstill.

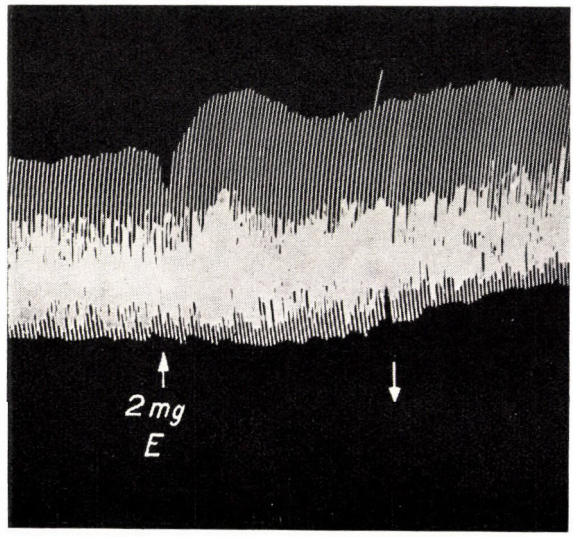

Fig. 9. Effect of elastase on frog heart. E = Elastase 24 U/mg

Effect on coronary flow

20 U of elastase produced slight coronary constriction on the rabbit heart isolated according to LANGENDORFF. This effect is demonstrated in Table IV.

Table IV

Effect of elastase on coronary flow in the isolated rabbit heart

Number of experiment	Control values in ml/min									
	1′	2′	3′	4′	5′	6′	7′	8′	9′	10′
1.	7.8	7.8	7.8 ↓	7.1	6.85	6.6	6.45	6.10	5.95	6.3
2.	6.0	6.1	6.0 ↓	6.1	5.8	5.6	5.9	5.85	5.9	5.9
3.	5.4	5.5	5.4 ↓	5.1	5.05	4.90	4.9	5.1	5.2	5.4
4.	5.9	5.9	6.0 ↓	5.6	5.5	5.5	5.6	5.9	6.0	6.0
5.	5.0	5.2	5.1 ↓	4.3	4.1	4.0	3.7	3.7	4.2	4.4
Mean ml.	6.05	5.98	6.05	5.65	5.46	5.32	5.31	5.33	5.45	5.6

↓ 20 U. elastase

Table V shows the influence of elastase on the coronary spasm of intact animals. 5 minutes following the administration of 100 U/kg elastase, posterior pituitary total-extract caused elevation of the T-wave in 50 per cent, whereas in 100 per cent of the controls. At the same time, 2 × 10 mg/kg papaverine hydrochloride inhibited the pituitary effect by 41 per cent.

Table V

*Effect of elastase on coronary spasm in the rat**

Number of animals	Substance investigated	mg/kg i. v.	Occurrence of coronary spasm, per cent
10	0.9% NaCl	0.2 ml/kg	100
12	Elastase	1 mg (= 100 E. U.)	50
11	Papaverine HCl	1 × 10	64
12	Papaverine HCl	2 × 10	59

* Coronary spasm was induced by rapid intravenous injection of 1 U/kg posterior pituitary total-extract. The substance investigated was given 5 minutes prior to the administration of the hormone, or, when given twice, 10 and 5 minutes before.

From these data it has been concluded that elastase produced a slight coronary spasm in the isolated mammalian heart while in intact animals it somewhat inhibited the coronary spasm induced by posterior pituitary total-extract.

Effect on smooth muscles

JAQUES and SCHÄR [28] demonstrated that trypsin evoked specific contractions of the excised uterus of rats in oestrus. Similarly, the enzyme increased the tone of excised rabbit intestine. On guinea-pig intestine contractions were evoked only at toxic concentrations which then decreased the sensitivity to histamine.

The effect of elastase was investigated on infantile rabbit uterus, rabbit intestine, guinea-pig intestine and the excised uterus of rats sensitized by diethylstilboestrol. In most experiments the effect of elastase was compared to those of trypsin, kallikrein, as well as of organospecific drugs.

