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EFFECT OF NARCOTICA AND HYPNOTICA ON RESPIRATION VOLUME AND FREQUENCY.

WITH 8 FIGURES IN TEXT.

By F. E. SZONTÁGH M. D.
PÉCS.

PRESENTED BY G. MANSFELD, MEMBER OF THE ACADEMY.
(RECEIVED FOR PUBLICATION 15. 3. 1948.)

I. INTRODUCTION.

The quantitative examination by *Mansfeld* et al. (1) of the correlation between concentration and effect of narcotic drugs have shown that the nervous system both central and peripheric reacts to narcotic drugs in a way differing from the response of other organs. The function of the latter diminishes proportionally to the concentration of the drug. In a contrast to this, the function of the nerve cell (excitability, stimulus conduction, and automatic centre function alike) remains unimpaired until the narcotic drug is applied in the lowermost efficacious concentration. When, however, this concentration is applied the nerve cell ceases to function i. e. it does not react to the strongest stimuli either. (*Mansfeld's* „All or none principle of anaesthesia".) The evidence of this principle has been yielded first of all by the change of the respiration of the anaesthetised animals (2). In 29 experiments 14 various concentrations of aether were inhaled by the dogs. 8 of these concentrations did not result in a decrease of respiration though their inhalation lasted for 4 hours. In the cases of the 6 higher concentrations the frequency of respiration began to decrease and respiration stopped within 2 minutes after this. The same results have been obtained by *Kärber* and *Lendle* (3) who observed in rabbit experiments, besides the respiration rate, its volume, further the concentration of the aether in the arterial and venous blood also. In the series consisting of 45 experiments neither the rate nor the volume of respiration changed when 1 Liter of air did not contain more than 160 mgr. of aether whereas the application of higher concentrations invariably resulted in respiratory stop. The practical importance of these experiments should be evaluated in surgical anaesthesia. In any case, it is conceivable that fatal accidents occur during operations in general anaesthesia.

It has been further shown that curare, paralysing heat in frogs, and anoxaemia, interfere with the nervous functions in a manner resembling the effect of anaesthetic drugs. This fact favours the view that the paralysis of the nervous functions occurs like the so-called phase changes (melting of metals or boiling of fluids) i. e. suddenly when due temperature is conferred to the system.

Later on, *Mansfeld* (4) and his co-workers examined the relation of function to oxygen consumption in the nerve cell and muscle tissue during anaesthesia. In muscle cells the change of the concentration of the anaestheticum and that of oxygen consumption were rather parallel whereas in the nerve cell complete paralysis may occur without a change of oxygen consumption. A slight reduction of the oxygen consumption (20 to 30 per cent.) could not be attained unless at least a tenfold concentration of the anaestheticum was employed. It has been inferred from this phenomenon that there is a process sensitive to anaesthesia in the nerve cell, for the paralysis of which considerably lower concentrations of the anaestheticum suffice than for the paralysis of oxygen consumption, further that it is the paralysis of this process occurring in accordance with the all or nothing principle which brings the nervous functions to a stop.

The question has been raised in which manner the function of the nerve cell will change if the oxydising processes are primarily impaired e. g. through lowering the temperature. In these experiments diminished oxydations were attended by depressed function which was, in a contrast to the paralysis due to anaesthesia, rather constant. Thus reduced oxydation was, in *Mansfeld's* experiments, the only means to evoke a constant reduction of the function of a nerve cell without bringing about its complete paralysis. Yet there is a fact forming a controversion to the results reported above i. e. the effect of the barbituric compounds lowering the respiratory rate which may, f. i. in cases of intoxications, persist for several days. One of the co-workers of *Mansfeld, E. Mészáros*, examined the effect of phenobarbital on the isolated spinal chord of frogs and found that its paralysis occurred, like under the action of any other anaestheticum, in full accordance of the all or nothing principle. In order to settle this controversion *Mansfeld* et al. (5) examined the central regulation of breathing. In the course of these investigations they revealed the existence of an inhibitory centre lying between pons and medulla oblongata. This centre has, when the function of the higher centres has been eliminated, a depressing effect owing to which depressed respiration may persist without a paralysis like in anaesthesia.

From these results the essential difference between the effect of anaesthetica and hypnotica may be seen: general anaesthetica paralyse

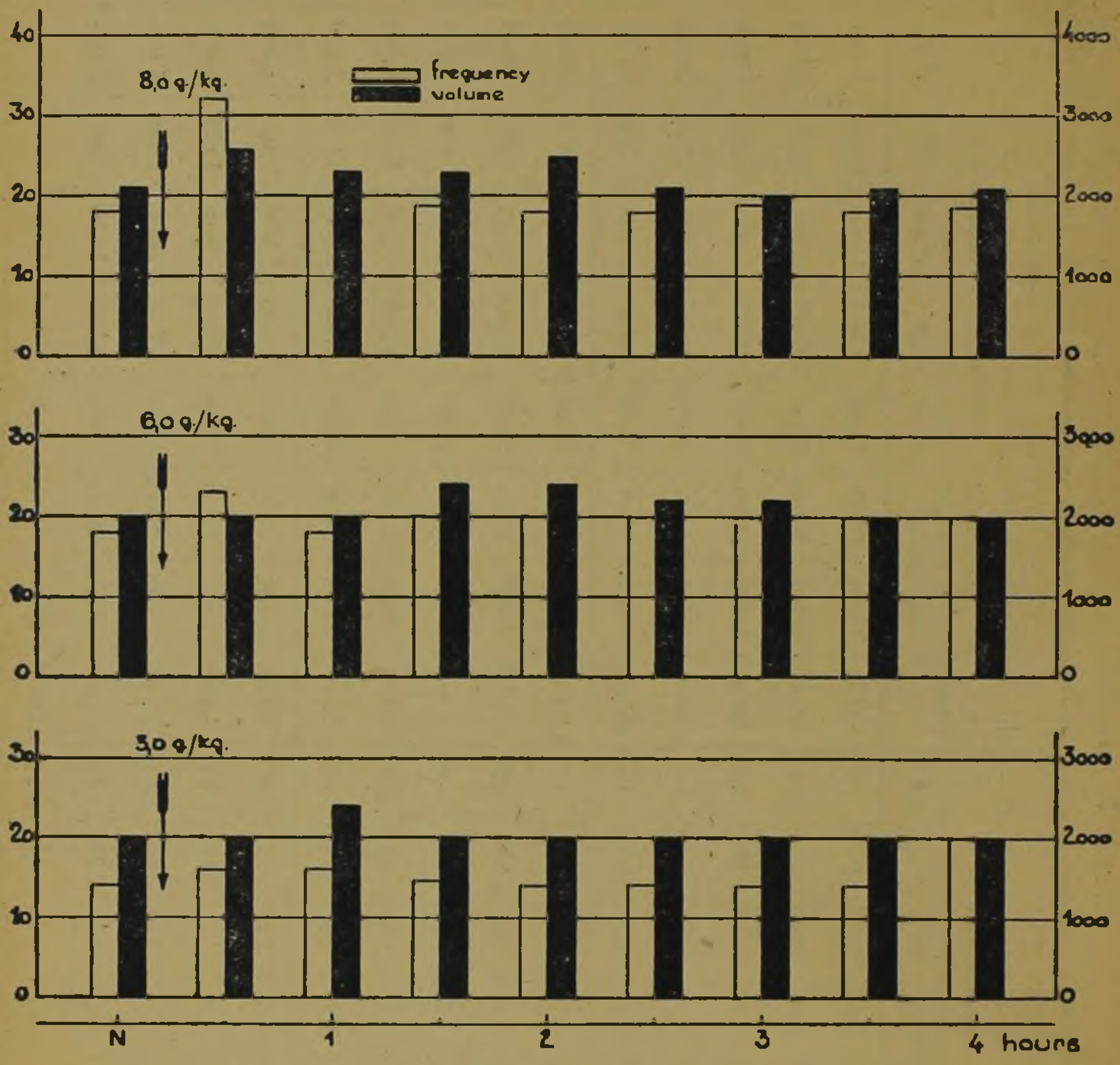
first of all the inhibitory centers (as seen from the initial irritation) whereas hypnotic drugs have a greater affinity to the active centres whereby the inhibitory ones become prevalent. This is the case during natural sleep as commonly known by *Economo's* (6) histologic investigations and the experiments of *W. R. Hess* (7) referring to the centre of sleeping.

The diminished function of the nerve cell due to the effect of the inhibitory centres is essentially similar that produced by diminished oxydations, since *Mansfeld* (8) has demonstrated that hypnotic drugs reduce the oxygen consumption of the nerve cell whereas in anaesthesia its function ceases in the society of unchanged oxygen consumption. Thus anaesthesia and sleeping are thoroughly different processes: anaesthesia is associated with complete paralysis of the nerve cell unaccompanied by reduced oxydations whereas during the action of hypnotic drugs (and probably during natural sleep also) oxydations are reduced by the inhibitory centres whereby the function of the nerve cell also diminishes.

First we had to examine the difference between anaesthesia and hypnotic effect with a larger material. We aimed to find a method by which one could tell whether a compound having paralysing effect should be applied to higher organisms as an anaesthetic or as a hypnotic drug. A special attention was to be called to the alcohol the action of which is apparently disfavouring the statements and conclusions drawn from the above experiments. Evidently, alcohol may be considered a representant of the anaesthetic group it never does, however, give rise to a sudden paralysis of respiration (as f. e. aether, chloroform, urethane). As it may be observed in patients intoxicated with alcohol, its effect consists, in a contrast to the all or nothing principle of *Mansfeld*, in a gradual decrease of the respiratory rate and volume. Well, it has been stated by our preliminary experiments *that the depression of respiration is accompanied by the lowering of the temperature* owing to which the oxydations of the nerve cell and its function also are depressed. If we kept the temperature of the animals intoxicated with alcohol at a constant level the respiration did not change, despite the general anaesthesia ensued. If the intoxication was fatal the paralysis of respiration occurred in accordance of the all or nothing principle.

Starting from these observations we examined the effect of two anaesthetica, alcohol and urethane, *at a constant temperature*, further two hypnotic drugs, phenobarbital and evipane, from the same aspect: their action on the rate and volume of respiration.

FIG. 1.



noea lasted for $2^1/_2$ minutes. Two dogs were given 10,0 Gr./kilo alcohol. Both animals have been killed by this dosis..

The diagramme taken after the administration of 8,0 Gr./kilo may partly be seen on fig. 2.

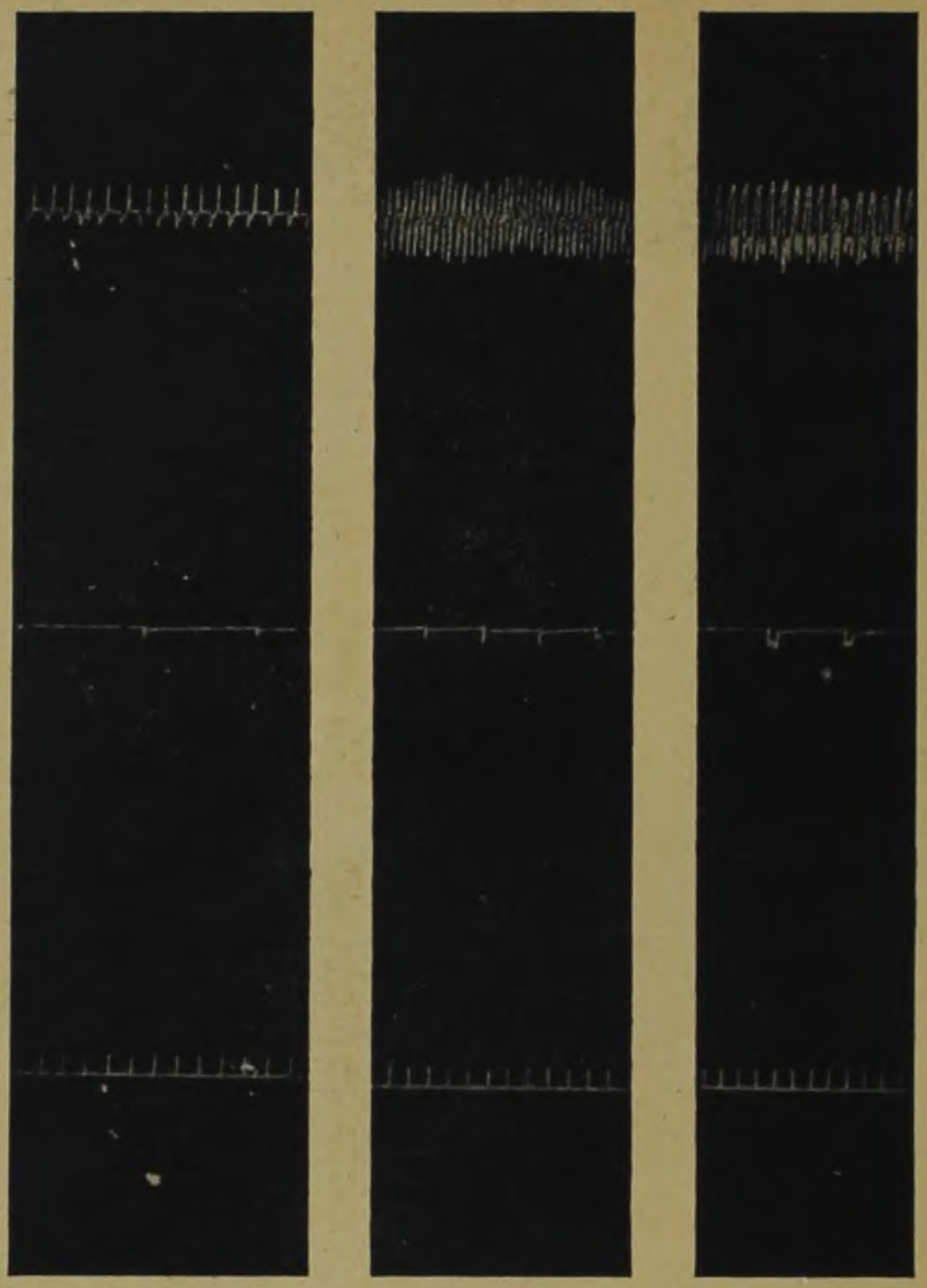
It may be seen from the results that even such alcohol doses as being nearly fatal do not suffice for a lasting depression of the respiratory rate. Apart from the tachypnoea observed before the awakening of the animals the rate of respiration was, during the whole experiment, slightly above the normal value. The volume of respiration was still more increased.

B) *Evipan*. The drug was administered after a fasting of 12 hours by a gastric sound. Per kilo weight 0,05, 0,10, and 0,20 Gr. respectively were given (table II and fig. 3).

TABLE II. *(Evipan.)*

Dosis.	Exp. N°	Frequency of resp./min.									Volume of resp. in ml./min.								
		Norm.	Time after the administration								Normal	Time after the administration							
			30'	1^h	1^h 30'	2^h	2^h 30'	3^h	3^h 30'	4^h		30'	1^h	1^h 30'	2^h	2^h 30'	3^h	3^h 30'	4^h
0,05 g pro kg	1.	14	17	32	30	19	15	15	15	15	1700	1900	3000	2200	1900	1900	1700	1700	1700
	2.	19	19	33	23	24	19	19	19	19	2100	2200	2800	2400	2500	2200	2100	2100	2100
	3.	16	20	35	33	29	19	18	18	18	1800	2600	2700	2300	2300	2100	1900	1900	1800
	4.	16	19	29	28	26	16	17	17	17	1900	2300	2600	2600	2500	2000	1800	1900	1900
	5.	20	25	41	36	22	21	21	21	21	2500	3000	3900	3500	2800	2800	2600	2500	2500
0,10 g pro kg	1.	24	19	19	19	19	26	27	25	25	2600	2100	2000	2100	2100	4000	3900	3600	3600
	2.	19	19	18	19	19	22	28	22	22	1800	1800	1800	1700	1700	2700	2400	2100	2000
	3.	19	17	17	17	17	31	25	20	20	1700	1600	1700	1600	1600	4200	3900	2600	2700
	4.	17	15	16	16	16	27	27	18	18	1600	1600	1800	1700	1600	3600	3800	2900	2900
	5.	21	20	19	19	19	34	33	25	24	2300	1900	1700	1900	2000	4500	4000	3800	2800
0,20 g pro kg	1.	18	3	3	3	2	17	42	76	77	2100	700	800	700	900	2500	3900	3900	4200
	2.	26	6	6	6	6	30	52	98	116	2600	1200	1000	1100	1500	3000	4200	4600	5100
	3.	21	4	4	4	4	24	39	91	103	1800	800	800	800	900	2200	3000	3200	3300
	4.	17	5	5	5	5	17	32	69	76	1300	800	800	800	1200	1400	2900	3300	3600
	5.	18	2	2	2	2	22	35	76	77	1200	500	600	600	500	1900	3500	3500	3800

Fig. 2.

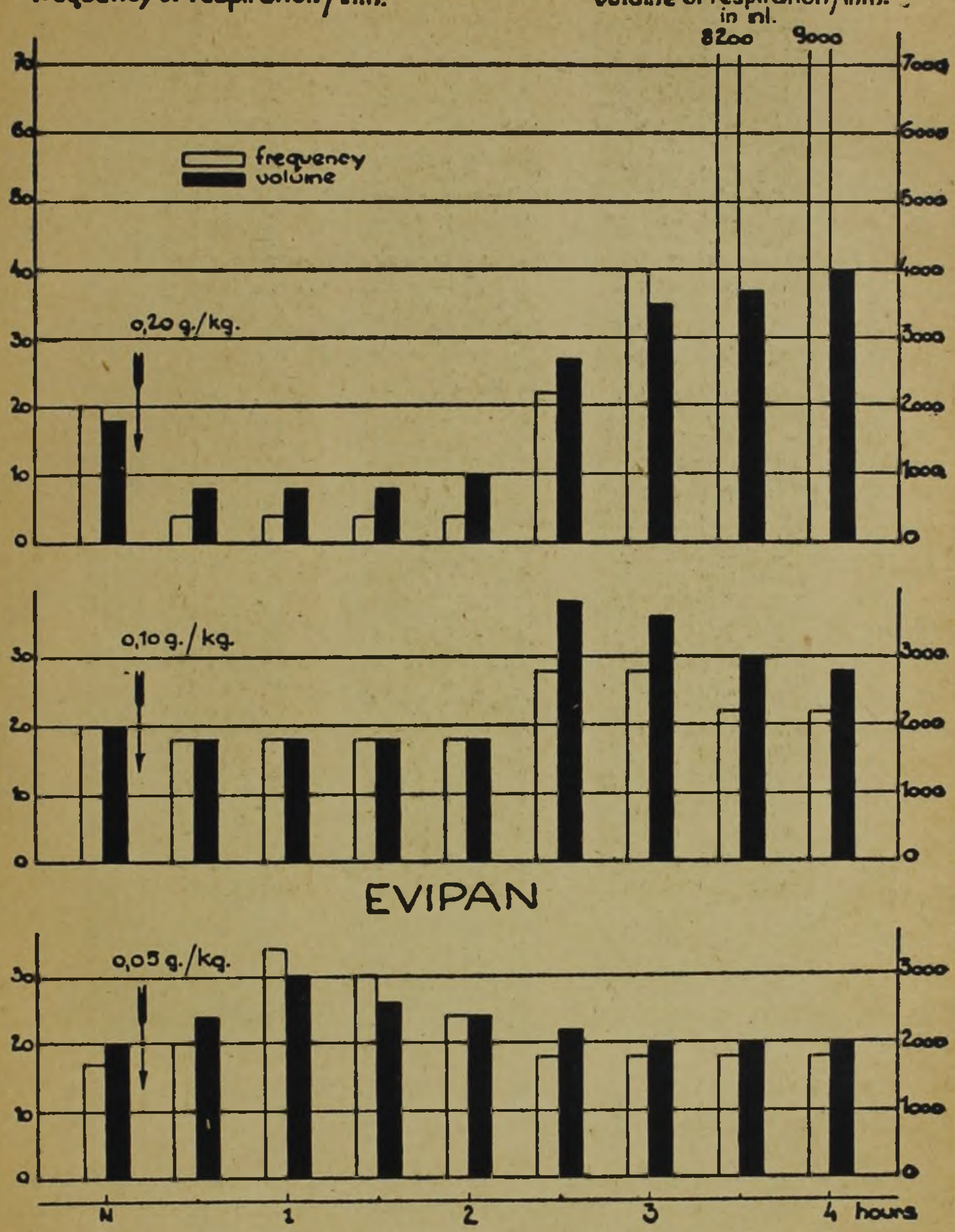


10 to 20 minutes after the lowest dosis a deep general anaesthesia occurred without corneal reflex. The effect lasted for 25 to 40 minutes. After 2 to $2^1/_2$ hours the animals were completely recovered. Aside from the tachypnoea preceding the awakening no change in the respiration could be observed.

If 0,10 Gr./kilo were given the anaesthesia lasted longer. During the anaesthesia both the rate and volume of respiration were slightly decreased until the tachypnoea pointing to the onset of the awakening ensued and the reflexes simultaneously returned. Full awakening occurred after 6 to 7 hours.

This impairment of respiration was considerable if 0,20 Gr/kilo had been administered. The decrease of the rate of the respiration was particularly marked. Recovery did not occur before 12 to 14 hours though

FIG.3.