2.4 U/ml elastase, 0.1 mg/ml trypsin or 0.2 U/ml kallikrein failed to evoke contractions of the rabbit uterus, nor did they influence the contractions caused by 0.1 μg/ml adrenaline.

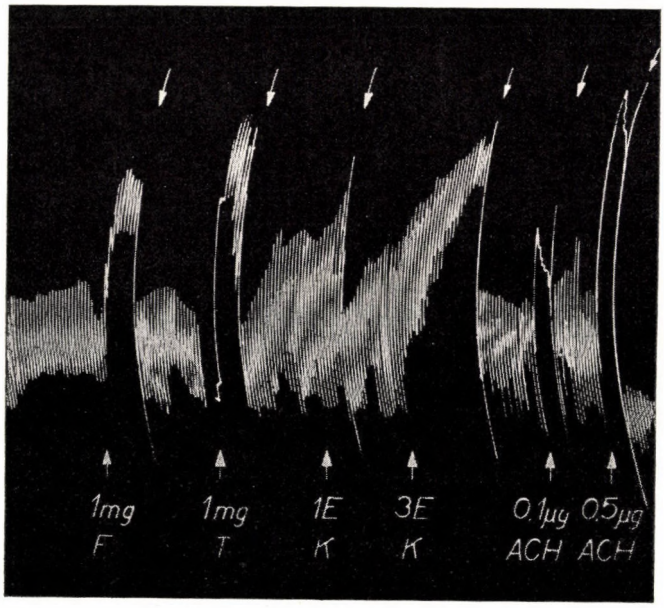

Fig. 10. Effect of elastase, trypsin, kallikrein and acetylcholine on rabbit intestine. The doses refer to 10 ml. E = Elastase 24 U/mg. T = Trypsin, K = Kallikrein, ACh = Acetylcholine

On excised rabbit intestine, 0.05 to 0.2 mg/ml (1.2 to 4.8 U/mg) elastase first decreased peristalsis and then caused an easily reversible rise in tone. Similar effects were produced by 0.05 to 0.1 mg/ml trypsin. 0.1 U/ml kallikrein was ineffective, while 0.2 to 0.3 U/ml increased the tone of the intestine. This effect is shown in Fig. 10. The sensitivity to acetylcholine of the organ was not influenced by 0.1 mg/ml (2.4 U/ml) elastase.

When 0.05 to 0.4 mg/ml (1.2 to 9.6 U/ml) elastase was added to guinea-pig intestine, only the higher doses initiated a minimum increase in contraction and peristalsis. The effect resembled that of 0.1 U/ml kallikrein. 0.1 mg/ml trypsin caused marked contractions; this effect was difficult to stop by washing the intestine. The results are shown in Fig. 11.

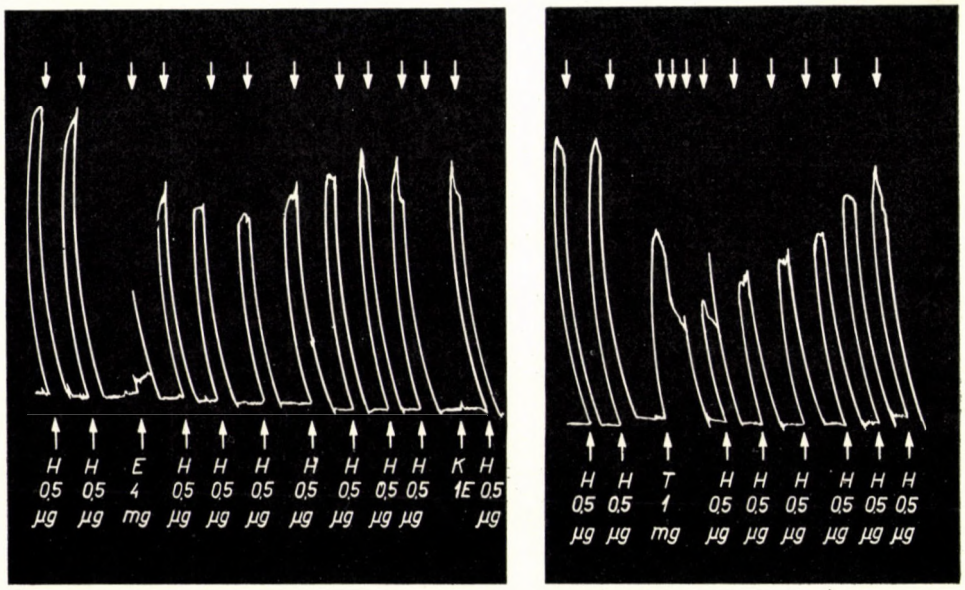

Fig. 11. Effect of elastase, trypsin and kallikrein on guninea-pig intestine. The doses refer to 10 ml. E = Elastase 24 U/mg, K = Kallikrein, T = Trypsin, H = Histamine dichlorhydrate

In the case of elastase, the change in the sensitivity to histamine proved to be a function of both the dose and the incubation time. Fig. 12 shows for instance the effect of 0.1 mg/ml (2.4 U/kg) elastase on the histamine sensitivity at various incubation times. Incubation for 1 minute did not influence the histamine sensitivity, while incubation for 3 minutes induced first a short rise, then a decrease in sensitivity. The effect was even more marked after incubation for 6 minutes.

On the other hand, 0.4 mg/ml (9.6 U/ml) elastase decreased the contraction evoked by the standard histamine dose already after incubation for 1 minute (Fig. 11). 0.1 mg/ml trypsin after incubation for 1 minute, also lowered the sensitivity, while kallikrein was ineffective after incubation for 1 to 3 minutes. In the case of elastase and trypsin, the decrease of histamine sensitivity was probably the consequence of a toxic effect and not that of some specific action.