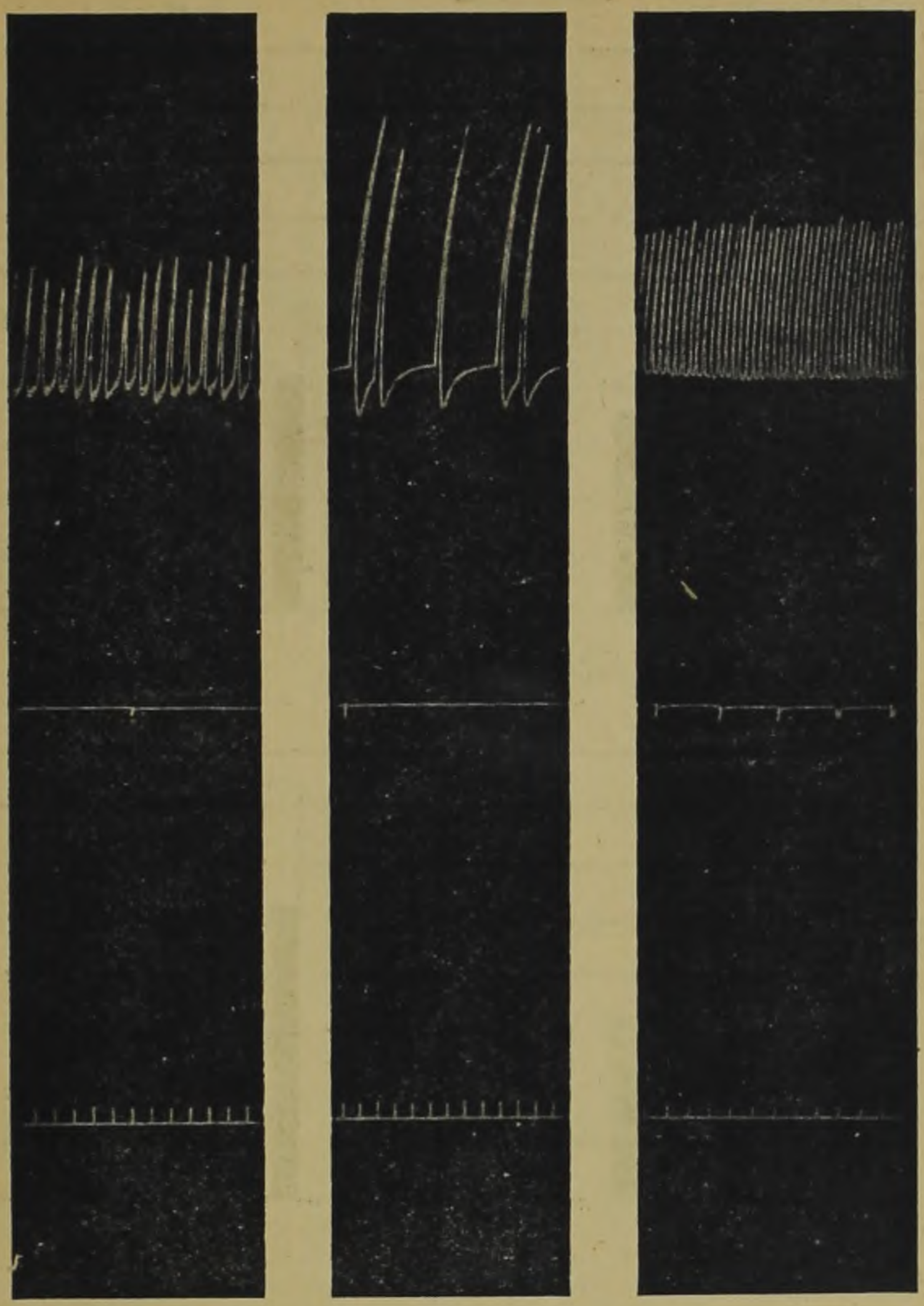
the onset of awakening could be observed as early as after $2^1/_2$ to 3 hours. None of the animals has perished.

The details of a curve taken after the administration of 0,2 Gr./kilo have been recorded on fig. 4.

TABLE III. *(Urethan.)*

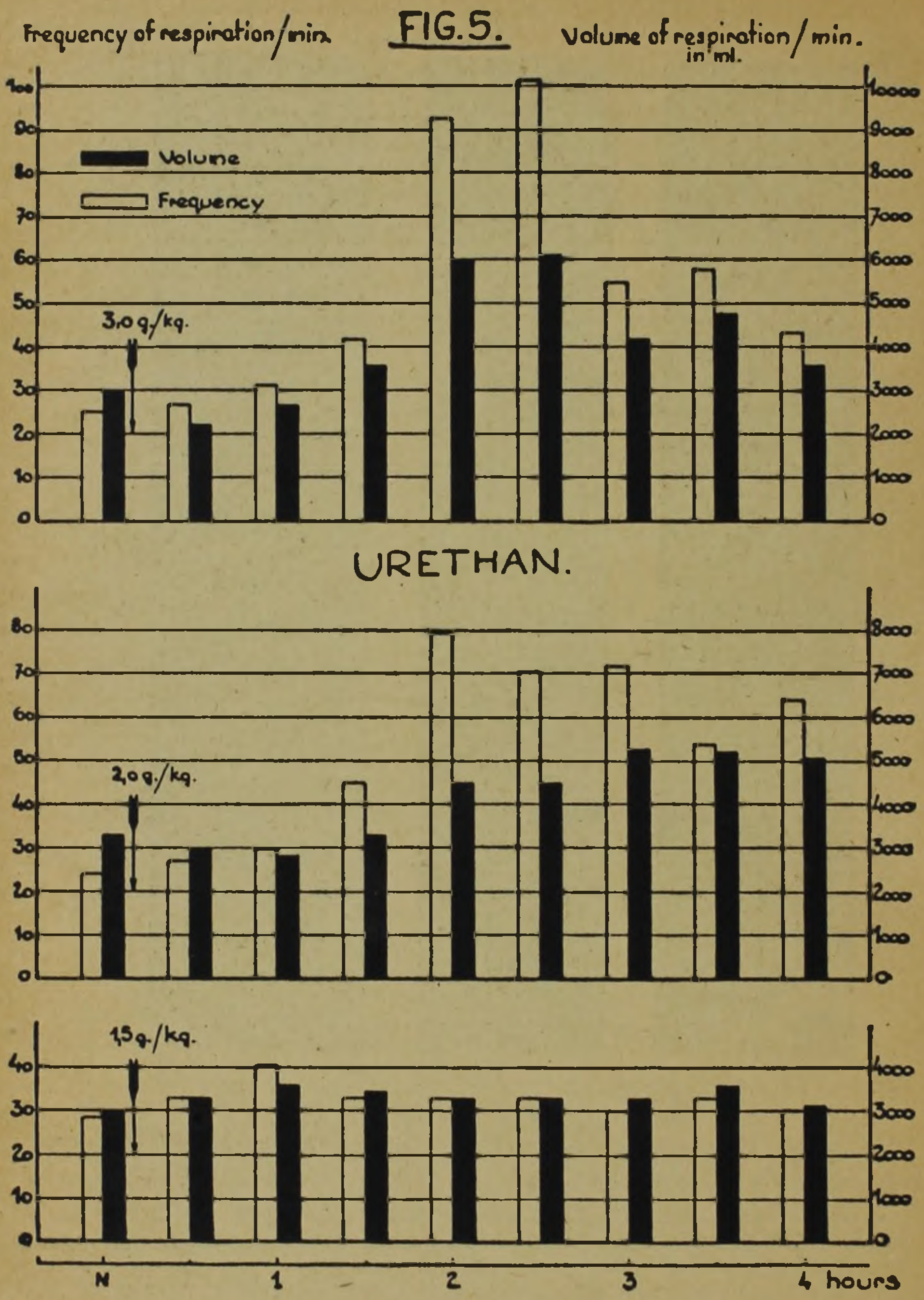
Dosis.	Exp. N°	Frequency of resp./min.									Volume of resp. in ml./min.								
		Norm.	Time after the administration								Normal	Time after the administration							
			30'	1^h	$1^h 30'$	2^h	$2^h 30'$	3^h	$3^h 30'$	4^h		30'	1^h	$1^h 30'$	2^h	$2^h 30'$	3^h	$3^h 30'$	4^h
1,5 g pro kg	1.	20	22	27	23	22	20	20	20	20	2000	2200	2400	2200	2200	2200	2300	2300	2200
	2.	16	20	21	20	20	17	16	16	16	1800	2300	2300	2300	2300	2200	2300	2500	2300
	3.	21	21	22	21	21	21	21	22	21	1900	2000	2500	2200	2000	2100	2000	2000	2000
	4.	23	24	30	24	23	23	23	23	23	2400	2400	2500	2400	2400	2500	2400	2400	2400
	5.	20	23	30	23	22	20	20	28	20	1900	2100	2300	2100	2100	2000	2100	2400	2100
2,0 g pro kg	1.	18	19	22	32	61	58	57	46	46	2600	2200	1900	2400	3900	3400	4200	4200	3600
	2.	14	18	21	25	48	40	45	34	49	1900	1800	1700	2100	2600	2600	3300	3300	3300
	3.	16	16	18	34	52	46	46	37	40	2100	2100	1700	2000	2300	2300	2600	2600	2600
	4.	17	20	20	29	43	43	43	24	30	2300	2100	1800	2300	3800	3600	4100	3900	3900
	5.	15	17	19	30	56	43	48	34	45	2100	1800	1800	2200	2900	3100	3300	3300	3300
3,0 g pro kg	1.	15	17	18	27	54	67	38	36	17	1700	1800	1900	3000	3600	4000	2900	2900	2800
	2.	21	22	22	30	71	71	47	50	39	3000	1700	2000	2300	5200	5000	3800	5000	3000
	3.	16	16	20	23	63	70	32	30	23	1900	1500	2000	2100	2900	3100	2100	2100	1700
	4.	17	19	22	35	69	68	29	38	34	1700	1700	1800	2700	4300	3900	3000	3800	2700
	5.	16	16	23	25	53	64	39	39	32	1700	800	1300	1900	4000	4000	3200	2200	1800

Fig. 4.



It is fair to infer from these results that the respiratory rate of the experimental dogs and the volume of respiration can be constantly lowered by the administration of due doses of evipan (0,1—0,2 Gr. per kilo body weight) whereas these doses are never fatal for the animals.

C) *Urethane.* The effect of this drug was examined in the 3rd series of our experiments (table III and fig. 5). Deep anaesthesia could not be attained by the subcutaneous injection of 1,0 Gr./kilo credited with a narcotic effect; the corneal reflex was never missing and the animals reacted to strong stimuli. The awakening of the dogs began 1 to $1^1/_2$ hours



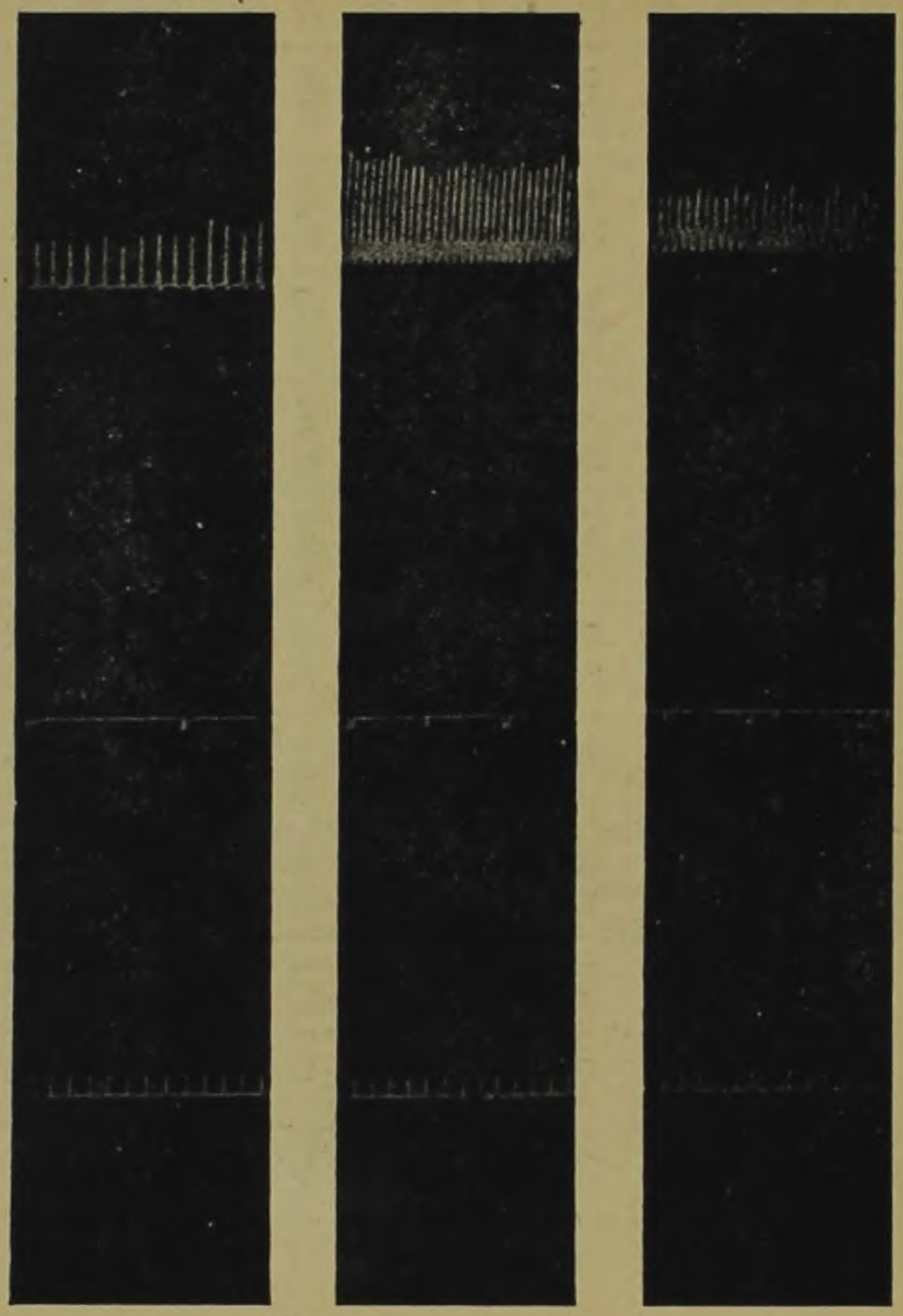
of sleep. The preconscious tachypnoea was moderate and no considerable change could be observed in the frequency and volume of respiration.

If 1,5 Gr./kilo had been administered the corneal reflex did, except the experiments 2 and 4, cease after 35 to 40 minutes and it was missing

TABLE IV. *(Luminal.)*

Dosis.	Exp. N°	Frequency of resp./min.									Volume of resp. in ml./min.								
		Norm.	Time after the administration								Normal	Time after the administration							
			30'	1^h	$1^h30'$	2^h	$2^h30'$	3^h	$3^h30'$	4^h		30'	1^h	$1^h30'$	2^h	$2^h30'$	3^h	$3^h30'$	4^h
0,05 g pro kg	1.	19	26	37	48	48	48	49	43	43	2000	2000	2000	2000	2000	2000	2000	2000	2000
	2.	21	34	42	51	50	50	49	48	48	2400	2200	2400	2400	2300	2400	2500	2400	2400
	3.	19	24	30	39	41	41	40	39	39	1900	1900	1900	1900	2000	1900	1800	1900	1900
	4.	22	37	43	57	57	57	57	49	49	2600	2200	2600	2400	2600	2600	2600	2600	2500
	5.	19	29	38	45	45	45	45	41	41	2100	2100	2100	2300	2100	2100	2100	2100	2200
0,08 g pro kg	1.	18	19	41	67	71	58	58	58	37	2100	2100	2100	2200	2300	2200	2100	2100	2100
	2.	20	20	36	48	52	46	47	46	35	2300	2300	2300	2300	2300	2300	2300	2300	2300
	3.	16	20	40	52	55	46	46	45	39	2000	2000	2000	2000	2300	2000	2000	2000	2000
	4.	22	23	47	49	54	47	42	47	46	2800	2800	2800	2800	3100	2800	2800	2800	2800
	5.	14	18	36	44	48	43	46	43	33	1800	1900	1900	1900	2000	1800	1800	1800	1800
0,12 g pro kg	1.	18	17	17	14	16	16	20	30	58	1800	1600	1500	1400	2100	2100	2600	2800	3100
	2.	14	10	9	9	10	13	15	18	39	2100	1500	1500	1500	1700	1800	1900	1900	2400
	3.	19	19	12	8	14	14	14	21	54	2300	1700	1200	1200	2500	2200	2200	2800	2400
	4.	22	21	12	10	10	16	26	31	102	2800	2100	1800	1800	1900	2100	3000	3200	3300
	5.	17	13	10	9	10	11	20	20	37	2000	1100	1000	1100	1800	1800	2300	2300	2300

Fig. 6.



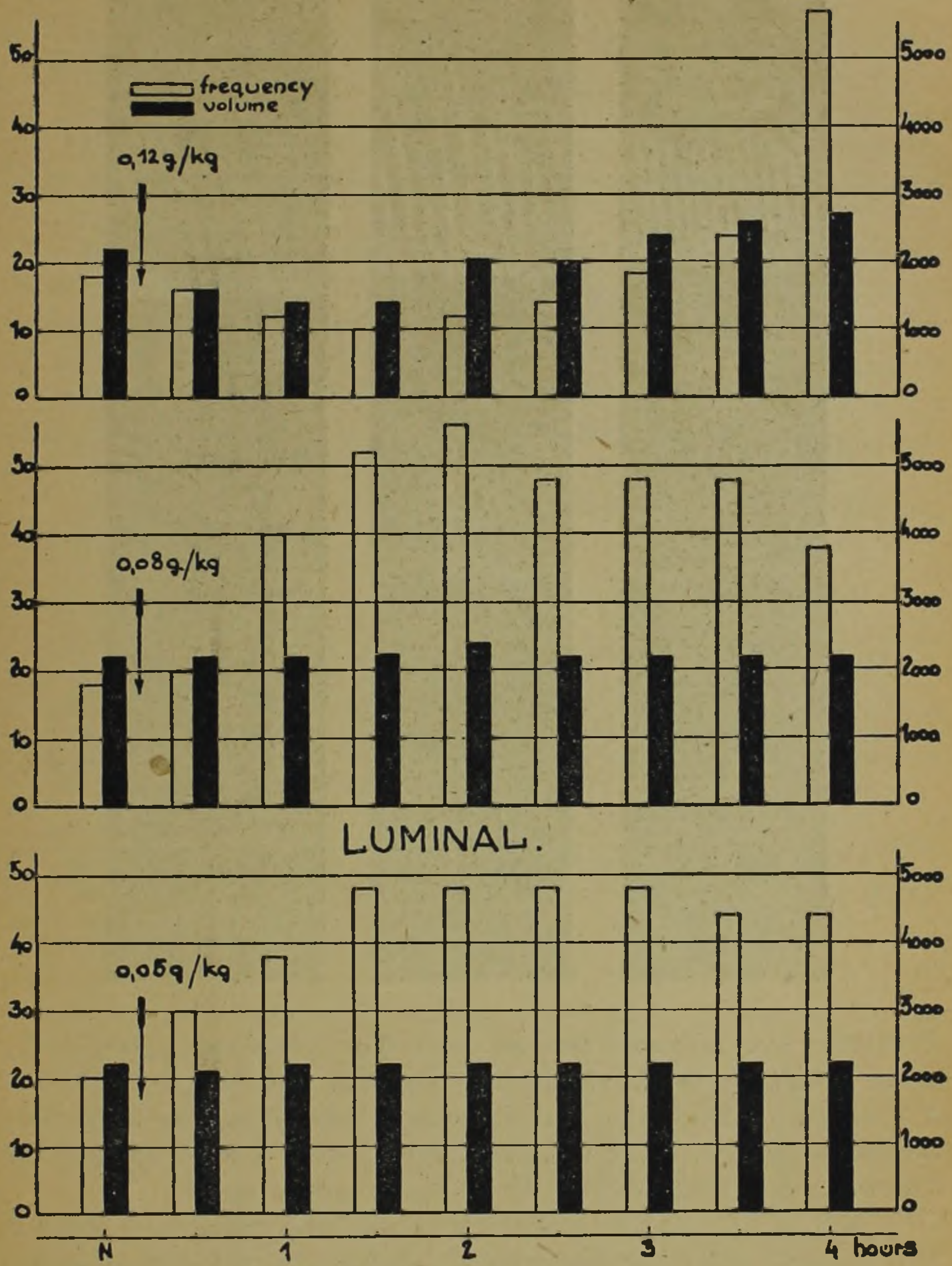
for $1^1/_2$ hour on an average. Then tachypnoea ensued and lasted until full consciousness has been regained after 6 to 9 hours. During the deep anaesthesia the respiratory volume was slightly decreased whereas the frequency was somewhat higher than normally.

In the third series 3,0 Gr./kilo had been injected subcutaneously. Deep anaesthesia occurred in all cases. After 30 to 40 minutes none of the reflexes could be elicited. Otherwise, the animals behaved like after the dosis of 1,5 Gr./kilo with the difference that full consciousness has not been regained before 17 to 22 hours, after a long tachypnoic period.

Not a single animal has been killed by the administration of urethane. One part of the diagramme of respiration taken after the administration of 3,0 Gr./kilo may be seen from fig. 6.