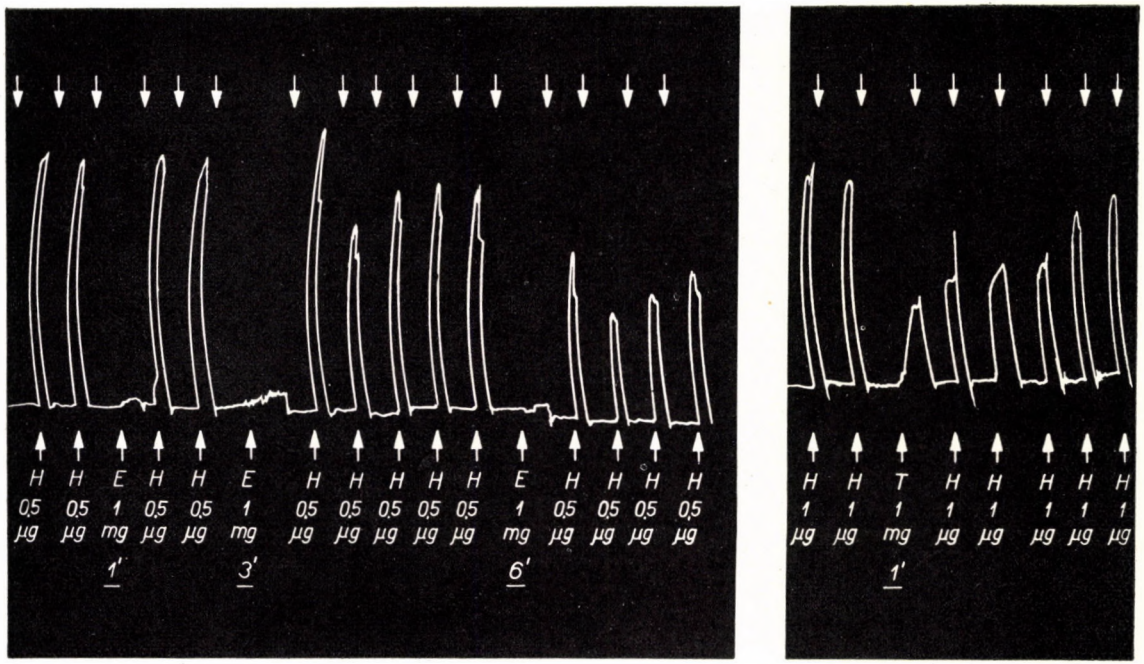

Fig. 12. Effect of elastase on histamine contraction at different incubation times. The doses refer to 10 ml. H = Histamine dichlorhydrate, E = Elastase 24 U/mg, T = Trypsin

On the rat uterus, 5 to 10 μg/ml (0.12 to 0.24 U/ml) elastase evoked, after a latency of 10 to 15 seconds, marked contractions. The height of the contractions was the function of the dose employed. The spasmogenic effects of different doses of elastase and trypsin are shown in Fig. 13.

Fig. 13 shows further that about 10 μg/ml (0.24 U/ml) elastase produced an effect comparable to that of 0.5 μg/ml trypsin. It seems that the uterus of rat

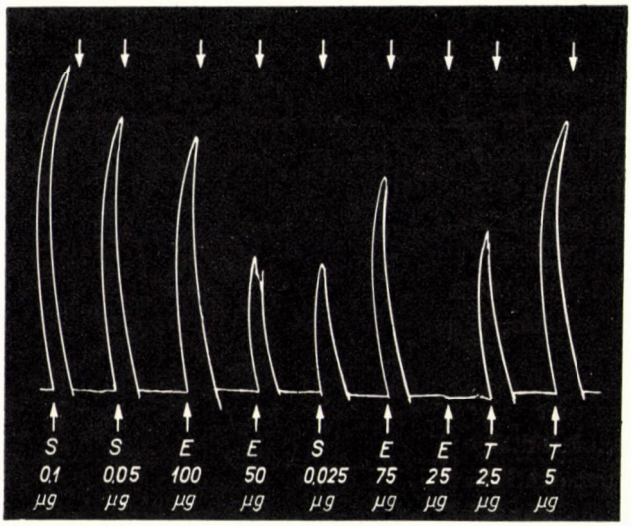

Fig. 13. Effect of elastase, trypsin and serotonine on the uterus of rat. The doses refer to 10 ml. S = Serotonine creatinine sulphate, E = Elastase 24 U/mg, T = Trypsin

in oestr us represents the smooth muscle organ most specific for proteolytic enzymes. 0.1 U/kg kallikrein and also histamine were namely ineffective on this organ.

Discussion

Fro m among the pharmacological properties of elastase investigated in the present study, the toxicity of preparations with varying elastolytic activity was found to depend on the number of elastolytic units contained. The higher the unit number, the more toxic was the preparation. When the DL_{50} values of the individual preparations were converted from mg into elastolytic unit, the intravenous LD_{50} of the enzyme turned out to vary in the mouse between 2960 and 4240 U/kg, with a mean value of 3455 $\pm$ 207 U/kg. Toxicity was the same in the rat.

After the intravenous administration of the enzyme, the symptom characteristic of its toxic doses was the appearance of bloody froth discharge from the snares and mouth of the animals. Post mortem a marked hyperaemia

of the lungs as well as an increase in pulmonary weight was found. The weight of the lungs was augmented by 54 per cent and so did the pulmonary index. At the same time, the weight of the liver and spleen did not change and intestinal hyperaemia was rarely observed. BALÓ *et al.* [18] administered elastase to rats intraperitoneally and observed hyperaemia of the intestines due to the capillary dilatation evoked by the enzyme. This phenomenon rarely occurred in our animals which had been treated intravenously and died within a short period of time. In the latter case toxic manifestations were restricted almost exclusively to the lungs. Pulmonary tissue rich in elastic fibres, seems to be particularly sensitive to the enzyme and therefore the capillary damage appears earlier in the alveoli than in other organs.