These experiments show that the rate of respiration cannot be

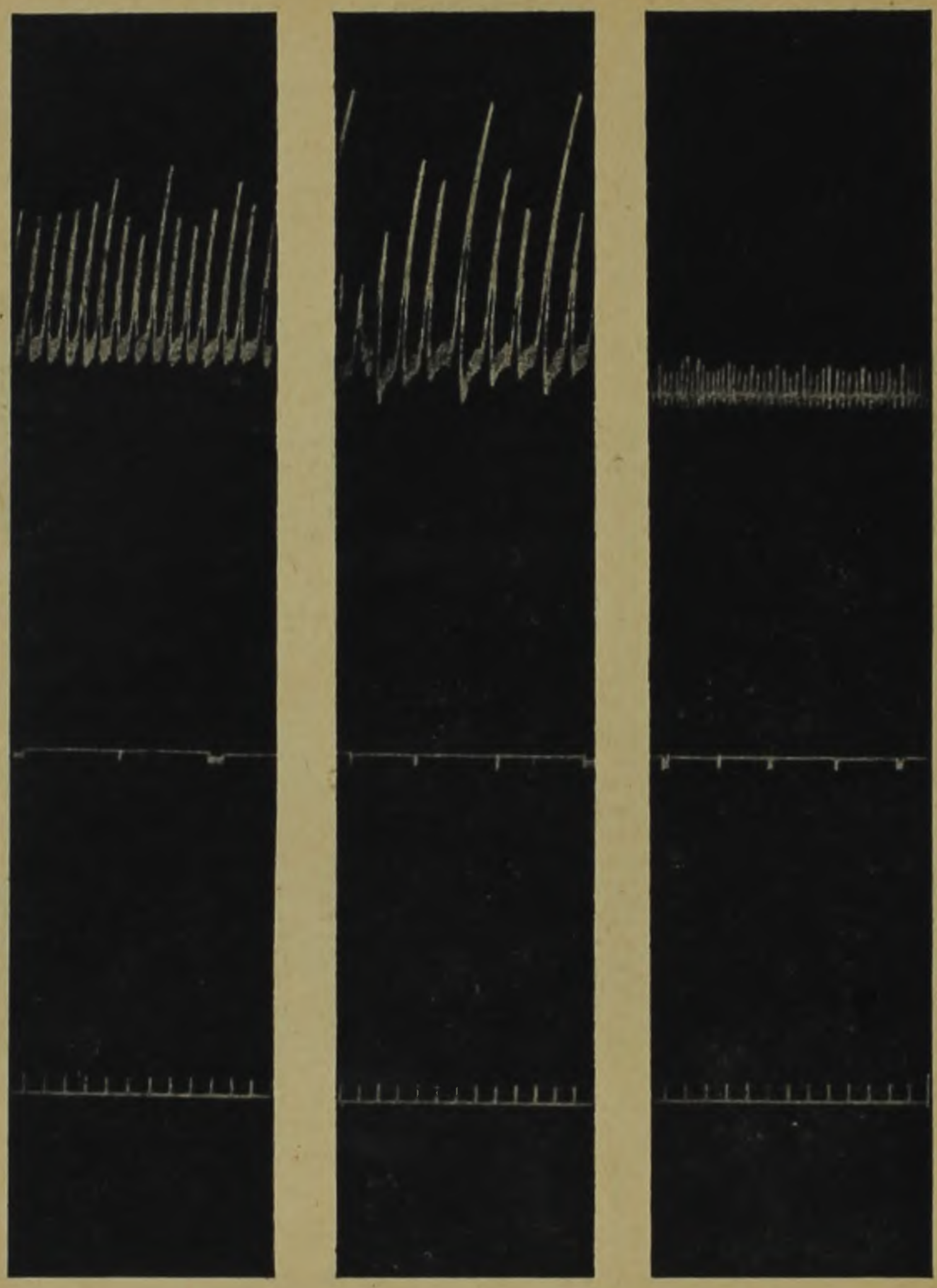
FIG. 7.



lowered by urethane, enormously high doses though may be given to them. The effect exerted on the volume of respiration is not significant.

D) *Luminal* (phenobarbital). The dosis of 0,05, 0,08, and 0,12 Gr./kilo respectively was given intravenously (table IV and fig. 7).

Fig. 8.



Immediate sleep occurred after all doses. The animals were sleeping for 25 to 30 minutes or less than 1 hour after the dosis of 0,05 Gr./kilo or 0,08 Gr./kilo respectively. The higher dosis was followed by tachypnoea lasting rather long. Full awakening occurred after 12 to 16 hours in the first series and after 14 to 20 hours in the second series.

If 0,12 Gr./kilo were injected the anaesthesia lasted for 3 hours in the course of which the frequency and intensity of the respiration was markedly decreased. The tachypnoea was longlasting and intensive and full consciousness did not return before 21 to 26 hours. One of the animals has been lost.

One curve of the luminal experiments has been recorded on fig. 8.

The results show that an adequate dosis of phenobarbital is followed by a lasting and considerable decrease of the rate and frequency of respirations. The effect exerted on the volume is less marked than that exerted on the frequency.

SUMMARY.

1. In 60 experiments performed with dogs the action of two narcotic drugs and two hypnotic ones exercised on the respiration of the animals was examined.

2. It has been demonstrated that there is an essential difference between these two groups of paralysing drugs. If provisions are made to keep the temperature of the experimental animals on a constant level the rate and volume of respiration is not diminished by alcohol and urethane even if their dosis is near the letal one. On contradistinction to this, the administration of phenobarbital or evipan is attended by a lasting (sometimes for several hours) impairment of respiration.

3. This method may lend itself to distinguish narcotic (anaesthetic) and hypnotic drugs.

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A SIMPLE METHOD FOR ISOLATION AND CRYSTALLIZATION OF FIBRINOGEN FROM CATTLE-PLASMA.

WITH 1 FIGURE IN TEXT.

BY D. BAGDY

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PRESENTED BY F. B. STRAUB MEMBER OF THE ACADEMY.

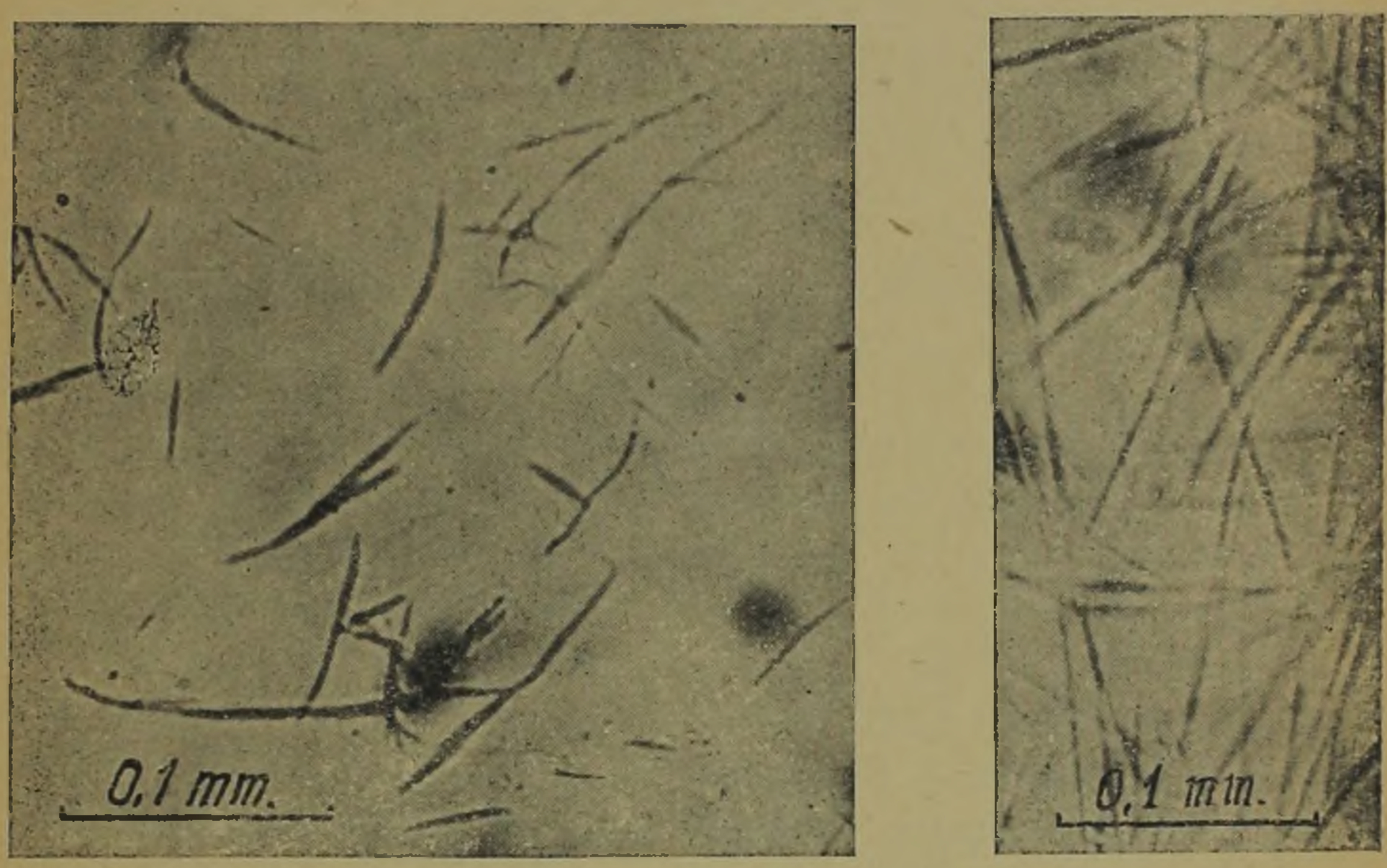
(RECEIVED FOR PUBLICATION 18. 3. 1948.)

Fibrinogen was precipitated from fresh oxalated plasma with $^1/_3$ volume of saturated ammonium sulphate at room temperature. The precipitate was centrifuged and dissolved in 0,9% sodium chloride solution corresponding to $^1/_5$ of the starting plasma volume. The insoluble part and suspended solid components — mainly red corpuscles — were centrifuged out and the solution made up to the starting plasma volume with distilled water.

The solution cooled at 0 C°, acidified with *M*/10 hydrochloric acid to *pH* 5,3 and the flocculated fibrinogen centrifuged for a short time at 0 C°. The white, tough precipitate was transfered with a glass-stick into a glass beacker and an amount of distilled water corresponding to 1/10 of the initial plasma volume was added in portions of 25 ml. The *pH* of the solution was adjusted to 7,5—8,0 with a few drops of 12% ammonium hydroxide. The fibrinogen was slowly dissolved by pressure of glass rod. From time to time *pH* of the solution was controlled by universal indicator paper (Merck) and was kept at the mentioned value with ammonium hydroxide.

In these fibrinogen solutions showing a considerable Tyndall-effect the clottable protein determined with the method of *K. Laki* (1) forms 75—85% of the total protein. The amount of the total protein was determined by means of trichloracetic acid precipitation. On standing at room temperature ammonia slips away and the *pH* of the solution shifts to the acid side. A few hours later the solution becomes turbid and on stirring shows a silky glittering. Under the microscope partly isolated needle-shaped, partly star-shaped crystals forming a net-work can be seen. After 1—2 hours on the appearance of turbidity, the crystals were centrifuged out and the supernatant liquid conserved with some thymol crystals

at room temperature. A few hours later the translucent and slightly opalescent solution gradually gets turbid and becomes milky as a result of separation of crystals in a mass. After centrifugation the settled crystals were dissolved in weak alkaline distilled water (alkalinized with ammonium hydroxide to *pH* 7,5—8,0) to water-clear appearance. Buffered with *M*/5 phosphatbuffer of *pH* 6,8 the solution clots with thrombin. The solutions thus prepared contain an average of 85—96% of clottable protein. The crystals or the solutions prepared from them also may be conserved at 0 C⁰ or in a frozen state for months, but at room temperature, they loose



Fibrinogen crystals.

their coagulability within a few days even in sterile conditions. The disapperance of the coagulability may be due to the action of a proteolytic enzyme which has come down with fibrinogen, already present in plasma (called by *Christensen* and *MacLeod plasmin*) (2). The exact circumstances to avoid the contamination of the fibrinogen preparations with plasmin were not found. In very few cases the fibrinogen solution becomes clear losing its power of coagulability before crystallization.

The proteolytic enzyme is present in plasma as plasminogen — an enzyme precursor which seems to be present in our all preparations. It is not yet clear whether the rapid clarification of the preparations is due either for a greater degree of plasminogen or for its accentuated activity.

Experiments to eliminate the effect of plasmin and to isolate a preparation of plasmin of high purity are in progress.

I am specially grateful to Prof. *K. Laki* for his help and good advices and much indebted to Prof. *M. Gerendás,* who kindly prepared the photograph of the plate.

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(2) *Christensen* and *MacLeod,* J. Gen. Physiol. 28. 363, 559. 1945; 30. 149. 1946.

SEASONAL STRUCTURAL CHANGES IN THE ADENOHYPOPHYSIS OF GUINEA PIGS.

WITH 10 FIGURES IN TEXT.

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INTRODUCTION.

The functioning of the organism is greatly influenced by external and internal factors rhythmically changing in course of the year. The vital functions have to be in constant harmony even under these changing circumstances, this inner harmony is safeguarded by the nervous and hormonal system. Since the varying functional state of the incretory glands manifests itself in their finer structure and since these functional changes probably occur in order to maintain the harmony of the organism as a function of external influences, there are rhythmical changes to be expected in the histological structure of these glands which might permit conclusions about the functioning of these organs.

Subject of this paper are the seasonal changes of the adenohypophysis of guinea pigs. The guinea pig is one of the most used laboratory animals, which is often chosen for the evaluation of hormone-preparates, which made it desirable to investigate what seasonal differences there are in the quantity, quality and grouping of cells, in the quantity and consistency of colloid, in vascularisation etc., so that the characteristic structure of every month might be established as a basis for histological evaluation of hormone-preparates.

Seasonal changes in structure of the thyroid of guinea pigs have been described by *Mödlinger* (9), those in the adrenal glands by *Stohl* (16). Since there is a synergism between these glands it was expected to find some connexion in the structural changes also.

In the past years several authors described rhythmical changes of structure in the pituitary of different animals in connexion with gonadic processes, vital cycle, season and time of day. Seasonal changes in the pituitary of mammals were investigated only on hibernating animals.

Gemelli (4, 5) found, that in the pituitary of hibernating marmots there is a considerable decrease in the number of basophil cells, these cells appearing in great number in spring. His observations were confirmed by *Rasmussen* (11) on the pituitary of *Marmota monax L.* and *Coninx-Girardet* (2) who besides found a difference in the grouping of cells in summer and winter, the winter regrouping taking place in October.

METHODS.

Male guinea pigs were used, which were dissected from 4. 1941 to 5. 1942 with an additional section in 7. 1946 and 2. 1947. Male animals were chosen because the oestrus of females taking place more than once in a year would have influenced the picture. The animals were of the same breed, lived under identic conditions, received the same food and were approximately of the same age. They were always dissected about the middle of the month at 11 a. m. The pituitaries were fixated in Susa's mixture, embedded after the method of Péterfy and sections of 5—7 μ were prepared out of them. The slides were stained with Heidenhain's azan-staining, Mallory's method, Ehrlich's haematoxyline-eosine and Heidenhain's ironhaematoxyline.

GENERAL DESCRIPTION OF THE PITUITARY OF GUINEA PIGS.

Of the three pituitary lobes the anterior is the largest, sitting ventrally and reaching laterally upwards on both sides (Plate I. Fig. 1). There are chromophobe and chromophil cells in this lobe, the latter consisting of three types: α-(acidophil, eosinophil), β-(basophil) and δ-cells.

α-cells are round to oval, their borders very distinct but sometimes they might form a plasmodium too. Their average size is 11,71 μ × 8,90 μ. The diameter of the nucleus is 6,39 μ. The size and grouping of the chromatine-granula depends on the functional state of the cells.

β-cells are also round to oval, but oftener we find angular forms as well. Its borders are well visible, but they might form plasmodium too. Their medium size is 12,45 μ × 8,94 μ. Their granula are coarser than those in the cytoplasm of eosinophil cells. The nucleus is stained better than in the α-cells, its size is 6,15 μ. The structure of the nuclei in the β-cells also depends upon the functional state of the cell.

On both cells the liquefaction of hyperchrome cells is perceivable. In the course of liquefaction the granula become denser and denser until they are transformed into a colloid-like substance. The chromatine of the nucleus becomes coarser and denser, while the nuclear membrane disap-

pears. The cells become smaller, of an elongated spindle-shape. There are much more β-cells than α-cells liquefying as it was observed also on human pituitaries by *Farkas* (3).

δ-cells are very few and rare. They are round-multangular, their border is not well visible. Liquefaction may take place here too, in the end these cells are not spindle-shaped but ramified.

Borders of the chromophobe or γ-cells are invisible. Their nucleus is bigger than that of the chromophil cells (7,23 μ) and undergoes a peculiar change in course of the year. Among them there are often big cells (20,32 μ × 14,34 μ) whose borders are clearly visible. Size of the nuclei in these cells is 8,41 μ.

γ-cells may be found in greater number on the ventral side and in the chromophobe zone [Romeis (12)]. In these regions there are some scattered α-, β- and δ-cells also to be seen, same as there appear some γ-cells, singly or in small groups among the chromophil cells in the dorsal and central part of the anterior lobe.

The cells of the anterior lobe are arranged by argyrophil fibres to strangs and groups the formation of these depending upon the functional state of the gland. The strangs and groups consist of cells of the same or of different sorts. The cells may form pseudofollicles as well.

Between the cell-groups there are sinus-like capillaries [Romeis (12)] springing mostly from the upper hypophyseal arteries and to a smaller extent from the lower ones. The width and blood-content of these is changing parallel to the functional changes of the gland (Tab. I.). The literature cites different opinions about the definition of sinuses. These investigations point in the way that in case of incretory glands sinuses and capillaries are not different anatomical substrates, but different states of the same vessels according to the degree of function of the gland. The capillaries are always close-walled, the endothelium cells well visible. In some months a granular secretion or colloid droplets may be seen in them.

In the chromophobe zone of the anterior lobe there run arterioles, the media of which is transformed into epitheloid cells put in a row like a string of pearls (Plate I. Fig. 2.). These media-cells being contractile elements we think it possible that the bloodsupply of the anterior lobe is adjusted to its different needs because of functional changes of the cells, by varying the diameter of these vessels.

Contrary to the findings of *Vanderburgh* (17) who has not investigated annual cycles, I found during almost the whole year colloid in the anterior lobe, sometimes in pseudofollicles, mostly in cysts. The cysts were always to be found in the same place, near the ventral margin of the anterior lobe directly at the caudal end of the pituitary cavity that separates the anterior

lobe from the medial lobe. *Vanderburgh* (17) also describes cysts in the pituitary of guinea pigs, but he intended that they occur only „occasionally" in the anterior lobe and are characteristic for the medial lobe. In the latter region I was never able to find a cyst, they occured always only in the beforesaid region. The diameter of these cysts underlies seasonal changes. Their wall is lined by columnar epithelium, the height of which varies between 4,86μ and 20,75μ. On their free surface the cells have kinocilia. The basal corpuscules are well visible. If there are more cysts in this region then — as can be seen from complete serial sections — they turn out to be branches of a tubular system. The cysts contain colloid of changing quality. Conclusions about its consistency can be drawn from its staining; the colloid stained red with the azanmethod mostly forms lumps, this shows its being more consistent, the colloid stained blue is strongly vacuolized, that is, thin. With Mallory's method the colloid is stained with growing consistency yellow, blue, red (in the future we shall refer to them only as azan-red, azan-blue, Mallory-yellow, Mallory-blue or Mallory-red colloids). In the colloid there are almost always to be found some nuclei, cells or cell-fragments.

The pituitary cleft separating the anterior and medial lobes I found without exception on every guinea pig, contrary to *Romeis* (12), who described its early disappearance. This fissure has a ventro-caudal direction, reaches the surface on both sides and ends cranially at about the border of the infundibular lobe and the pars tuberalis. Its diameter varies (9,73μ—82μ), in its caudal end at the folding over of the paraneural and distal wall it mostly contains colloid. The distal wall is lined here with high columnar ciliated epithelium (17,0μ—22,62μ), these cells resemble the columnar epithelium of the ciliated cysts, but are higher than these. Among them there are some narrow cells to be seen, their nuclei are rod-shaped, packed with chromatine. Sometimes such a nucleus is seen to wander into the colloid of the cleft, pressed out by the two neighbouring cells. In accord with *Vanderburgh* (17) I also found the distal wall of the pituitary cleft communicating with the ciliar cysts (Fig. 1.). Thus it seems probable that these cysts are created by evagination of the distal wall of this cavity and may become tied off later on.

The medial lobe is well developed as on rodents generally [*Romeis* (12)]. The cells are aligned like epithelium, there are no groups separated by argyrophil fibres. Some rows of connective tissue can be seen but insufficient to part the lobe into strangs.

This lobe contains no chromophil cells. There are three sorts of cells to be found: 1. typical- or lightcoloured-nuclei-cells, these are similar to the γ-cells of the anterior lobe, their boundaries not well visible, at the most

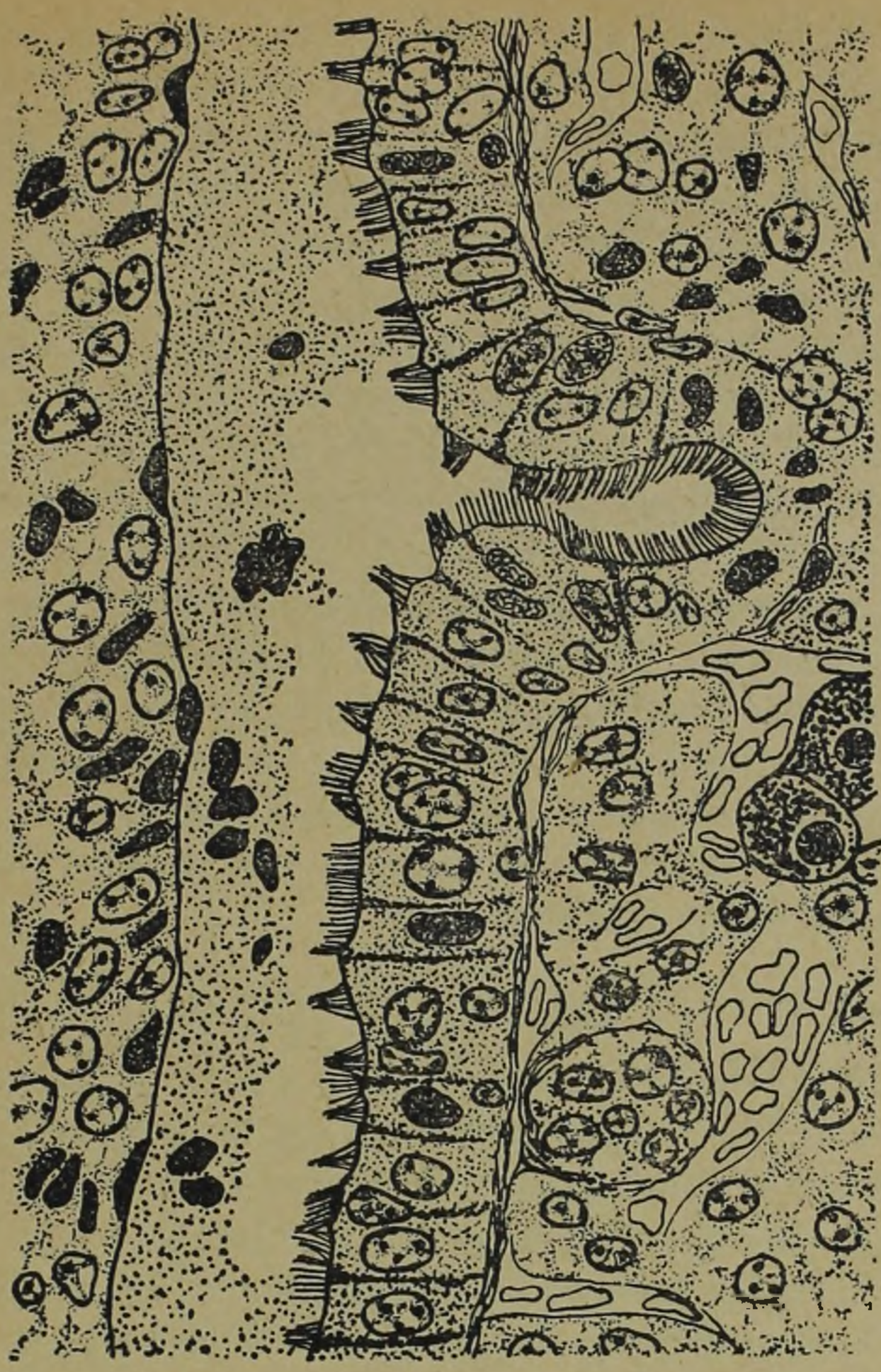


Fig. 1. — Distal wall of the pituitary cleft communicating w h a ciliated cyst. 19. 11. 1941. Susa. Azan. Ok. 9. Obj. 40.

on the margin of the lobe. The size of the nucleus is 6,90μ and undergoes the same changes as that of the chromophobe cells. 2. Cells with dark-coloured nuclei, very similar to the previous one, with the exception that the nucleus is smaller (4,96μ), it contains the chromatine-granula stuffed together and is stained therefore darker. 3. Hyperchrome or crumpled-nuclei cells resembling the liquefying α- and β-cells of the anterior lobe, spindle-formed, the cytoplasm stained dark violet by azan- and Mallory-staining, with an angular nucleus (4,67μ). The chromatine-content is coarsely dispersed or lumped together („Knitterkern“ „crumpled“ nucleus), the nuclear membrane is well visible, contrary to the liquefying cells of the anterior lobe. The hyperchrome cells form elongated bundles and groups around the cranial and caudal end of the pituitary cavity.