The intraalveolar septum is known to contain collagenic tissue, rich in elastic fibres. It can be assumed that, on account of its elastolytic activity, the enzyme affects the state of the collagen and this results in an increased permeability of the capillaries. The wall of blood vessels thus becomes permeable for exudate and even for cellular elements.

PRASAD [29] pointed out that, apart from H-substances, certain enzymes are also involved in the genesis of pulmonary oedema. According to our findings, elastase with its elastolytic and proteolytic activity is capable readily to increase capillary permeability in pulmonary tissue. The results of our rabbit experiments concerning capillary permeability also revealed the enzyme to enhance the adsorption of dye to the tissues as well as to increase capillary permeability. Our findings, together with the results of BALÓ *et al.* [18], indicate that elastase produces capillary dilatation on both intravenous and intraperitoneal administration. In mice and rats treated intravenously this effect is, however, restricted almost exclusively to the lungs.

If the animals had survived the intravenous administration of a high dose of elastase the situation was considerably changed. Apart from intestinal hyperaemia an extreme increase occurred in both the spleen weight and the quotient spleen : lungs. This latter was 1.0 in normal animals ; 0.64 in those which had died shortly after elastase administration and 2.05 in those that had survived the intervention. In addition, the weight of the liver rose by some 30 per cent. This increase, though lesser than that of the spleen, still greater than that of the lungs. It was remarkable that on administration of subtoxic doses of elastase, the effect of the enzyme manifested itself not in the lungs but rather in another mesenchymal organ rich in elastic fibres. To establish the cause of this phenomenon, further investigations are needed.

As to the biological effects of elastase, 120 to 480 U/kg of it evoked hypotension in the cat. In most cases this decrease in blood pressure was transient and did not resemble the effect of either acetylcholine, histamine or serotonine. The fall of blood pressure was not inhibited by vagotomy or by atropine, a fact indicating that the hypotension was not due either to

an excitation of the sensory endings of the vagal nerve (BEZOLD-reflex) or to that of the parasympathetic nerve endings.

The elastase-induced hypotension was not abolished by antihistaminic drugs. This observation excluded the possibility that an eventual contamination of the preparation with histamine had caused the blood pressure to lower. Another finding contradicting the histamine action was that elastase caused spasms on the uterus of rats in oestrus, while histamine was ineffective on this organ.

Elastase resembled serotonine in bringing about contractions of the rat uterus. But the blood pressure decreasing effect was not attributable to contamination with serotonine, since the serotoninolytic BOL—148 (bromo lysergic acid diethylamide) inhibited hypotension caused by serotonine but not that evoked by elastase.

Kallikrein, the hormone isolated from the pancreas, is known to exert a hypotensive action [24—27]. Our experiments revealed that kallikrein eventually present in the preparation had no role in the blood pressure decreasing effect of elastase. Kallikrein inhibited namely the adrenaline-induced elevation of blood pressure, while elastase transiently potentiated the vasopressor effect of both adrenaline and noradrenaline.

The findings that elastase increased the blood flow in the isolated hind limb of the cat, and that on intracutaneous application to rabbits it evoked capillary dilatation, indicate the mechanism of the vasodepressor action to be based mainly on the dilatation of the peripheral blood vessels. However, as the response to the compression of the carotid arteries was inhibited by the enzyme to 20 to 50 per cent, the possibility should not be overlooked that the inhibition of the vasomotor centre was also involved into this effect.

As to the effect of elastase on the vessels of the heart, in isolated mammalian hearts the enzyme was found somewhat to constrict the coronaries. In intact animals it inhibited to some extent the coronary spasm induced by posterior pituitary extract. On the frog heart, 24 to 48 units elastase augmented the amplitude while greater amounts led to diastolic standstill.