It seems probable that the second type of cells is a transitory state between the first and the third type. Between the cells and in smaller and greater fissures we often find colloid. The paraneural wall of the pituitary cavity consists of the same cells as the whole medial lobe, the hyperchrome cells forming long bundles. The medial lobe is characterized by its weak vascularisation too.

In the infundibular lobe the cells are ordered by a few argyrophil fibres in round groups and bundles. They look like the chromophobe cells of the anterior lobe and the typical cells of the medial lobe. The cell-boundaries are seldom visible, the average size of the nuclei is 6,85μ. Follicles containing colloid are frequent. They have to be considered true follicles and no pseudofollicles since the bordering cells are arranged circularly or elliptically, like epithelium. In the dorsal as well as in the ventral tract of the infundibular lobe there are one or two major cysts to be found, lined with columnar, cubical or squamous epithelium in which colloid of varying consistency, nuclei and cell-fragments are to be found. Several arteries and veins run through the lobe, between the cell-groups a few capillaries are to be found.

REPORT ON THE SEASONAL STRUCTURAL CHANGES.

15. April 1941 (Prep. 1, 4 & 5.).

In the anterior pituitary lobe there are basophil and chromophobe cells, the former in much greater number. β-cells do not form sharply delined groups, this may rather be seen in case of γ-cells.

There are three sorts of β-cells: those with light nuclei, with dark nuclei and those liquefying. The nuclear membrane of the dark nuclei cells throws folds, the finely granulated chromatine-content is stuffed together. These nuclei are by 1,42μ smaller than the light ones. The cytoplasm of the dark nuclei cells is also stained darker, these cells can therefore safely be considered as a transitory state of liquefaction. β-cells of the first and second type are in about equal numbers present, liquefied cells are fewer. Among the γ-cells there are great cells too, in the nuclei of these the coarse chromatine is clogged together in the middle, the cytoplasm contains azan-red droplets.

No ciliated cysts are to be found, the capillaries are wide, but only moderately filled with blood-corpuscles.

The pituitary cleft contains a little azan-blue colloid.

In the medial lobe light-nuclei cells predominate along with some dark-nuclei cells and a few hyperchrome ones.

In the infundibular lobe between the cells and in some follicles a considerable quantity of azan-blue, Mallory-blue colloid is stored.

In the anterior lobe one of these April animals we found only light-nuclei β-cells. The nuclei of γ-cells contain 6—8 strongly refracting azan-red colloid droplets. These can also be found in the cytoplasm. A cyst also developed, lined mostly with squamous epithelium and some ciliated cubical cells, containing few colloid and crumbling nuclei. In the capillaries granular secretions are found in some places.

20. May 1941 (Prep 17/2), *16. May. 1942* (Prep 89/1.).

In the anterior lobe basophil cells are to be found in still greater number than the month before. On the ventral margin there are now β-cells too, γ-cells are found in little groups, bundles and singly scattered among them.

Both cell-types form well discernible groups marked off by argyrophil fibres. The light-nuclei β-cells have a darker colour than they had in April, there are considerably more dark-nuclei cells. Liquefying cells are to be seen frequently.

In May there is a distinct tendency towards formation of pseudofollicles built jointly by the different cell-types. The colloid. in their cavity is stained azan-red, on the rim azan-blue and Mallory-yellow or-blue.

The ciliated cysts are in this month very frequent and well developed (Plate I. Fig. 3.). From the caudal end of the pituitary cavity they can be traced far along the ventral margin or else they lie right on the border of the anterior and medial lobe. They are lined with columnar epithelium (height: 20,75μ), sometimes replaced by cubical or squamous epithelium. The cavities are large, with a diameter up to 150μ × 81μ. They contain ample colloid either of thin consistency, highly vacuolized, or lumped together. Round the nuclei which are frequently found in the colloid there is always a small vacuole. The cysts which belong to the ramified ducts (those which lie along the ventral margin) always contain thin colloid. The colloid in the cysts on the border of the two lobes may be of varying consistency, which is closely related to the quality of the cells lining the cysts. When the cyst contains thin colloid, the cells are high and ciliated, while in the cysts where the colloid lumps together the epithelium might decrease to become squamous and the cilia disappear. A peculiar draining of these cysts can be observed. The wall of the cysts containing thin colloid becomes interrupted somewhere on the side towards the medial lobe and the contents stream visibly with aid of the cilia between the cells of the medial lobe (Plate II. Fig. 4.).

The blood-supply of the anterior lobe is very generous, the wide capillaries are almost stuffed with blood-corpuscles.

The ciliated cells of the pituitary cleft are high (17,08μ). The caudal portion is filled with thin vacuolized colloid containing big lumps as well.

The proportion of the typical and dark-nuclei cells in the medial lobe differs individually, while the hyperchrome cells form only small groups at the caudal end of the pituitary cavity, grouping around the colloid flowing from an opened cyst. The vessels of the medial lobe are wider than the average and contain more blood-cells.

In the infundibular lobe there are many follicles, formed by low columnar epithelium cells, the cement list [„Kittlinie" (Heidenhain)] well visible on their free surface. They contain thin colloid.

19. June 1941. (Prep 52/1).

The cells of the anterior lobe are arranged this month also into groups and bundles bordered by argyrophil fibres. On the surface of the lobe there is about equal amount of basophil and chromophobe cells, but advancing towards the center of the lobe, the γ-cells, ordered into long bundles, become dominant. The β-cells undergo a peculiar transformation. There are only few light-nuclei cells, stained still lighter as compared with the month before. Most of the β-cells lost their granula and seem to be in the state of exhaustion, their frontiers can only be deducted from the space left by the bordering cells. The cytoplasm is completely void of granula, highly vacuolized or stretched web-like between the next cells or found in fragments only. The chromatine-content of the nuclei is perfectly clogged together, the individual chromatin granules can only be discerned with strong magnifying. Seen with weaker magnifying the nucleus looks like a bright red, (azan- and Mallory-staining) compact globule. (Fig. 2.) These pyknotic nuclei are by 2,1μ smaller than the light nuclei. The exhausted, vacuolized cells being β-cells is proved by transitory forms where commencing vacuolisation and nucleo-pyknosis is found along with a few basophil granula. The described β-cells are characteristic for this month, seen with low magnifying the anterior lobe is „spotted" by the pyknotic nuclei. Liquefying β-cells are only rarely encountered. Once again there are only few large γ-cells. In the chromophobe zone there is a cyst lined with cubical epithelium, non-ciliated. Several whole nuclei may be seen in the viscous colloid with an aera of thinner colloid around them. On the rim of the whole mass there are big vacuoles with tiny pyknotic nuclei.

Pseudofollicles are often found this month as well, they are just like those from the month before. On their customary place we find some ciliated cysts, their diameter (100μ × 70μ average) and the thickness of their walls (12,76μ) diminished compared with the previous month. They contain viscous colloid.

The capillaries are much narrower than the month before.

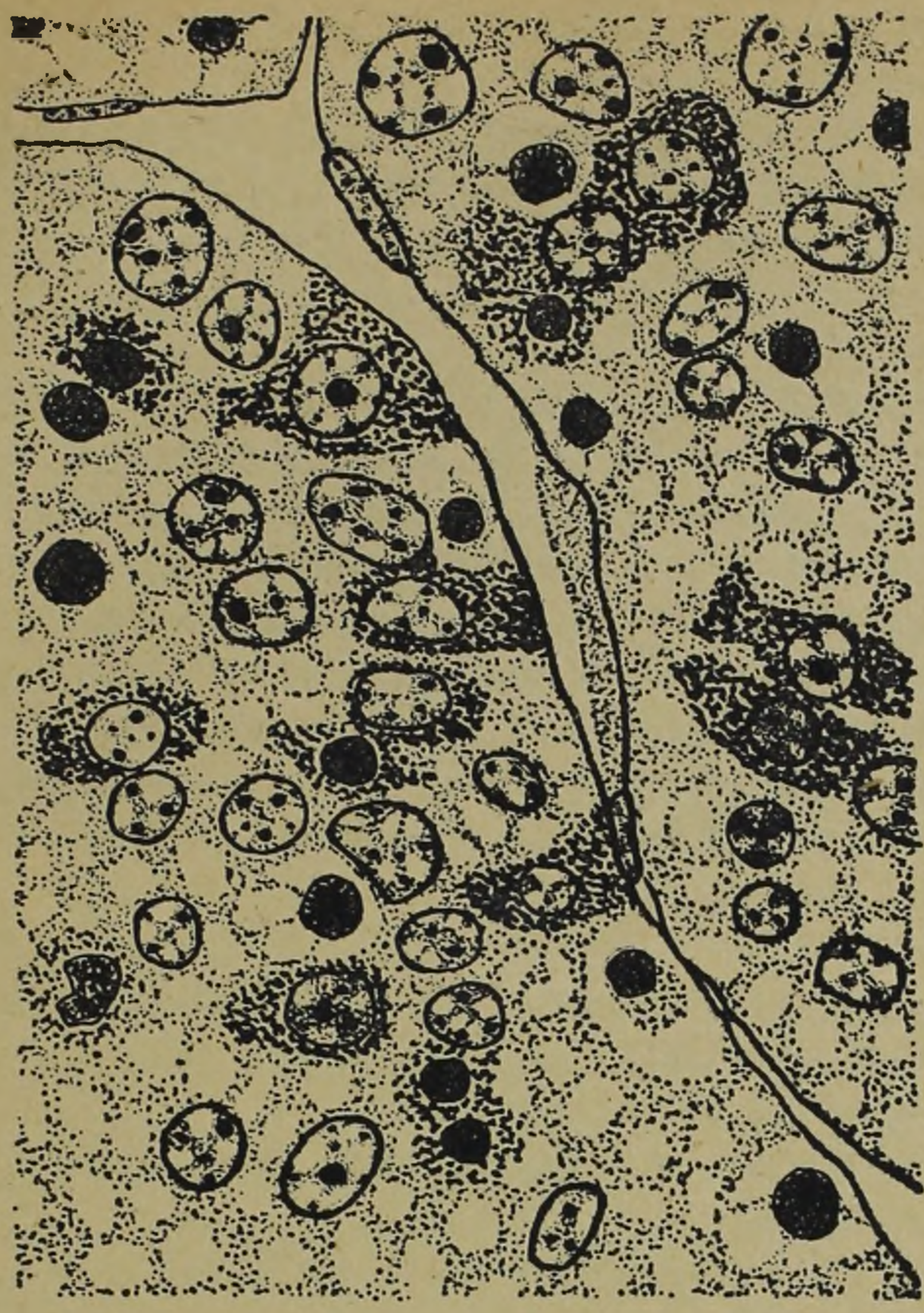


Fig. 2. — Exhausted basophil cells with pyknotic nuclei.
19. 6. 1941. Susa. Iron-haematoxyline — eosine. Ok. 9. Obj. 90.

The pituitary cleft is remarkably wide (82μ), the columnar epihelium of its distal wall is high (22,62μ). The caudal end of the cavity contains much thin colloid.

In the medial lobe there are few light-nuclei cells, the crumpled-nuclei ones are dominant. These are not always hyperchromes, their cytoplasm is often vacuolized and remains unstained. The crumpled nuclei accumulate along the paraneural wall beside the colloid substance of the pituitary cavity and getting mixed up with it they crumble to a red debris (azan- and Mallory-staining). In two places a major quantity of thin colloid accumulates between the cells, containing azan-red colloid droplets and nuclei too. Here we also see round the colloid crumpled nuclei.

Pyknotic nuclei are also to be found in the infundibular lobe. A great amount of colloid is stored between the cells and in the follicles. On the ventral part of the lobe there are cysts to be found lined with cubical or

squamous epithelium, containing some nuclei in their Mallory-blue colloid.

This month the pituitary of a female was also examined. The anterior lobe contains still more degranulated β-cells and there are only single typical cells among the dominant crumpled-nuclei ones in the medial lobe.

17. July 1941 (Prep 55/I).

On the surface of the anterior lobe the proportion of basophil and chromophobe cells changed in favour of the former. Although the number of γ-cells increases as we move towards the center of the lobe but, it still remains less than that of the β-cells. The pronounced structure resembles that found in May but the capillaries running between the cell-groups are not so wide. Only some of the β-cells have visible borders, most of them form plasmodia. They are stained with about the same intensity as the light-nuclei β-cells of June. Scattered exhausted β-cells and small groups of liquefied ones may also be seen. There are some large cells among the γ-cells.

In the cranial part of the anterior lobe scattered pseudofollicles are formed by β- and γ-cells. On its usual place there is a cyst lined with columnal epithelium bearing disorderly, clammy cilia. It contains a little thin colloid and some viscous lumps.

The pituitary cleft is still rather wide (58μ), rich in thin, in some places viscous colloid.

Typical cells are the most frequent in the medial lobe, followed by hyperchrome cells. The dark-nuclei ones are the rarest. The crumpled nuclei of the hyperchrome cells are of such a compact structure, that chromatine granula can only be discerned under strong magnification. In some places minor accumulations of colloid are found.

The infundibular lobe contains less colloid. On the ventral side a large cyst developed, its cubical epithelium partly ciliated, containing little colloid only.

30. July 1946 (Prep 104/I).

The pituitary of the August animal was missing from the material collected. As later I was also unable to get guinea pigs in this month, I examined the pituitary of an animal dissected on July 30. Since the dissections always took place around the middle of the month, this should replace to some extent the August specimen.

In the anterior lobe there are about equal number of basophil and chromophobe cells, mixed with one another, the γ-cells forming independent groups in places. The structure is much less pronounced than it was in the middle of the month. β-cells form plasmodia or are sharply delined.

The latter are stained with the same intensity as in May. These well-delined, granulated cells appear mostly along the capillaries. Their pole touching the capillary is more intensely granulated than the opposite one. The dark-nuclei β-cells seen in April and May appear once again in places and quite a number of liquefying cells is found singly or in groups. Scattered degranulated, exhausted β-cells may also be seen. One or two large γ-cells are found too.

There are no pseudofollicles, a cyst is formed, lined with medium high columnar epithelium (10,09μ), the cells lying disorderly in some places. It contains very little colloid with some nuclei.

The blood-supply of the anterior lobe is very generous, the capillaries full of blood-corpuscles have sinus-like width (12,16μ).

The pituitary cleft is less wide (46μ) than it was middle of the month containing a small quantity of thin colloid.

The medial lobe contains typical and dark-nuclei cells.

The infundibular lobe contains still less colloid than in the middle of the month. Some cells with pyknotic nuclei may again be seen.

15. September 1941 (Prep 59/I).

The chromophil and chromophobe cells are so mixed up, that seen with weaker magnification the anterior lobe appears „marbled" (Plate II. Fig. 5.). On the ventral side of the lobe and in the chromophobe zone the cells are divided into groups by argyrophil fibres, this structure becomes blurred around the center of the lobe. The colouring of the chromophil cells in this month is remarkable. Stained with Mallory's method they look rather basophil, with the azan-method they assume a more acidophil character. The number of liquefied β-cells is remarkably great.

The nuclei of the γ-cells appearing in great number around the ventral margin of the lobe vary in a great degree. There are nuclei containing beside the clumped chromatine some nucleoli, in others the chromatine is finely dispersed and in the middle there is a strongly refracting formation, resembling colloid droplet produced by fusion of the nucleoli (stained azan-red). And lastly angular, pyknotic nuclei are also to be found. In this same region groups of small hypoeosinophil cells (10,03μ $\times$ 7,60μ) and typical eosinophil cells may be seen as well. Some γ-cells have around their nucleus fine acidophil granula, this phenomenon may be connected with the origin of α-cells. Large γ-cells appear in greater number compared with previous months. In the chromophobe zone some δ-cells appear this month. Normal cells are rare, they mostly show different grades of liquefaction.

Between the γ-cells azan-red colloid is to be met in several places, but in the anterior lobe neither pseudofollicles, nor ciliated cysts are formed.

The capillaries are much narrower than the month before and contain less bloodcorpuscles.

The pituitary cleft is narrow, the ciliated columnar epithelium-cells are discernible only on a small part of it.

In the medial lobe typical cells are the most frequent. The dark-nuclei cells form closed groups in great number at the caudal end of the pituitary cavity and along the paraneural wall. Their cytoplasm is stained light violet this month with azan- and Mallory-staining. Hyperchrome cells appear only rarely. Some small accumulation of colloid is to be seen between the cells.

In the infundibular lobe the accumulation of colloid increases again, the colloid appearing in follicles and cysts or between the cells.

15. Oktober *1941* (Prep 62/1 & 62/I).

Chromophil and chromophobe cells still mix strongly on surface of the anterior lobe but in the inside of the lobe acidophil cells become dominant, γ-cells are found singly or in small groups among them. The structure of the anterior lobe is much less pronounced than in the beginning of summer. There are light-nuclei and dark-nuclei α-cells, the difference between the size of the nuclei of both types is smaller (0,26μ) than in the corresponding β-cells. The dark-nuclei α-cells are usually more intensely granulated. In the nuclei of γ-cells there appear several nucleoli, these transform into strongly refractive homogenous colloid droplets stained bright-red by azan- and Mallory-staining, or black by ironhaematoxyline lining along the inner surface of the nuclear membrane (Fig. 3.). In these γ-cells the cytoplasm usually also contains similar colloid-droplets, these wander towards the capillaries and penetrate into them. There are other cells, where the chromatine-content of the nucleus and the nuclear membrane completely disappear leaving only a large colloid-accumulation on its place.

In the anterior pituitary lobe of both animals examined one ciliar cyst has been found, lined by medium columnar epithelium (12,68μ) and cubical epithelium. They contained azan-blue colloid with azan-red globules sticking to the end of the cilia.

The capillaries are narrower still than the month before. They contain in places greater azan-red colloid droplets. To the wall of a capillary there clings an intensely granulated dark-nuclei α-cell, at the point of contact a secretory blastule projects into the capillary.

The pituitary cleft is rather narrow (29μ), the ciliated columnar epithelium high: 21,83μ. In the cavity a little thin colloid containing nuclear debris can be found.

PLATE I.

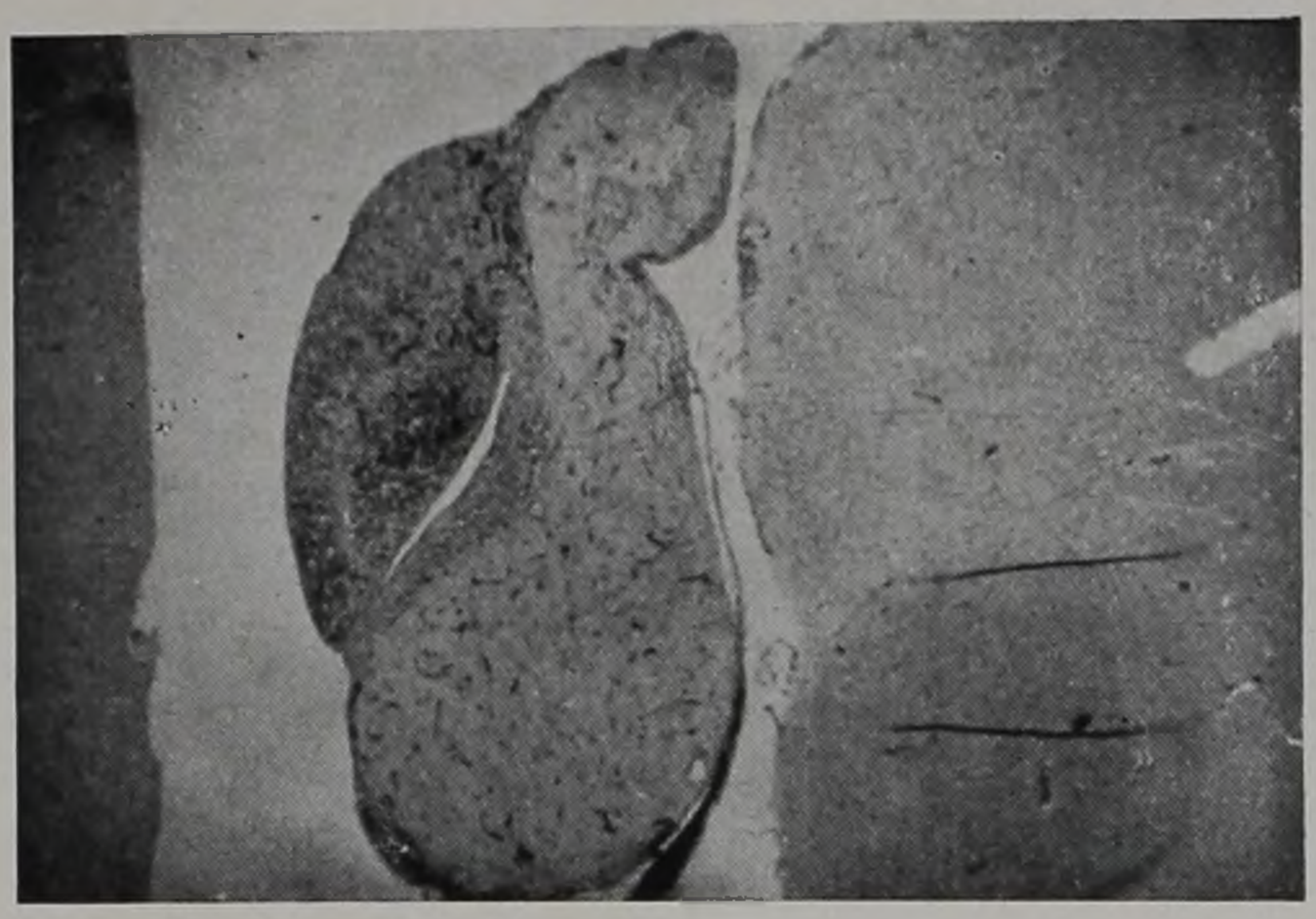


Fig. 1. Sagittal section of the pituitary. 15. 9. 1941. Susa. Ironhaematoxyline — eosine. Ok. 9. Obj. 2.

Fig. 2. Longitudinal and transversal section of an arteriole on border of the infundibular and anterior lobe. Epitheloid media-cells in the vascular wall. 15. 9. 1941. Susa. Azan. Ok. 9. Obj. 40.

Fig. 3. Section of three ciliated cysts in the anterior lobe. 20. 5. 1941. Susa. Mallory. Ok. 9. Obj. 40.

PLATE II.

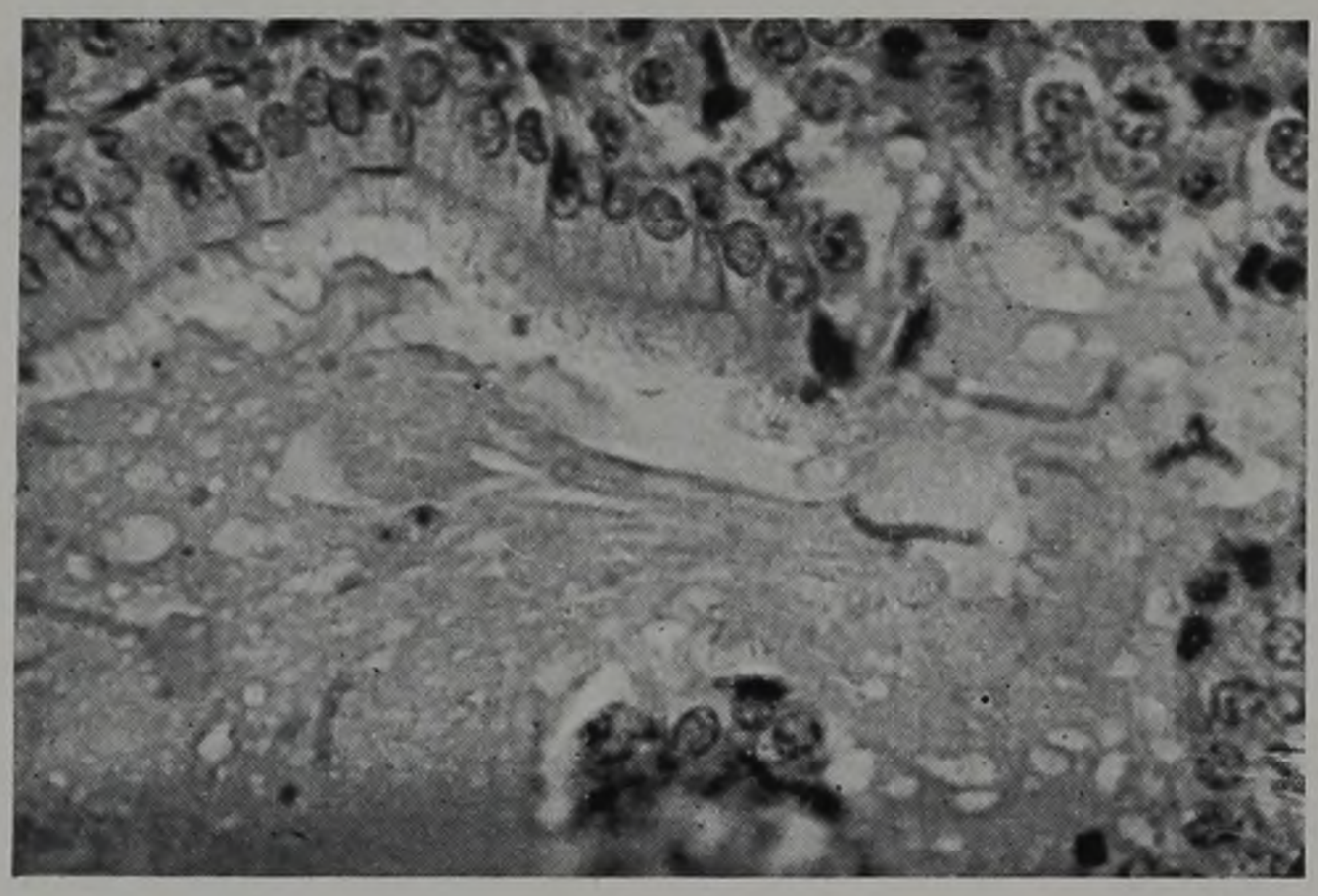


Fig. 4. Content of a ciliated cyst streaming into the medial lobe. 20. 5. 1941. Susa. Haematoxyline — eosine. Ok. 9. Obj. 40.

Fig. 5. „Marmorated" anterior lobe. Chromophil and chromophobe cells strongly mixed. 15. 9. 1941. Susa. Mallory. Ok. 9. Obj. 8.

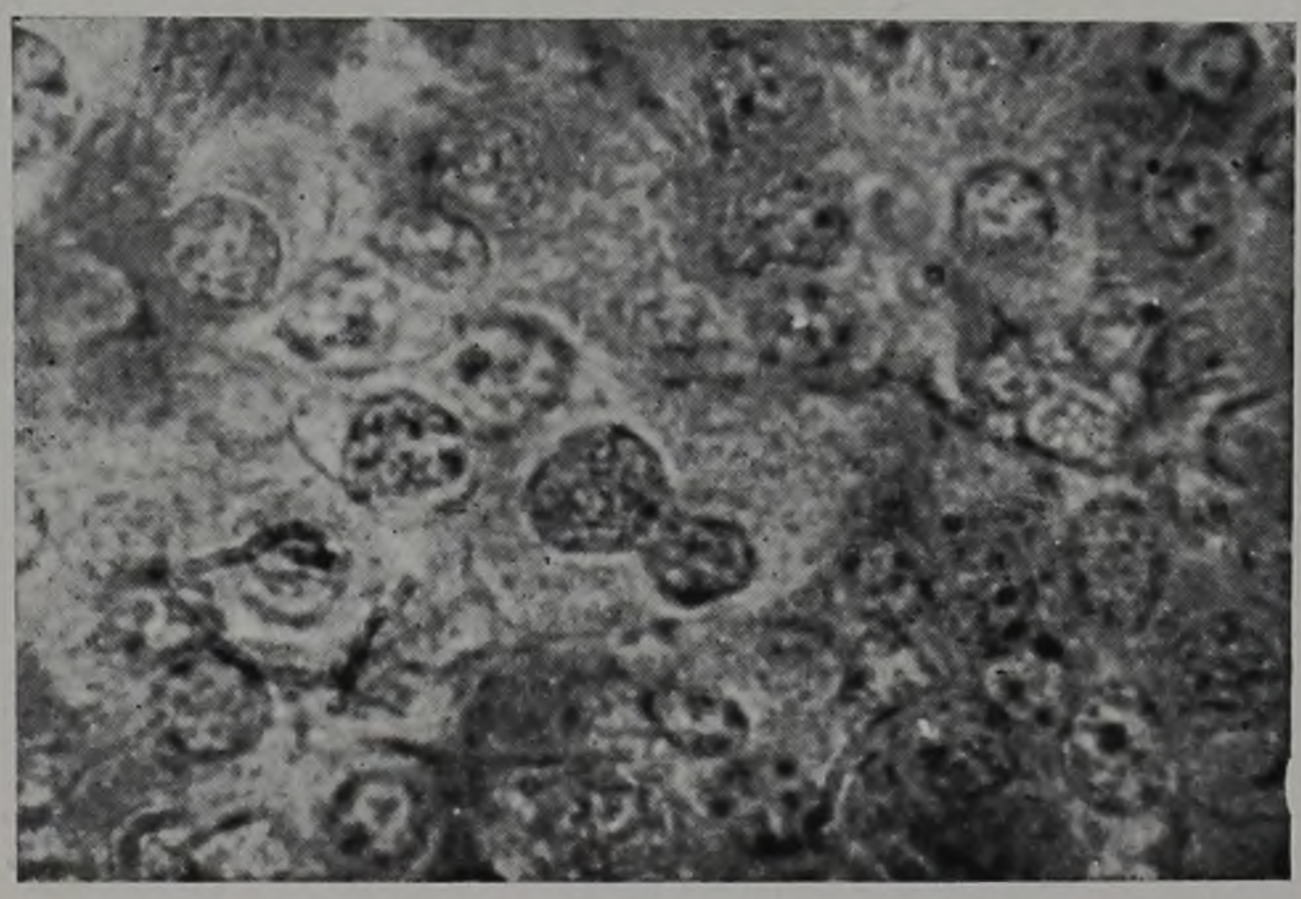


Fig. 6. Large chromophobe cell dividing amitotically. 15. 12. 1941. Susa. Azan. Ok. 9. Obj. 90.

Fig. 1.

Fig. 2.

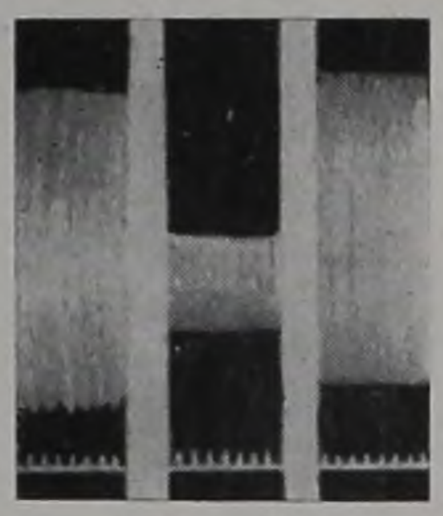


Fig. 3.

Fig. 1. Left: Isolated frog heart in Ringer (*pH* 7, 2). Middle: after washing 6 times every 2 minutes with $6{,}10^{-5}$ quinine. Right: the same concentration of quinine in Ringer solution buffered to *pH* 5. Time marker on all tracings: 5 sec. Fig. 2. Left: Isolated heart in normal Ringer. Middle: washed 7 times every 2 minutes with $6{,}10^{-5}$ quinine. Right: the same concentration of quinine in Ringer containing a 175% excess of *Ca* ions. Fig. 3. Left: Isolated heart in normal Ringer. Middle: washed 8 times every 2 minutes with $6{,}10^{-5}$ quinine. Right: the same concentration of quinine applied afterwards in a Ringer without any *K* ion.

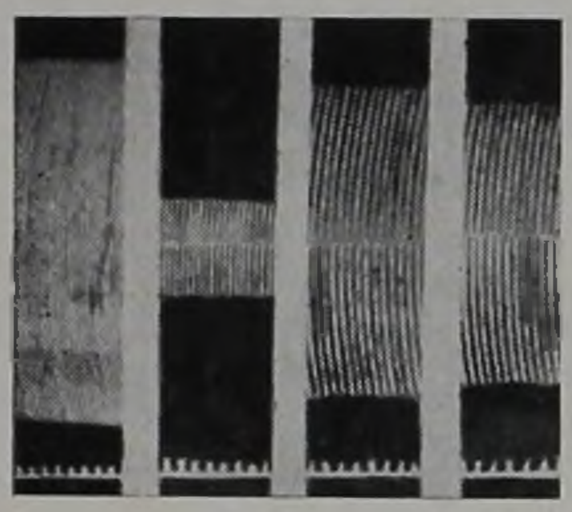


Fig. 4.

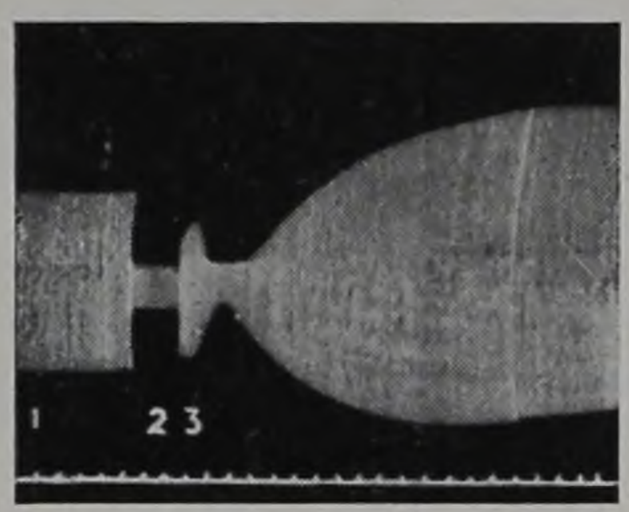


Fig. 5.

Fig. 4. From left to right: Isolated heart in Ringer; after 7 washings every 2 minutes with 10^{-6} quinine; washing with the same quinine containing 10^{-9} adrenaline; the effect after 10 minutes; extreme right: the amplitude after 45 minutes with no change of bathing fluid. Fig. 5. 1: isolated heart in Ringer 2: after 8 washings every 2 minutes with 6,10- quinine, at 3: the quinine solution changed to a solution containing the same quinine and in addition 3,6 μ g of ATP per ml.

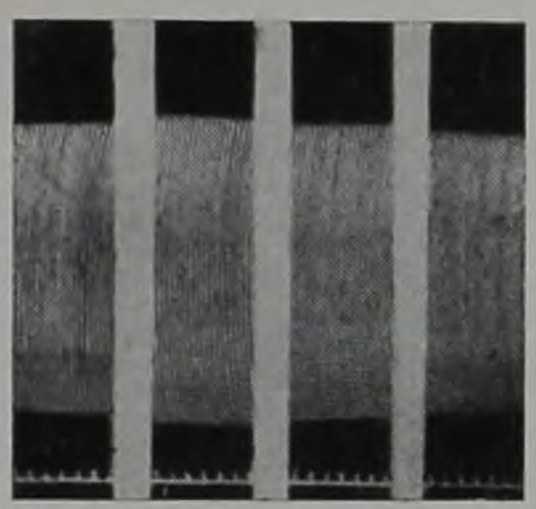


Fig. 6.

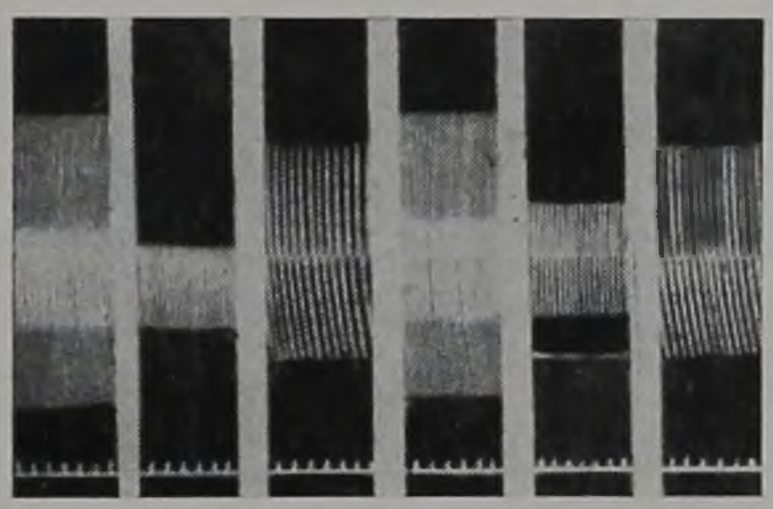


Fig. 7.

Fig. 6. From left to right: isolated heart in Ringer, in presence of 5, 50 and 500 μ g ATP resp. Fig. 7. From left to right: 1. isolated heart in Ringer, 2. after 7 washings every 2 minutes with $6{,}10^{-5}$ quinine, 3. after the addition of 1,5 μ g of ATP in presence of the same concentration of quinine, 4. after washing with Ringer, 5. after 6 washings every 2 minutes with $6{,}10^{-5}$ quinine in presence of 10^{-5} ergotamine, 6. after the addition of 1,5 μ g per ml. ATP in presence of the same quinine and ergotamine as before.

This plate belongs to Lichtneckert-Straub's paper:
The Actioa of Adenosinetriphosphate on the Isolated Frog Heart.

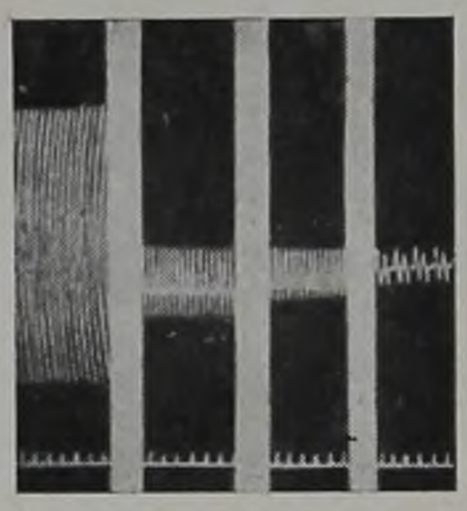


Fig. 8.

Fig. 9.

Fig. 10.

Fig. 8. From left to right: 1. isolated heart in Ringer, 2. after washing 6 times every 2 minutes with $6{,}10^{-5}$ quinine, 3. 1 μg adenylic acid in presence of the same quinine, 4. 5 μg adenylic acid and the same quinine as before. Fig. 9. From left to right: 1. isolated heart in Ringer, 2. after 7 washings every 2 minutes with 10^{-6} quinine, 3. the same quinine as before in presence of 1 μ g per ml of pyrophosphate. Fig. 10. From left to right: 1. isolated heart in Ringer, 2. after 6 washings every 2 minutes with 10^{-6} quinine, 3. changing the solution to the same quinine concentration but containing 1 : 200 diluted muscle extract.

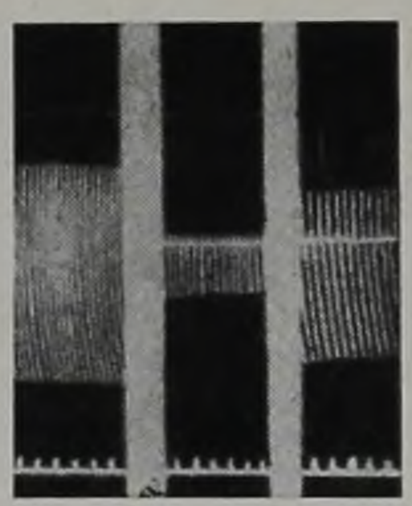


Fig. 11

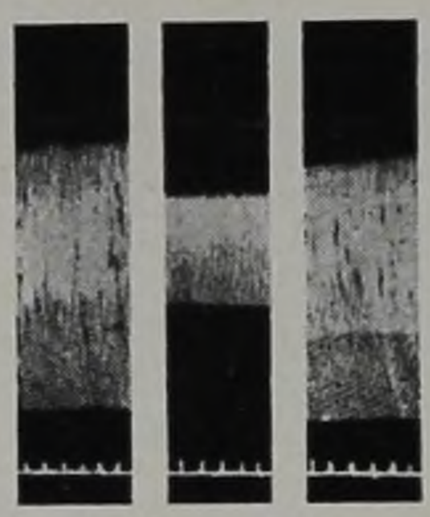


Fig. 12.

Fig. 11. Left isolated heart in Ringer, middle: after 5 washings with a Ringer containing 0,002% $CaCl_2$, right: addition of 0,6 μg ATP per ml in a Ringer containing 0,002% $CaCl_2$. Fig. 12. Left: isolated heart in normal Ringer, middle: after 7 washings every 2 minutes with a Ringer containing 0,002% $CaCl_2$ only, right: the same deficient Ringer solution in presence of 10^{-6} adrenaline.

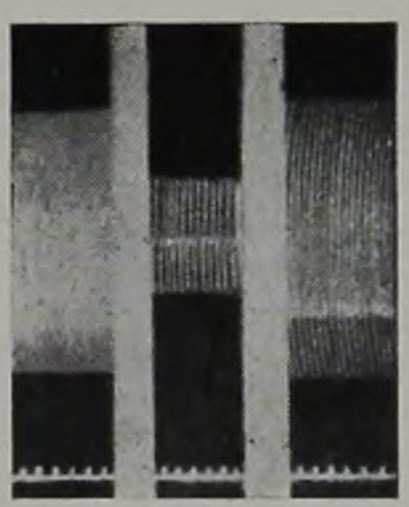


Fig. 13.