The results obtained on various smooth muscle organs showed the enzyme to behave in these tests in a manner similar to crystalline trypsin. Of the four smooth muscle organs investigated it was the uterus of the rat in oestrus which responded to elastase by specific contractions. The contractions of this organ depended on the dose and did not damage the organ. A similar effect of trypsin has been demonstrated by JAQUES and SCHÄR [28]. Like trypsin, elastase first decreased the peristalsis, then enhanced the contractions of the rabbit intestine. On the guinea-pig intestine the spasmogenic action was weaker than that of trypsin. The sensitivity to histamine was decreased by elastase on account of its toxic effect, after prolonged incubation.

The effect of elastase on isolated organs differed from that of kallikrein. The uterus of the rat in oestrus seemed namely to represent the smooth muscle organ specific for testing elastase and trypsin ; kallikrein failed to induce its contractions. In addition, kallikrein led to contractions of the rabbit intestine only at high concentrations and it failed to decrease the sensitivity to histamine of the guinea-pig ileum even after prolonged incubation. Thus, from the findings on these isolated organs it could be concluded that the elastase present in the organism showed a biological behaviour similar to that of the proteolytic but not elastolytic trypsin. This resemblance has been further stressed by the observations of KOVÁCS and BAGDY [30], who found the local application of elastase to be just as effective in the treatment of human pulmonary abcesses as that of trypsin.

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Recensio

The Physiology and Pathology of the Cerebellum

By Robert S. Dow, and Giuseppe Moruzzi

The University of Minnesota Press, Minneapolis, 675. p. 185 illus. USA. $ 12.50

This important book is not only an excellent summary of the almost incomprehensible wealth of information gained in the past twenty years by the development of modern neurophysiological methods about the functions of the cerebellum, but also an attempt to relate these to older data, as well as to our knowledge of clinical symptomatology and pathology. Part I, devoted to the physiology of the cerebellum, was prepared jointly by both authors, Giuseppe Moruzzi contributing the major share, while Part II, containing the clinical symptomatology and pathology, was written by R. S. Dow. — After a short, but most interesting historical introduction (Chapter 1), the results of ablation experiments (Chapter 2) are very carefully and systematically reviewed. In the last paragraph of this chapter an excellent analysis of the influence of the cerebellum on postural tonus, reflex and voluntary movements is found. The third chapter deals with the results of stimulation experiments. In the concluding paragraph the results are correlated with anatomic data on efferent cerebellar systems and a fairly good agreement is reported. The second part of this paragraph contains a lucid discussion of the phenomena related to cerebellar rebound. In the fourth chapter the vast material of electrophysiological experiments of the past few decades is reviewed, with special regard to the spontaneous activity of the cerebellar cortex, the representation of different sensory modalities, localization of functions and the somatotopic distribution of afferent projections. These questions are discussed at length in the concluding paragraph of the chapter, from which the most interesting treatment of the problem of somatotopic arrangement, found in anaesthetized and missed in unanaesthetized preparations, must be mentioned. In the 5th chapter the relations between the cerebellum and other central structures are summarized; relations to the spinal cord, the labyrinthine system, the vegetative functions, the cerebral cortex and different sensory functions being the main topics. In the paragraph of general considerations, the three main problems treated are the cerebellar regulation of postural tonus, of voluntary movements and the influence on sensory functions. Especially in the second and third functions does the significance of cerebellar influence on the "γ"-fibre system neuromuscular spindles receive due consideration. The 6th chapter devoted to developmental physiology, deals with the important question, how far histological differentiation of the cerebellar cortex can be correlated with the development of functions. The neuroanatomist must, however, draw attention to the fact that although during histogenesis myelinization and the disappearance of the outer granular layer in consequence of the migration towards the depth may be striking features, they in themselves do not necessarily indicate stages of development of the main synaptic systems of the cerebellar cortex. If the appearance of the different kinds of synapses, especially the cerebellar glomeruli, but also of the pericellular baskets, climbing fibres, etc. would be correlated to the development of functions, more decisive conclusions could very probably be reached. In the 7th chapter the authors present their general views on the functions of the cerebellum, the intensity and continuity of cerebellar control of posture, voluntary movements, sensory and autonomic functions are considered to be probably de-