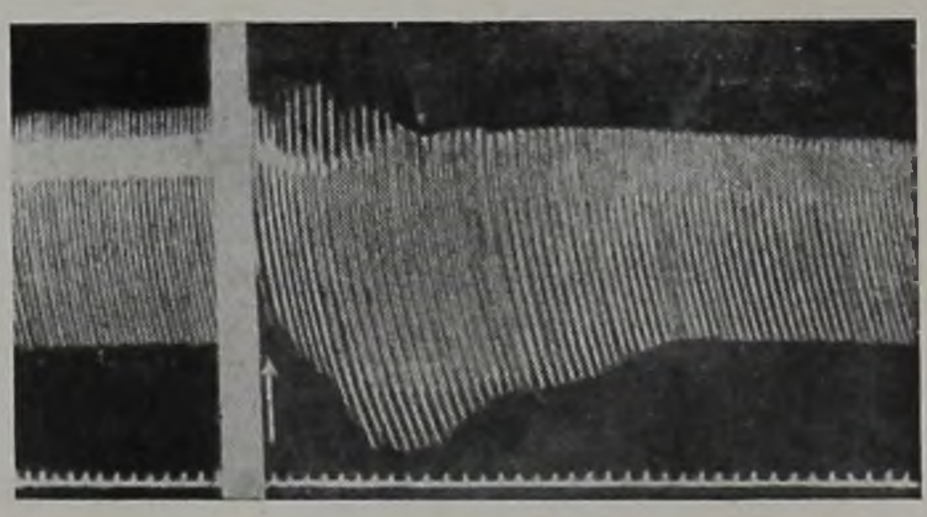


Fig. 14.

Fig. 13. Heart in situ. Left: normal, middle: 5 minutes after the intravenous injection of 15 μ g quinine per g frog, right: 3 minutes following the intravenous injection of 60 μ g ATP per g frog. Fig. 14. Frog heart in situ. Left: normal. Right: injection of 50 μg ATP per g frog at arrow.

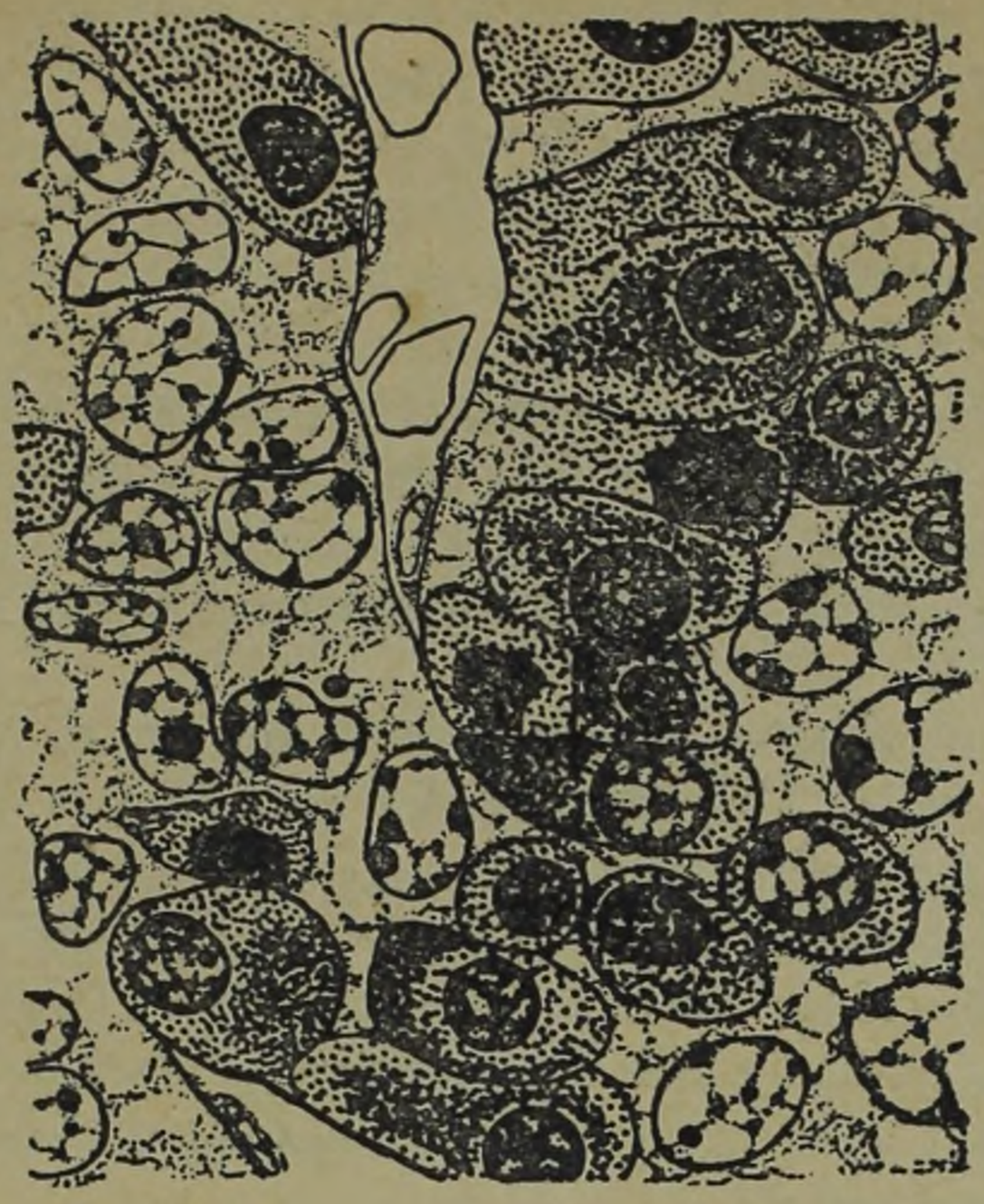


Fig. 3. — Light-nuclei and dark-nuclei acidophil cells and chromophobe cells in the anterior lobe. The nuclei and cytoplasm of the chromophobe cells contain colloid-droplets. 15. 10. 1941. Susa. Azan. Ok. 9. Obj. 90.

The medial lobe is dominated by light-nuclei cells, azan-red colloid droplets appearing in their nuclei and cytoplasm as well. The few hyperchrome cells form elongated bundles along the paraneural wall.

In the infundibular lobe three wide cysts (46μ—32μ) are found bordering the chromophobe zone. Their wall is of cubical epihelium. There is a similar cyst in the ventral part of the lobe as well. They contain thin colloid with some nuclei.

19. November 1941 (Prep 65/1 & 65/I).

In the anterior lobe there is no definite structure same as the month before. The number of γ-cells increases. They form into a big bundle running along the middle and into small groups between the α-cells. Once more there are light-nuclei and dark-nuclei α-cells, but there is a greater number of liquefied cells too. The dark-nuclei and at the same time more intensely granulated α-cells observed this and the previous month are probably precursory states of liquefaction. The colloid droplets in nuclei and cytoplasm of the γ-cells are less frequent than the month before. There are γ-cells to be found where the nucleus is replaced by a single big drop of colloid the size of which (3,70μ) is about half of that

of the average nucleus. The nuclei of the large γ-cells scattered in the chromophobe zone are often of compact structure, stain intensely.

This month a great number of cysts is formed. Their cavity is not big ($54\mu \times 49\mu$). The ciliated columnar epithelium is of medium height ($11{,}55\mu$). They contain thin or viscous colloid with nuclei and — in one of the cysts — a granulocyte.

The diameter and blood-content of the capillaries is same as the month before. Here and there they contain azan-red colloid.

The hypophyseal cleft is somewhat wider (34μ), than the month before. Some nuclei of the ciliated columnar epithelium wandered into the thin colloid of the cleft.

In the medial lobe there is a great number of hyperchrome cells beside typical cells. They are ordered into long bundles. Nuclei and cytoplasm of the typical cells again contain azan-red colloid droplets. The nuclear membrane of some of the nuclei bulges and the colloid droplet sits in this bulge. Between the cells there are larger droplets as well.

In the infundibular lobe there are several cysts and follicles formed, the former lined with sqamous and cubical epithelium and containing highly vacuolized colloid.

15. December 1941 (Prep 67/1a.).

In the anterior lobe acidophil and chromophobe cells are strongly mixed. The number of the latter increase compared with last months, they dominate especially on the ventral side. The cells of the anterior lobe form groups well delined by argyrophil fibres. There are only light-nuclei-cells, their borders blurred, hardly granulated at all (hypoeosinophil cells). Liquefying-cells are rare. The nuclei of γ-cells contain a great number of colloid droplets, up to twelve. Occasionally there are cells to be found, the nuclei of which are „burnt out" and contain only a very small amount of powderized chromatine. There appears a surprisingly great number of large γ-cells in the chromophobe zone. Their cytoplasm may also contain colloid droplets, their great nuclei, almost stuffed with chromatine, are stained intensive. In γ-cells of this type amitotical cell-division may be observed (Plate II. Fig. 6.). The cytoplasm of some of the large γ-cells contains round the nucleus fine acidophil granula, similar to those seen in September.

There is one ciliated cyst, its proportions being $53\mu \times 34\mu$. The ciliated columnar epithelium is of medium height ($11{,}24\mu$). It stores a small amount of thin, resp. viscous colloid.

The capillaries are narrower still than in the previous months, their blood content is small too, they rarely contain an azan-red colloid droplet.

The pituitary cleft is narrow (9,73μ) containing no colloid.

In the medial lobe there are once more typical and hyperchrome cells. The latter form bundles in still greater numbers and their cytoplasm is stained darker. In the cytoplasm of the hyperchrome cells there are colloid droplets.

In the infundibular lobe a cyst developed, lined with cubical and columnar epithelium, the latter in places ciliated. It contains thin colloid.

16. January 1942 (Prep 71/1 & 71/I).

The formation of groups and bundles among the cells of the anterior lobe is conspicuous. The acidophil and chromophobe cells are mixed as in the previous months. The number of α-cells again increased sharply at the cost of the γ-cells, forming a strong independent bundle in the middle of the lobe.

In the usual place a big ciliated cyst developed (156μ $\times$ 97μ). The columnar epithelium measures 14,99μ. The cyst is rich in thin colloid containing some viscous lumps as well.

The capillaries are extended sinus-like full of blood. They transport no colloid.

The pituitary cleft is much wider than before, but almost perfectly empty.

In the medial lobe conditions are the same as in November and December, but colloid droplets are seen only in few nuclei and cytoplasms. Between the cells an accumulation of azan-red and Mallory-yellow colloid can be seen, round one of these clusters hyperchrome cells are lined up, their poles in contact with the colloid staining darker.

In the infundibular lobe there developed follicles and on the ventral side a cyst lined with cubical epithelium, the wide (58μ $\times$ 53μ) cavity containing colloid of varying consistency.

18. February 1947 (Prep 108/1).

This month also the cells of the anterior lobe are ordered into groups sharply delined by argyrophil fibres. This demarcation is underlined by the blood-filled sinuslike capillaries running between the groups. The number of chromophobe cells excedes that of the chromophil ones. The α-cells have mostly dark nuclei, they aggregate near the distal wall of the pituitary cavity. The cell-borders are not too well visible, the cytoplasm is stained acidophil but there are no granula visible. The nuclei of γ-cells mostly contain finely dispersed chromatine and one or two nucleoli, in others we find colloid-droplets characteristic for the preceding months. Colloid droplets in the cytoplasm are rare. There are some large γ-cells to be seen. In the chromophobe zone and along the ventral surface of the lobe

there appear β-cells among the γ-cells, singly or forming plasmodia. A few γ-cells are to be seen here too, in normal or liquefied form. At the caudal end of the pituitary cavity hitherto unknown cells accumulate. They unite into plasmodia, their cytoplasm remains unstained. The nuclei are small (5,13μ) containing finely dispersed chromatine and one or two large nucleoli. This cell-type may be regarded perhaps as undifferentiated cells.

Two ciliated cysts developed, lined with rather high columnar epithelium (14,89μ), containing thin colloid.

The distance between the walls of the pituitary cleft is 31μ. The ciliated columnar epithelium cells can be followed for a long distance. The cavity contains little thin colloid.

In the medial lobe typical and dark-nuclei cells become mixed, some hyperchrome cells form a bundle along the paraneural wall. In several typical cells a cluster of colloid droplets may be seen in place of the nuclei and there are minor accumulations of colloid between the cells.

In the infundibular lobe follicles are frequent, four cysts lined with cubical epithelium are also found. They contain azan-blue colloid with some azan-red droplets in it.

16. March 1942 (Prep 79/I).

The structure of the anterior lobe is same as before. The chromophobe cells prevail over the chromophil ones. The γ-cells form everywhere closed groups surrounded by α-cells. The borders of the latter are blurred, they are moderately granulated, their nuclei are stained light. They give an impression of exhausted cells. The nucleus and cytoplasm of γ-cells contain no more colloid droplets. Among the chromophobe cells we find hypobasophil ones as well, their cytoplasm is granulated only around the nucleus.

In the anterior lobe several ciliated cysts are found, lined with epithelium cells of varying height (17,02μ—4,86μ), filled with thin colloid and a few colloid lumps. There are droplets sticking to the end of the cilia.

The capillaries are narrow, but contain a great number of blood-corpuscles. In the ventral part of the lobe the capillaries are wider and almost empty, directly beside them there is a fibrous substance stained light blue (azan- and Mallory-staining).

In the caudal portion of the pituitary cleft the distal and paraneural walls almost touch, the medial and cranial portion is much wider (51μ).

The medial lobe is dominated by typical cells, hyperchrome cells form elongated bundles.

There are no follicles or cysts in the infundibular lobe, only some azan-blue colloid accumulating between the cells.

COMPARISON OF THE SEASONAL STRUCTURAL CHANGES AND THE FUNCTION.

A short survey of the histological conditions throughout the year suffices to prove that the adenohypophysis of the guinea pig undergoes seasonal, rhythmical structural changes. Like in every other incretory gland here also the structure represents a specific functional state.

The great aestival hormone-producing period extends over April, May and ends after a month's interval in July. In April there is no storable colloid yet, the increasing granulation, the appearing of dark, dense nuclei and the liquefaction of β-cells all point to the beginning of secretory production. The substances needed for this purpose reach the cells through the capillaries which are in April extended (Tab. I., Fig. 3.). They become wider still in May and are full of blood corpuscles. At that time they probably transport already a part of the produced secretion. In May the β-cells are stained darker still, there are more dark nuclei cells than in April, the secretion reaches its peak. The frequent appearance of pseudofollicles and cysts is also in connection with the intense secretory function, they store the secretion which has not been transported by the capillaries. From the appearance of great number of liquefying β-cells in April, their decrease in May and the simultaneous formation of pseudofollicles one may draw the conclusion that the colloid of these pseudofollicles is derived from the liquefied β-cells, that is to say it is a product of a holokrin secretory mechanism. The colloid of the ciliated cysts is almost certainly not produced by the pseudofollicles as those are to be found in the next two months as well. It is possible that it is a product of the non-liquefied β-cells but the route of this secretion can not be traced with our present methods. The β-cells become exhausted after two months of secret-productive activity, in June most of them loose their granula and the normal cells are also smaller and less-stained than in May (Tab. III.). The exhausted cells then regenerate, in mid-July they are still small (Tab. III.) and contain few granula, at the end of the month they are bigger already (Tab. III.), though not as big as in May, and intensely granulated. The liquefying cells appearing in great number at the end of July also point to a recommenced secretory activity. At that time there are no pseudofollicles left, only a small cyst developed, containing few colloid only. The produced secret seems to take its way through the greatly widened capillaries. The fact that the pole of the β-cells wich is in contact with the capillaries is more intensely granulated points in this direction as well, though actual passing of the secretion through the capillary wall could not be observed. The generous blood-supply of the cells in this period to prepare them for the September rearrangement and the building of new

Fig. 4. — Width of capillaries in the anterior lobe.

α-cells is also task of the widened capillaries. In September no storable colloid is produced, this may be the cause of the narrow and empty pituitary cavity and the total lack of cysts. The minor colloid accumulations certainly originate from liquefied β-cells.

In October begins the secretory activity of α-cells similar to that of the β-cells. The nuclei become denser, the cytoplasm more granulated, in November we already find a considerable number of liquefying cells. The produced secretion is stored in October in one ciliated cyst, in November there are several ones already. The blood-stream seems to transport only small amounts of secretion, the capillaries remain narrow till January (Tab. I.). Till December the α-cells become exhausted, this is shown by their small size (Tab. V.) and slight granulation. Their second secretory period is in January, when the size of the α-cells reaches its peak (Tab. V.). The liquefying cells reappear and a large cyst becomes filled with colloid. January and especially in February the anterior lobe is traversed by wide capillaries (Tab. I.) which probably transport part of the produced secretion and in February bring the cells substances needed for the spring-regrouping and the formation of new β-cells.

The autumnal transformation of the anterior lobe takes then place in September, the spring transformation in February—March. The β-cells stain more intensely with Mallory's method than with azan-staining in both periods using the same fixative. The cells are stained by

the aniline-blue component of both dyes, the only difference between them being that Mallory's mixture contains oxalic acid, the azan-dye on the other hand acetic acid. According to *Pischinger* (10) the tissues quickly lose their dye-retaining properties at a certain *pH* of the dye varying individually for each tissue the limits of reaction depending upon the isoelectric point. The *pH* of aniline-blue in the two methods used is different because of the different acids. The isoelectric point of the transformed or newly produced β-cells surely changes during the autumnal and spring transformation so that these cells become more sensitive against the *pH* of the dyes and conditions in Mallorys mixture better comply with the conditions of positive adsorption. The perfect clearing up of these conditions would only be possible by the use of dyes with exactly defined *pH*.

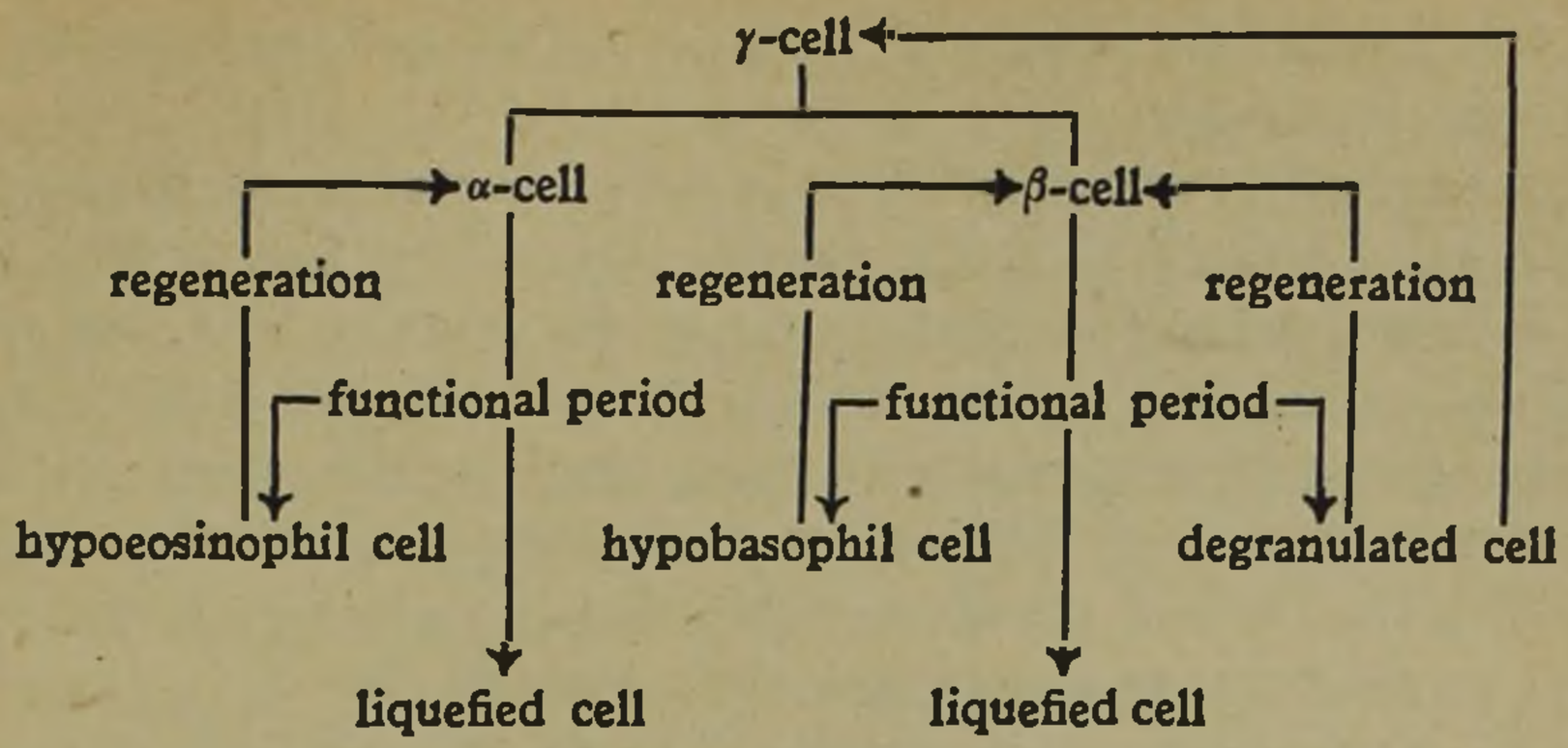
The secretory function of the chromophobe cells is done by the nucleus. This nuclear activity begins in the autumn months and continues throughout the winter months till February inclusively, we found colloid-droplets even in the γ-cells of an April animal. Nucleoli or their transformed products were observed to leave the nucleus in different animals and in different organs by several authors. In the pituitary of guinea pigs only the nuclei of chromophobe cells of the anterior lobe and typical cells of the medial lobe show any secretory activity. The colloid-droplets reach the cytoplasm in two ways. They may accumulate in the center of the nucleus in a berry-like heap and the nuclear membrane gradually dissolves around them. The chromatine content disappears and only the colloid remains to be seen in the cytoplasm. It is more usual however that the colloid-droplets aligne along the inner surface of the nuclear membrane and bulge the membrane in several places. The actual transition of these droplets I could never observe they were either out or in the nucleus. The droplets retain their staining properties, form and refraction in the cytoplasm as well, they tend out of the cell and either unite outside the cells with droplets from other cells or — in the anterior lobe —, make their way directly into the capillaries. The droplets leaving the nuclei can not be considered as artificial products since they appear only in a certain phase of the year and they leave the cells not always on the same side, but in different directions. The fact that I could not observe the droplets actually leaving the nucleus may certainly be explained by the pituitary having a daily functional rhythm too [*Apor & Stohl* (1)] and the transition of these formations taking place in another time of day than when the investigations took place.

There is a strict relation between the staining of the erythrocytes in the capillaries of the anterior lobe and the nuclear secretion of the γ-cells.

With azan-method the erythrocytes stain yellow with orange *G*, while with Mallory's method they are stained red by acid fuchsin. According to *Seki* (14, 15) the membrane of the erythrocytes has small pores, therefore they are stained by the highly dispersed, small-molecule orange *G* and acid fuchsin. In those months, when there are colloid-droplets produced in the γ-cells, the erythrocytes are stained red with the azan-method by the great-molecule, coarsely dispersed azocarmine. The colloid getting into the capillaries surely becomes adsorbed to the erythrocytes and this thin layer is stained by azocarmine. With Mallory's method the erythrocytes have a violetreddish hue probably because the great-molecule aniline-blue also partakes in the staining of the adsorbed colloid layer.

Having become acquainted with the function of the cells of the anterior lobe there remains the problem of the genetic connection between these cells. The functional period of α- and β-cells are — as we have seen —, so similar that I am convinced they being diversely differenciated forms of the same cell, the „pituitary cell". The secretion of β-cells is done with greater intensity as shown by the more frequent appearance of liquefied forms that indicate a holokrin type of secretion and the degranulated, exhausted cells, which never appear among α-cells they losing only part of their granula and becoming hypoeosinophil cells. Both α- and β-cells transcur two functional periods one after the other, but while the intensity of function of β-cells reaches its peak in the first period, the second period of the α-cells excedes the first one in intensity. In the time between the periods (June, December) the exhausted β-cells and the hypoeosinophil cells regenerate, new cells are produced to replace the liquefied ones out of the chromophobe cells appearing in great number at that time. These cells appear in masses also during the autumnal and spring transformation (September and February—March) the new α- and β-cells are produced out of them. The γ-cells are thus the ancestral cells of the chromophil cells, this explains that cells in amitotic division may be seen among them (December) (Plate II. Fig. 6.). The division of chromophil cells I could never observe.

The systematic examination throughout the year brings us closer to the physiological significance of the pituitary cysts as well. Ciliated cysts are always to be seen in the anterior lobe of the pituitary of guinea pigs with the exception of two months (April and September) when there is no storable colloid resp. the cells of the lobe are regrouped. The observations indicate that the cysts appear when secret is being produced, and the more intense this function becomes, the more and the wider cysts are formed (May, November, January). If the stored colloid becomes of



Genetic connexions between cells of the anterior lobe.

tougher consistency it can not move on and must therefore be diluted. This is very probably done by the cells and nuclei which are frequently found in the colloid, since they are always surrounded by a halo of thin colloid or a vacuole. These cells and nuclei produce perhaps a ferment and perish in turn. Such shrunken cells and cell-fragments are seen in the colloid of large cysts. In the cysts of the infundibular lobe and in the pituitary cavity similar activity of the cells was also to be observed. The problem remains what happens to the perished cells and nuclei. They may dissolve in the colloid but it is also possible that they are removed by leukocytes since we found a granulocyte in the cyst of a November animal.

The colloid stored in the cysts may take three ways. It may reach the bloodstream through the surrounding epithelium cells, the marginal vacoules seen in some places point to this way of resorption. But it also can get into the pituitary cleft that often communicates with the cysts. After the abundance of colloid in the cysts in May great quantities of colloid are found in the pituitary cavity (June). Finally as we saw, in May the wall of the cysts may burst and the colloid streams out. In the two latter cases the cilia play surely an active part in transferring the colloid.

The cilia of the columnar epithelium of cysts are produced apparent similar as in the oviduct of man and rabbit, described by *Mihálik* (6, 7). In the state observed the ciliar vesicle already opened up and the cilia were found on the concave surface of the cell. This state is pertained according to *Mihálik* for a long time, that is why we meet it so often.

The generous vascularisation of the anterior lobe points to the possibility, that not only the colloid stored in the cysts get into the capillaries, but they may also play a part in the direct transportation of secretions. In some of the months we actually found granulated secretion, secretory vesicle and azan-red colloid drops in the capillaries. The way

of the latter could be observed from the nuclei of chromophobe cells right to the capillaries.

In the medial and infundibular lobe there are not to be found seasonal changes as definite as in the anterior lobe. There is a striking parallelism between the chromophobe cells of the anterior lobe, the typical cells of the medial lobe and the cells of the infundibular lobe, regarding the staining and structure of cytoplasm, and nuclear structure. These similitudes made several histologues to suspect some relation between these cells. The cyclical examinations further two more evidences in this respect: comparative measurements show that all the three types gain their maximal size in the winter months (Tab. II, VII, VIII.) and the γ-cells show a functional conformity with the typical cells, their nuclear secretion proceeding parallelly. The colloid found in the medial lobe originates surely from the anterior lobe and, — in winter —, from its own secretory nuclei.

No secretory function could be seen throughout the year in the cells of the infundibular lobe. These cells must either be considered inactive or we have to suppose that they have a secretory function that can not be ascertained by present microtechnical methods. The colloid of the cysts and follicles of the infundibular lobe originate probably from the anterior lobe.

It is difficult to answer the question of the further destiny of the hormones produced by the adenohypophysis, but the small azan-red droplets that are sometimes seen in the infundibulum and interbrain suggest that the pituitary acts partly, through the vegetative centres of the interbrain, indirectly on other organs.

CONNECTION BETWEEN THE FUNCTION OF THE PITUITARY AND OTHER INCRETORY GLANDS.

The anterior lobe of the pituitary regulates by its glandotrope hormones the function of the other endocrine glands which in their part also influence by their hormones the function of the pituitary.

Extirpation of the pituitary of dogs leads to a marked atrophy of the suprarenal cortex while on rats feeding of the corticotrope hormone of the anterior lobe causes hypertrophy of the suprarenal cortex. According to *Stohl* (16) cellular activity of the glomerulous and fasciculated zone of the suprarenal cortex of guinea pigs becomes lively in May and June. This period coincides with the first great secretory activity of the basophil cells in the pituitary, which points to these cells producing the corticotropic hormone. It is also possible that the transitory thickening of the fasciculated zone observed in July is caused by the revival of the activity of the basophil cells. Pathological observations also favour this view. In *Cushing's* disease there are symptoms of the hyperfunction of the

suprarenal cortex even adenoms of the cortex have been described. All these symptoms are caused by the hypersecretion of the corticotropic hormone in connection with the basophil adenome found with *Cushing's* disease. The cholesterine-content of the suprarenal cortex of guinea pigs is largest during the winter months coinciding with its liveliest metabolic activity *[Stohl* (16)*]*. At that time there are acidophil cells in the anterior pituitary lobe which produce as we shall see the thyreotropic hormone. It seems as if there would be some connection between the cholesterine-household and the eosinophil cells.

Regarding the adrenalotropic hormone decisive proofs are not yet available. *Stohl* (16) described, that the greatest number of red nuclei are to be found in May and June in the suprarenal medulla of guinea pigs, indicating the peak of adrenaline-production. The functional period of basophil cells coincides with this increased adrenaline-production, the β-cells seem thus partake also in the production of the adrenalotropic hormone.

The suprarenal cortex and the gonads are in close connection regarding their development and their function. The disturbances created by deficiency of the cortex can just as well be eliminated by the administration of sexual hormones. Recently another substance, the androsteron has been identified in the suprarenal-cortex beside the cortine, this has the same character as the sexual hormones. Some pathological phenomena also point to a connection between gonads and suprarenal cortex; tumors of the cortex are accompanied by pubertas praecox in infancy while in women they lead to the appearance of secondary masculine sexual characteristics. These connexions make it probable that same as the corticotropic hormone, the gonadotropic hormone is also produced by the basophil cells of the anterior pituitary lobe. The decreased gonadic function during akromegalia caused by an acidophil adenome is explained by the lack of basophil cells and the consequent stop in the production of gonadotropic hormone. The basophil cells appear in great number in the pituitary of guinea pigs in the summer propagative period similar as *Rasmussen* (11) described on *Marmota monax* L. and *Coninx—Girardet* (2) on marmots, a fact also pointing to the production of gonadotropic hormone.

Thus the basophil cells produce several glandotropic hormones. That is probably why we see among the β-cells signs of exceptional secretory activity, a great number of liquefied cells and the appearance of degranulated, exhausted cells.

We know that the extirpation of the pituitary causes a marked decrease in the basal metabolism of the animals and we also know the

symptoms of administration of thyreotropic hormone produced by the pituitary in the thyroid and in other organs. All these symptoms appear only if there is a thyroid gland in the organism, after its extirpation the administration of thyreotropic hormone remains ineffective. In case of the guinea pig like in all other non-hibernating mammals thyroid function is according to *Mödlinger* (9) most intense in winter, its histological structure showing the same changes like after administration of thyreotropic hormone. This makes it apparent that the pituitary of guinea pigs produces this hormone in winter. The answer as to which cell may be regarded as producer of this hormone can be drawn from experiments and the study of some endocrine disorders. In case of acromegaly accompanied by acidophil adenome a thyroid-hyperfunction of the patients may often be observed, which is probably secondary caused by overproduction of the thyreotropic hormone. An increased quantity of thyreotropic hormone has been found in the blood of such patients. Pituitary nanism is mostly accompanied by thyroid-hypofunction. There is a lack of acidophil cells in the pituitary of these patients.

Conclusions drawn from some experiments made by *Sanchez—Calvo* (13) may bring us nearer to the solution of this problem. Rabbits and guinea pigs were kept in dark for 2, 6, 10, 24, 48 and 72 hours. The animals kept in dark for more than 24 hours showed the first signs of an effect of darkness: their α-cells became more granulated, were stained more intensely, the quantity of colloid in the capillaries increased, after 48 hours of darkness there appeared new hypoeosinophil cells and on some slides there were no basophil cells at all to be found. After 72 hours there developed an autocracy of eosinophil cells showing lively secretory activity while the β-cells disappeared. Experiments of *Mödlinger* (9) show that darkness induces a change in the thyroid showing the same histological picture as in the intense functional period of the normal annual cycle and as by administration of thyreotropic hormone. Darkness acts probably through the way: eye — vegetative tuberal nuclei — pituitary on the thyroid, that is through inducing the production of thyreotropic hormone in the pituitary. From the description of seasonal structural changes it is known, that acidophil cells are found exclusively in winter months in the anterior pituitary lobe and basophil cells are completely missing at that time. This phenomenon compared with cyclic thyroid-observations of the same animal *[Mödlinger* (9)*]* and the results of experiments of the two investigators mentioned above becomes well explained and furnishes a conclusive answer to the question of origin of the thyreotropic hormone.

SUMMARY.

1. On the anterior pituitary lobe of guinea pigs seasonal structural changes can be observed.

2. Chromophobe cells are found during the whole year, basophil cells in summer, acidophil cells in winter.

3. Basophil cells are more active than acidophil ones.

4. In the winter months colloid droplets are formed in the nuclei of chromophobe cells which get into the cytoplasm.

5. The anterior pituitary lobe contains the whole year long ciliated cysts, with the exception of two months (April and September) when its secretory activity diminishes. These cysts appear at the caudal end of the pituitary cavity, they store the colloid and further it later on to the pituitary cleft, the medial lobe or to the capillaries. The cilia play an active part in furthering the colloid to the pituitary cavity and the medial lobe.

6. The chromophobe cells of the anterior lobe, the typical cells of the medial lobe and the cells of the infundibular lobe can be regarded as morphologically identic, there is also a functional parallelism between the first two.

7. Histophysiological investigations on other endocrine glands of the same animals and the results of other experimental investigations

TABLE I.

Width of capillaries in the anterior lobe.

No	Date of section	Diameter (μ)	Average diameter (μ)
1.	1941. IV. 15.	8,40	8,66
5.	„ IV. 15.	8,91	
47/2.	„ V. 20.	10,40	10,40
89/1.	1942. V. 16.	10,40	
52/1.	1941. VI. 19.	—	5,23
55/I.	„ VII. 15	—	6,42
104/I.	1946. VII. 30.	—	12,16
59/I.	1941. IX. 15.	—	7,99
62/1.	„ X. 15.	7,10	7,47
62/I.	„ X. 15	7,83	
65/1.	„ XI. 19.	6,91	7,49
65/I.	„ XI. 19.	8,06	
67/1a.	„ XII. 15.	—	6,49
71/1.	1942. I. 15.	11,25	11,66
71/I.	„ I. 15.	12,06	
108/1.	1947. II. 18.	—	13,38
79/I.	1942. III. 16.	—	6,04

compared with my own results regarding the time of appearance of different pituitary cells point in favour of the basophil cells producing corticotropic, adrenalotropic and gonadotropic hormones, while the thyreotropic hormone seems to be produced by acidophil cells.

TABLE II.

Size of nuclei in chromophobe cells.

No	Date of section	Size of nuclei μ	Average size of nuclei (μ)
1.	1941. IV. 15.	7,36	7,23
5.	„ IV. 15.	7,09	
47/2.	„ V. 20.	7,45	7,26
98/1.	1942. V. 16.	7,07	
52/1.	1941. VI. 19.	—	6,91
55/I.	„ VII. 15.	—	7,15
104/I.	1946. VII. 30.	—	7,30
59/I.	1941. IX. 15.	—	7,25
62/1.	„ X. 15.	7,05	7,17
62/I.	„ X. 15.	7,28	
65/1.	„ XI. 19.	7,45	7,45
65/I.	„ XI. 19.	7,45	
67/1a.	„ XII. 15.	—	7,21
71/1.	1942. I. 16.	7,26	7,10
71/I.	„ I. 16.	6,93	
108/1.	1947. II. 18.	—	7,48
79/I.	1942. III. 16.	—	7,25

TABLE III.

Size of basophil cells.

No	Date of section	Size (μ)	Average size (μ)
1.	1941. IV. 15.	13,39 × 8,69	13,66 × 9,30
5.	„ IV. 15.	13,93 × 9,91	
47/2.	„ V. 20.	11.86 × 8,88	12,74 × 9,72
89/1.	1942. V. 16.	13.61 × 10,56	
52/1.	1941. VI. 19.	—	12,22 × 8,77
55/I.	„ VII. 15.	—	11,18 × 8,39
104/I.	1946. VII. 30.	—	12,00 × 8,84
59/I.	1941. IX. 15.	—	11,02 × 8,39
108/1.	1947. II. 18.	—	13,69 × 8,49
79/I.	1942. III. 16.	—	13,12 × 9,58

TABLE IV.

Size of nuclei in basophil cells.

No	Date of section	Size of nuclei (μ)	Average size of nuclei (μ)
1.	1941. IV. 15.	6,30	6,48
5.	,, IV. 15.	6,66	
47/2.	,, V. 20.	6,65	6,45
89/1.	1942. V. 16.	6,24	
52/1.	1941. VI. 19.	—	5,62
55/I.	,, VII. 15.	—	5,70
104/I.	1946. VII. 30.	—	6,23
59/I.	1941. IX. 15.	—	5,52
108/1.	1947. II. 18.	—	6,37
79/I.	1942. III. 16.	—	6,83

TABLE V.

Size of acidophil cells.

No	Date of section	Size (μ)	Average size (μ)
59/I.	1941. IX. 15.	—	10,03 × 7,61
62/1.	,, X. 15.	12,13 × 9,06	12,78 × 9,44
62/I.	,, X. 15.	13,43 × 9,81	
65/1.	,, XI. 19.	12.56 × 9,11	12,39 × 9,39
65/I.	,, XI. 19.	12,11 × 9,67	
67/1a.	,, XII. 15.	—	11,38 × 8,90
71/1.	1942. I. 16.	12,88 × 10,00	12,94 × 9,90
71/I.	,, I. 16.	13,00 × 9,79	
108/1.	1947. II. 18.	—	12,04 × 8,96
79/I.	1942. III. 16.	—	10,46 × 8,07

TABLE VI.

Size of nuclei in acidophil cells.

No	Date of section	Size of nuclei (μ)	Average size of nuclei (μ)
59/I.	1941. IX. 15.	—	4,89
62/1.	1941. X. 15.	6,47	6,42
62/I.	,, X. 15.	6,37	
65/1.	,, XI. 19.	6,60	6,56
65/I.	,, XI. 19.	6,51	
67/1a.	,, XII. 15.	—	6,36
71/1.	1942. I. 16.	6,70	6,95
71/I.	,, I. 16.	7,19	
108/1.	1947. II. 18.	—	6,71
79/I.	1942. III. 16.	—	6,83

TABLE VII.

Size of nuclei in typical cells of the medial lobe.

No	Date of section	Size of nuclei (μ)	Average size of nuclei (u)
1.	1941. IV. 15.	7,07	6,88
5.	„ IV. 15.	6,69	
47/2.	„ V. 20.	7,14	7,07
89/1.	1942. V. 16.	6,99	
52/1.	1941. VI. 19.	—	6,75
55/I.	„ VII. 15.	—	6,70
104/I.	1946. VII. 30.	—	6,57
59/I.	1941. IX. 15.	—	7,10
62/1.	„ X. 15.	7,45	7,36
62/I.	„ X. 15.	7,27	
65/1.	„ XI. 19.	7,39	7,24
65/I.	„ XI. 19.	7,09	
67/1a.	„ XII. 15.	—	7,15
71/1.	1942. I. 15.	6,75	6,92
71/I.	„ I. 15.	7,08	
108/1.	1947. II. 18.	—	7,14
79/I.	1942. III. 16.	—	6,97

TABLE VIII.

Size of nuclei in cells of the infundibular lobe

No	Date of section	Size of nuclei (μ)	Average size of nuclei (μ)
1.	1941. IV. 15.	6,84	7,15
5.	„ IV. 15.	7,46	
47/2.	„ V. 20.	6,57	6,73
89/1.	1942. V. 16.	6,88	
52/1.	1941. VI. 19.	—	6,73
55/I.	„ VII. 15.	—	6,82
104/I.	1946. VII. 30.	—	7,21
59/I.	1941. IX. 15.	—	7,10
62/1.	„ X. 15.	6,88	6,99
62/I.	„ X. 15.	7,09	
65/1.	„ XI. 19.	6,80	6,63
65/I.	„ XI. 19.	6,46	
67/1a.	„ XII. 15.	—	7,32
71/1.	1942. I. 16.	6,37	6,69
71/I.	„	7,00	
108/1.	I. 18.	—	6,43
79/I.	1942.	—	6,41

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THE ACTION OF ADENOSINETRIPHOSPHATE ON THE ISOLATED FROG HEART.

WITH 14 FIGURES IN TEXT.

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1. INTRODUCTION.

When the isolated frog heart is rendered hypodynamic, the addition of a number of compounds may restore it to normal. During the course of our work on the contractile proteins of muscle tissue, we have observed that their solutions improve the performance of the hypodynamic heart. The active principle proved to be thermostable and was found to be present in boiled extracts of heart and skeletal muscle.

It appeared to us that a substance, which readily increases the contractions of the heart and which is enriched in the protein fractions responsible for muscle contraction, must be of great importance in the normal, physiological mechanism of muscle contraction.

When we tried to isolate the active principle, we were faced with the same difficulty as a number of investigators before us. The hypodynamic frog heart is a complex system on which it is difficult to obtain quantitative data. A reproducible test, which gives a quantitative response, is the most important factor for the isolation of an active principle.

We have therefore tried a variety of treatments in order to find a test in which the amplitude of the heart is reversibly reduced without serious damage to other functions. Treatment of the heart with *Ca* deficient Ringer solution, chloroform, or chloralhydrate were tried and it was found that the muscle extracts are able to overcome their depressing action. These tests were either too cumbersome or the degree of treatment was not easily reproducible. We found that treatment with quinine under specific conditions satisfies our criteria. With this method, we were able to assay the potency of muscle extracts in restoring the function of the heart. The speed, with which the normal amplitude of the heart returns being proportional to the amount of active principle added, it was

possible to determine its relative concentration with an accuracy of 25%.

The quinine treated isolated frog heart recovers on addition of a 1 : 300 diluted boiled muscle extract (2 ml water per 1 g muscle), when this is added to the heart together with the same concentration of quinine as was used to render the heart hypodynamic.

While the isolation of the active substance is still in progress, we observed that two known substances act like the above muscle extracts: adrenaline and adenosinetriphosphate (ATP). Experiments to be described below, have shown that the action of the muscle extract cannot be ascribed to adrenaline, and it is more likely due to adenosine triphosphate.

2. THE EFFECT OF QUININE ON THE ISOLATED FROG HEART

Hungarian frog's (Rana esculenta) were used throughout this work. The excised heart was suspended on a Straub cannula holding 1,5 ml phosphate Ringer solution of *pH* 7 through which a steady stream of air was continually bubbled.

A stock solution of quinine contained 1 mg quinine sulfate per ml in a phosphate free Ringer solution.

It is well known that quinine decreases the amplitude of the isolated heart. At the level of more than 0,1 mg/ml it arrests the heart in diastole. According to our observations the toxic action of quinine is made up of several components. If it is applied carefully so that a dose which has no immediate effect is added in repeated washings, the amplitude of the heart decreases slowly, but with no great damage to other functions. In such a case adrenaline and ATP *in presence quinine*, promptly restore the normal function of the heart. If, however a dose is applied of quinine, which rapidly reduces the amplitude of the heart beat, the recovery is slow and much greater doses of adrenaline or ATP are needed to reverse its effect. In order to avoid this complication, the quinine treatment was routinely performed in the following way:

The freshly excised heart was treated with several washings of Ringer, then with a 1:15.000 solution of quinine in Ringer. After 1—2 minutes the solution was changed for a fresh lot of the same quinine solution. In most cases, when the washing was repeated every 1—2 minutes, first the tone of the heart was seen to diminish and then the amplitude began to decrease. Usually about 6—10 changes were made, by wich time the amplitude was reduced to 20—30% of the original. The heart was then ready for a test. If the amplitude dropped too fast so that 2—3 changes of quinine already brought the amplitude down, then the amount of quinine

was too great and the heart is not suitable for the test. In such case, the quinine solution was washed out with Ringer and the quinine treatment was started again with less quinine.