termined by the spatial and temporal patterns of the afferent input. The basic mechanism by which the cerebellar cortex controls the functions of other centres, cannot be exerted by a simple function, *e. g.* "keeping up" of an "optimum level" of activity, but undoubtedly by proper coordination of the discharges of Purkinje cells involved in the control of several mechanisms. The main difficulty in the problem of intracerebellar organization of activity is the almost complete lack of information concerning the events connecting the arrival of impulses at the afferent terminals with the response of Purkinje neurons. While being in complete agreement with the authors in that the multiplicity of afferent channels converging upon the same areas of the cerebellar cortex, as well as the many different possibilities of synaptic connections suggest that the spatial co-ordination of cerebellar functions takes place mainly at the level of Purkinje cells, the reviewer cannot share their views about intracerebellar association mechanisms. There is no evidence that Purkinje, or other neurits of cortical origin were running from one area of the cerebellar cortex to another. The Golgi type cells can connect at most to distances of a few millimeters. Considering spatial co-ordination at cerebellocortical level (cerebello-nuclear connections of course excluded), attention must be concentrated to hitherto not sufficiently analysed anatomical possibilities, as *a)* abundant branching of the same mossy afferents terminating in different folia and, very probably, also in different lobi ; *b)* the length of granular axons and the whole architecture of the parallel fibre system and its synaptic mechanisms ; *c)* the length and distribution patterns of the star cell axon system, especially of those terminating in the pericellular baskets of Purkinje neurons and *d)* recurrent Purkinje axon collaterals, terminating on neighbouring Purkinje cells. These are the fields in which joint endeavour of neurophysiologists and histologists — and, very probably, also of mathematicians, for the analysis of quantitative, especially statistic, aspects of synaptic relations, — might bring the most important results in the near future. — The thorough consideration given in this whole part to the anatomy of cerebellar pathways, especially to the investigations of the Oslo neuroanatomical group, is exemplary indeed. Perhaps, however, too little attention is paid to the synaptology of the cerebellar cortex.

Part II of the monograph gives in the 8th chapter an excellent and concise review of the clinical symptomatology of cerebellar lesions, the 10th with convulsive and hyperkinetic disorders and the 11th with the developmental anomalies of the cerebellum. The 12th chapter contains the description of atrophic changes of the cerebellum and its connecting pathways. In the 13th chapter, acute, in the 14th chronic, infections, in the 15th vascular diseases and in the 16th cerebellar injury, finally in the 17th chapter cerebellar tumours are discussed on an abbreviated "handbook" level. However highly the attempt of the authors may be appreciated to correlate modern cerebellar physiology with the more classical field of human cerebellar pathology, this synthesis is not entirely satisfactory. This is mostly due to the fact that the recent rapid advance of cerebellar physiology has not been followed at the same rate by human pathology. On the other hand, somewhat more consideration to neuronal histophathology *e. g.* in atrophic diseases, instead of treating systematically and with equal emphasis a large number of disorders with a much more limited bearing on cerebellar physiology, such as infections and vascular diseases and tumours, would have been probably rewarding.

These minor criticisms cannot, however, diminish our appreciation and in many respects admiration for the magnificient contribution of the authors to cerebellar physiology. This book will be an excellent aid to the neurophysiologist, as well as to the neuroanatomist and especially also to the clinical neurologist. The bibliography containing about 2030 references and the complete subject index are excellent. Printing and illustrations are a credit to the University of Minnesota Press.

J. SZENTÁGOTHAI

A kiadásért felel az Akadémiai Kiadó igazgatója

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