When an active solution is placed on the heart, the contractions become normal in spite of the continued presence of quinine. The heart can be rendered hypodynamic again and again by the same method of washing with quinine. One heart preparation can thus be used for the successive determination of the activity of 6—10 solutions. Quantitative estimation of the relative potency of extracts was made by comparing the action of suitable dilutions on the same heart preparation. It was observed, as a rule, that a heart used for several determinations in succession, became with time more and more sensitive to quinine, so that the quinine treatment had to be undertaken with ever diminishing concentration of quinine (down to 1 : 50.000). Occasionally we have encountered preparations which were not influenced by 1 : 15.000 quinine, even after prolonged washing. These were not used for our study, as higher doses of quinine, which would have diminished the amplitude of the heart beat, damage it to such an extent that its recovery is no more comparable with the average behaviour of the hearts.

When the treatment with quinine is performed as described above, the quinine becomes uniformly distributed in the heart and the amplitude becomes constant at a level inversely proportional to the cencentration of quinine. Two factors greatly influence the level at which the contractions become constant: the *Ca*/*K* ratio and the *pH* of the solution. More quinine is needed to obtain the same effect at an acid *pH* than at an alkaline one (Fig. 1.). This underlines the necessity of a strict *pH* control. On the other hand, it was pointed out by *Zondek* (1), that the action of quinine may be counteracted by the drastic incrase of *Ca* in the Ringer solution. Our observations confirm this point (Fig. 2.). The importance of the ion balance is strikingly brought out in an experiment recorded in Fig. 3. The heart was treated with quinine in the usual way and when the amplitude became reduced, the solution on the heart was replaced with a Ringer solution containing the same amount of quinine as before, but lacking any *K* ion. The effect is quite dramatic, as the contractions became quite normal in spite of the complete absence of *K* and the presence of an otherwise toxic concentration of quinine.

The effect of temperature on the quinine treatment interests us so far, that at higher temperature, parallel with a higher heart rate, smaller concentrations of quinine exert their action in a shorter interval of time and the necessary equilibrium is established much sooner. For this reason it is optimal to perform the experiments at 22° C.

There is practically no difference between the sensitivity of the isolated frog's heart toward quinine in summer and in winter frogs, provided the temperature of the heart during the experiment is the same.

3. THE EFFECT OF ADRENALINE ON THE QUININE TREATED ISOLATED HEART.

In spite of the wealth of information on the action of adrenaline on normal and hypodynamic isolated frog's heart, we found no indication in the literature that its action was ever studied under conditions similar to our studies. We have found that, when the heart is treated with quinine, as described above, and then the quinine-Ringer solution is replaced with a Ringer solution, containing the same amount of quinine as before and in addition 0,001μg of adrenaline/ml, the amplitude of the heart significantly improves within a few minutes and remains so for at least 45 minutes (Fig. 4.). With higher concentrations of adrenaline complete, and again, lasting recovery is observed. The speed and the extent of recovery is proportional to the concentration of adrenaline applied.

The sensitivity of this test object towards adrenaline is a very high one and we think it may be used for quantitative estimation of adrenaline, in the absence of ATP.

The action of adrenaline on the heart is tacitly assumed to be transient, as adrenaline was supposed to be decomposed within a short period of time. It appears from our studies that this is not the case, so far as the isolated heart is concerned.

The reaction to adrenaline is abolished by the presence of 0,01 mg/ml ergotamine, which alone has no influence on the amplitude of the quinine treated heart.

The quinine treated isolated heart reacts to adrenaline both in summer and in winter frogs.

4. THE EFFECT OF ATP ON THE QUININE TREATED ISOLATED HEART.

When the frog heart is treated with quinine, as described above, and the quinine-Ringer solution is replaced by a Ringer solution, containing the same amount of quinine as before and in addition a few μg of ATP/ml, the following reaction is observed, as shown in (Fig. 5.): Immediately on addition of ATP there follow a few strong contractions, then the amplitude first decreases, and then begins to rise at a rate, proportional to the amount of ATP added. 0,5 μg of ATP/ml is sufficient to restore the normal amplitude of the heart in 5 minutes, 2 μg/ml produces the same effect within a minute.

As in the case of adrenaline. the action of ATP is a lasting one. The amplitude of the heart falls off sometimes after 10—15 minutes, but

this is due to the presence of quinine, whose toxic effect increases during this time, as stated above.

Fig. 7. shows the effect of higher doses of ATP on the normal amplitude of the heart. 5 μg/ml gives a small increase, and even 500 μg/ml have no apparent harmful effect.

When ATP was added without quinine, sometimes a small but insignificant initial depression was observed and the amplitude soon became normal or even higher. Heart block was never observed.

As shown in Fig. 7. even the effect of a minimal effective dose of ATP is not abolished by 0,01 mg/ml ergotamine. On this ground the action of adrenaline and ATP can be differentiated.

The most surprising phenomenon about the action of ATP is its complete absence during the winter season. While the same reactions were observed during three successive summer seasons, no effect at all could be detected during the two intervening winters. There is a transition period in September-November and in March-April, when incomplete recoveries were observed and the effect was obtained only with higher doses of ATP. The best effects were observed from June to August. These extreme variations are most likely concerned with variations in the permeability of the heart. Such a control mechanism would prevent the loss of this very important substance during the winter season.

Neither adenylic acid, nor pyrophosphate have any effect on the quinine treated heart (Figs. 8. and 9.). Adenylic acid invariably inhibited the contractions. As the Ringer solutions always contain phosphate, this shows that there is no synthesis of ATP on the surface of the cells within an appreciable interval of time.

When ATP was hydrolysed in *N* hydrochloric acid for 7 minutes, or with baryta for 30 minutes, the activity was completely destroyed. Fractionation of the ATP preparation with *Hg*, *Ba*, acetone always yielded fractions, which had the same activity on the basis of their ATP content. Thus the action is not due to impurity but to ATP itself.

5. THE ACTION OF MUSCLE EXTRACTS ON THE QUININE TREATED ISOLATED HEART.

Muscle extracts were prepared from freshly excised rabbit, frog, horse skeletal muscle and from heart muscles. The tissue was minced and thrown into 2 volumes of boiling distilled water. The mixture was boiled for 10 minutes, then rapidly cooled and filtered clear. Such an extract of frog muscle, when diluted 1 : 200 with Ringer solution (1 : 600 dilution of the original wet weight of muscle) is able to restore the normal amplitude when added to the quinine treated heart in continued presence of quinine

(Fig. 10.). A similar extract of rabbit's muscle gave very good recovery in a 1 : 1000 dilution.

Contrary to the action of adrenaline, the action of the muscle extract is not inhibited by the presence of ergotamine. The quinine treated heart reacts to muscle extract only during the summer season.

The ATP content of skeletal muscle is of the order of 1-3mg/g muscle. The ATP content of the diluted muscle extract is therefore a few μ g per ml.

The action of the muscle extract may thus be attributed to its adenosinetriphosphate content.

6. THE EFFECT OF ADRENALINE AND ADENOSINETRIPHOSPHATE ON THE HEART IN PRESENCE OF CA DEFICIENT RINGER.

When the Ringer solution is replaced with one, wich is deficient in *Ca* ions (10% of the normal *Ca* being only present) the amplitude of the heart is reversibly decreased. If this *Ca* deficient Ringer is replaced by a similarly deficient Ringer containing 0,6 μ g of ATP/ml or 0,01 μ g adrenaline per ml, the amplitude of the heart is greatly improved. (Figs. 11. and 12.). This test is just as sensitive as the quinine test, but has the disadvantage that it can be used for the evaluation of the activity of biological materials only if their *Ca* content is negligible.

7. THE EFFECT OF ATP ON THE HEART IN SITU.

The frog (150 g) was anesthetized with urethane and the thorax opened, the heart was connected to the lever. Atropine was injected intravenously, followed by the injection of 3 mg quinine sulfate. The function of the heart deteriorated, as shown in Fig. 13. After the injection of 7 mg ATP the amplitude of the contractions returned to normal though the rythm remained halved.

In a similar experiment, when no quinine was applied and the function of the heart was normal, the intravenous injection of 6 mg ATP caused a few forced contractions but no heart block (Fig. 14.).

DISCUSSION.

Rothmann (2) has studied the effect of ATP on the electrocardiogramm of dogs and suggested that the beneficial effect of heart and muscle extracts on the strength of the heart muscle contraction is due to ATP. Later investigators have, however, not agreed with his conclusions.

Gillespie (3) in a review of older literature and based on his experiments, states that ATP only decreases the conduction and the amplitude of the heart and expressly states: „ATP was the most active of the adenyl group in producing heart block in the frog's heart". *Chen* and *Meining* (4)

have investigated the effect of ATP on the mammalian and amphibian heart and concluded that the beneficial effect of the drug is due only to the dilation of the coronary arteries.

From our experimental data, it is obvious that ATP exerts a positive inotropic action on the hypodynamic frog heart, similar to that of adrenaline. As with the latter substance, ATP also has no great effect on the normal heart. We are unable to agree with the findings of *Gillespie* that ATP produces a heart block.

It is known from the earlier work of this laboratory that ATP profoundly influences the physicochemical properties of the contractile proteins, causing dissociation of actomyosin at high salt concentrations and shrinking of the actomyosin gel at physiological salt concentrations. Therefore it is logical to find that it improves the mechanical function of the heart. That it has no action on the normal heart is understandable when we consider the high ATP content of muscle tissue.

Although ATP is best known as an energy reservoir, the chemical energy of oxidation and fermentation being stored in its form, yet it is difficult to visualize that the effects described in this paper are due to its presence or absence as a high energy compound. There is a hundred times as much ATP present in the frog's heart, as must be added to bring the amplitude of the heart back to normal. Moreover, under the same conditions, adrenaline exerts a similar action, so the necessary energy must be at hand and the fault is in the mechanism of the contraction. It appears that quinine acts on the surface, it blocks some process, which needs ATP and this ATP cannot be provided for from inside of the muscle fibers.

It is known from the work of *Buchtal* (5) that a few μ g of ATP when injected closearterially, will produce twitches in frog muscles.

Rózsa (6) has observed that a freshly minced rabbit muscle suspension in 0,5 *M KCl* can be brought to rapid contraction, when only 0,5 μ g ATP/ml are added to the suspension, although inside of the fibres there is a 500 times greater concentration of ATP.

It was suggested that this phenomenon might be explained by assuming that the ATP isolated is different from the ATP present in the muscle tissue. Alternatively it was supposed that all ATP inside the fiber is bound and therefore a small amount of free ATP added from without will start the mechanism of contraction.

Neither of these hypotheses is supported by experimental evidence.

It is more probable that the cause of the phenomenon is a property of the muscle cells. The process of contraction must start on the surface of the cells. There a chemical change in which ATP is an indispensable reactant, precedes contraction. High salt concentration in case of the

experiment with minced muscle, the presence of quinine or the absence of *Ca* in case of the hypodynamic heart, reduces the concentration or the availability of ATP. When this is replaced, the process is brought back to normal.

It is important to emphasise the close similarity between the action of adrenaline and ATP. To explain the connection between the two, chemically unrelated compounds, we suppose that adrenaline catalyses the change which is caused by ATP. This would explain why 500 times less molecules of adrenaline produce a similar response as ATP. We want to mention in this connection that adrenaline is most likely connected with the physicochemical change in the contractile protein actin, as under certain experimental conditions it enhances the polymerisation of actin. (7)

Again, the action of ATP and adrenaline is similar to that of *Ca* ions. This is significantly underlined by the experiments with quinine and by the experiments on the *Ca* deficient heart. In presence of adrenaline and ATP the heart appears to function with much less *Ca* ion.

SUMMARY.

The hypodynamic frog heart resumes normal activity when ATP (1 μ g/ml) or adrenaline (0,001 μ g/ml) is added to the bathing fluid which still contains the agent by which the function of the heart was depressed.

These experiments are interpreted by assuming that quinine reduces the effective concentration of a substance on the surface of muscle fibers, which substance is necessary for the initation of muscle contraction. ATP is either identical with this substance or a precursor of it. Adrenaline catalytically influences the formation of this substance. These processes are enhanced by *Ca* ions.

We want to express our thanks to Profssor B. Issekutz jun. for his ontinued interest and help.

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EFFECT OF DRUGS ON ACTIN.

WITH 6 FIGURES IN TEXT.

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INTRODUCTION.

The action of several drugs on the polymerisation of actin will be described in the present paper, with special reference to the relation of these actions to the ionic environment.

Polymerisation of actin is induced by the addition of salts. In earlier papers (1, 2) it was shown that monovalent ions (among them *K* and *Na*) and divalent ions (*Mg* or *Ca*) are both needed to obtain polymerisation. The process of polymerisation is regarded to be a series of reactions (2). An early reaction is catalysed by *Mg* ions and a later one by *K* and *Na* ions. The position of *Ca* ion is not yet clear, in some respects it may replace *Mg*, enhancing the rate of polymerisation. Under certain conditions, however, it has an inhibiting effect. As shown in an earlier paper, *Ca* and *K* ions are antagonistic at 0 C°: while both polymerise actin at a considerable rate when added alone, in presence of both ions the polymerisation is slow. In the present study, the polymerisation was studied at 24 C° and no such antagonism was observed.

METHODS.

Actin was prepared according to the method described (2). The polymerisation of actin was followed by the rise of viscosity of actin solutions. The rate of the reaction depends on the concentration of actin, and this was chosen so, that 50% polymerisation was reached in 5—30 minutes. The concentration of actin thus varied from 1—3 mg/ml. The salt free actin solution was mixed with the other ingredients at time 0 and the viscosity was repeatedly determined. 0,014 *M* veronalacetate buffer was present in all experiments (*pH* 7, *K* salts only). *K* and *Ca* ions, in variable amounts were added as chlorides.

RESULTS.

We have first determined the rate of polymerisation of actin as the function of the *K* : *Ca* ratio. In the experiments recorded in Fig. 1. the concentration of *Ca* ions was uniformly 0,002 *M*, whereas that of *K* ions

was varied from 0,014 *M* (buffer alone) to 0,114 *M*. In similar experiments, when the concentration of *K* was kept constant, and that of *Ca* varied, similar results were observed.

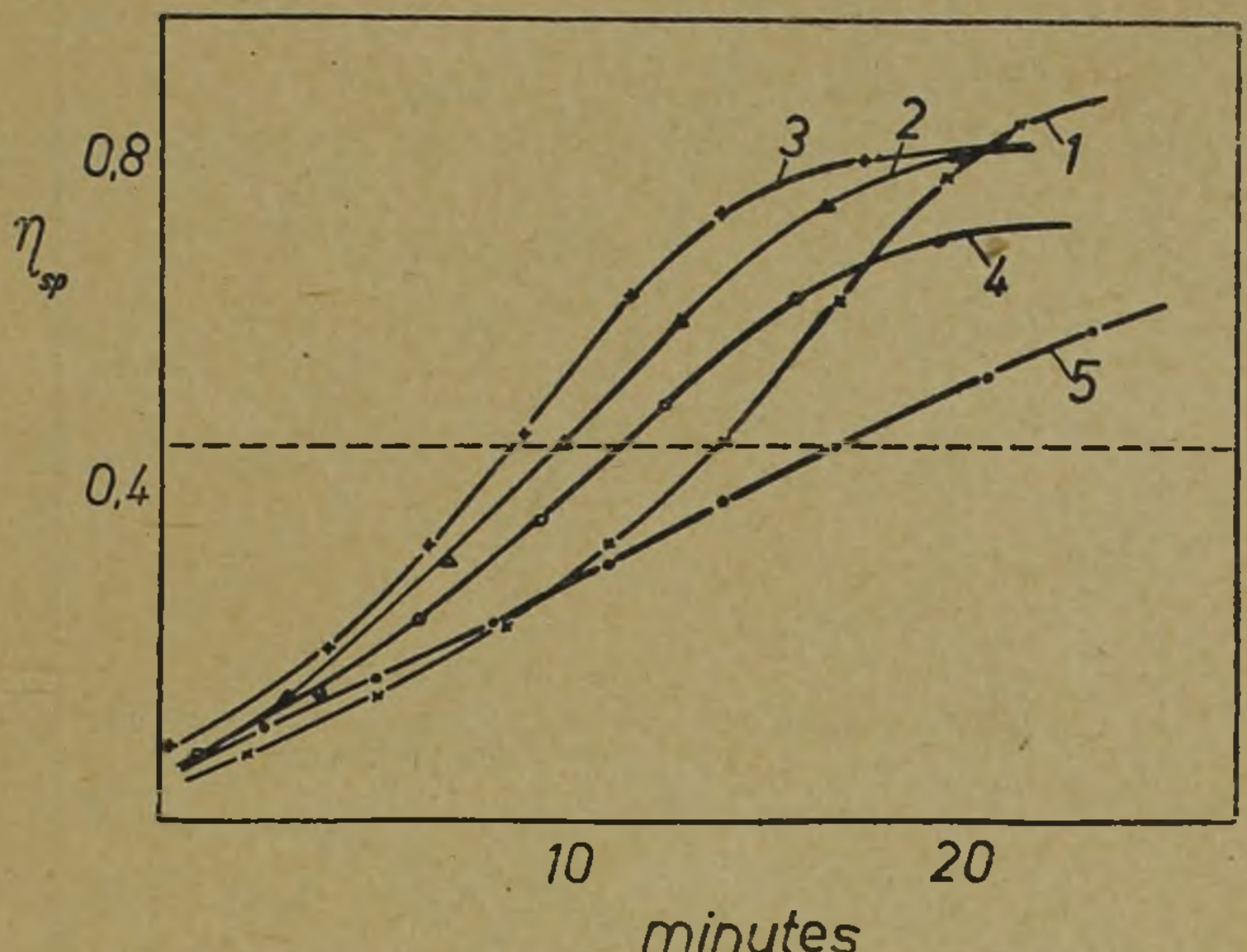

Fig. 1. Polymerisation of actin in presence of *Ca* and *K* ions. Concentration of $CaCl_2$ in every case: 0,001 *M*. Concentration of *KCl* for curves 1—5 : 0,114 *M*, 0,074 *M*, 0,054 *M*, 0,034 *M* and 0,014 *M* respectively.

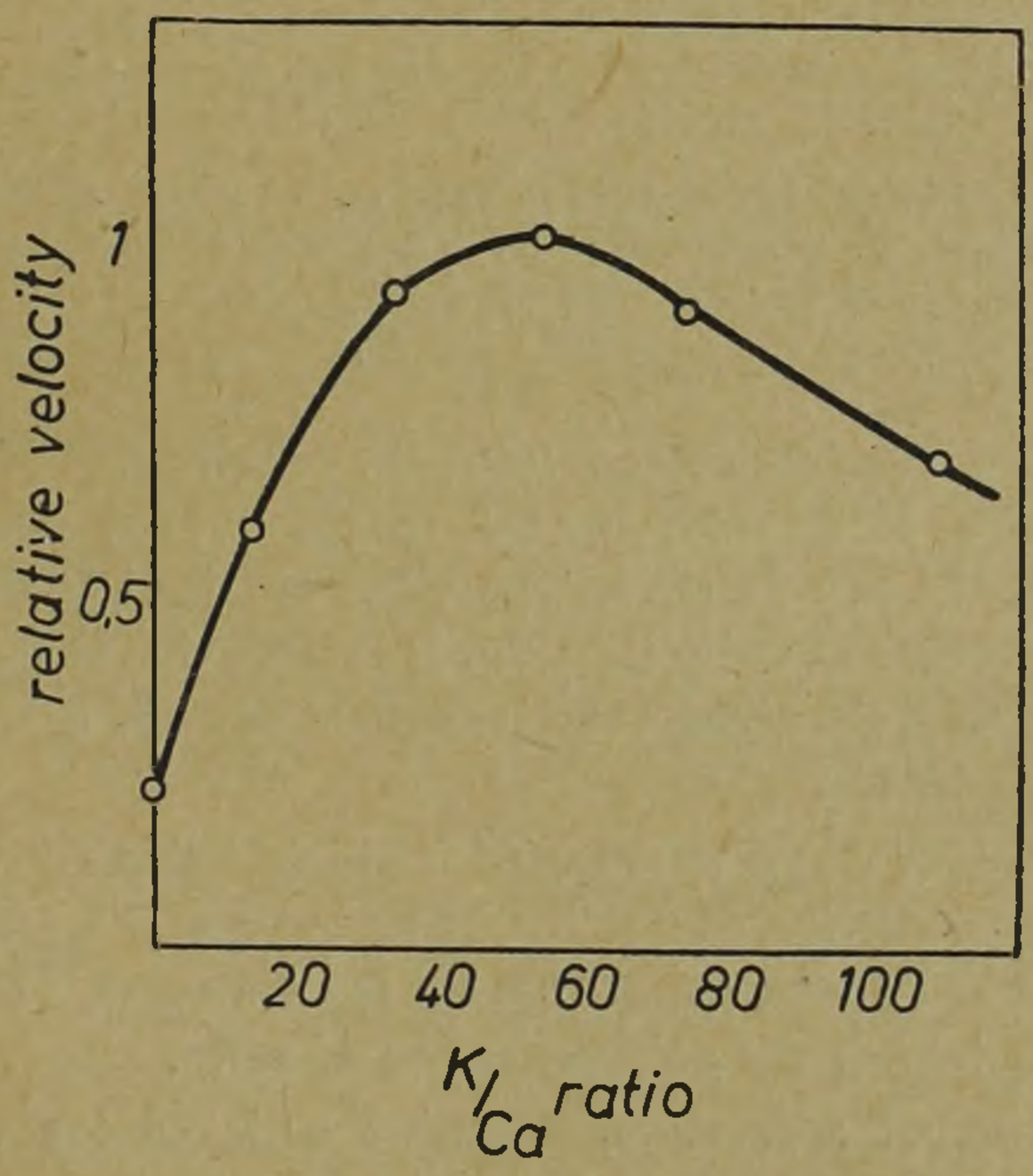

Fig. 2. Relative rate of polymerisation with varying *K* : *Ca* ratio.

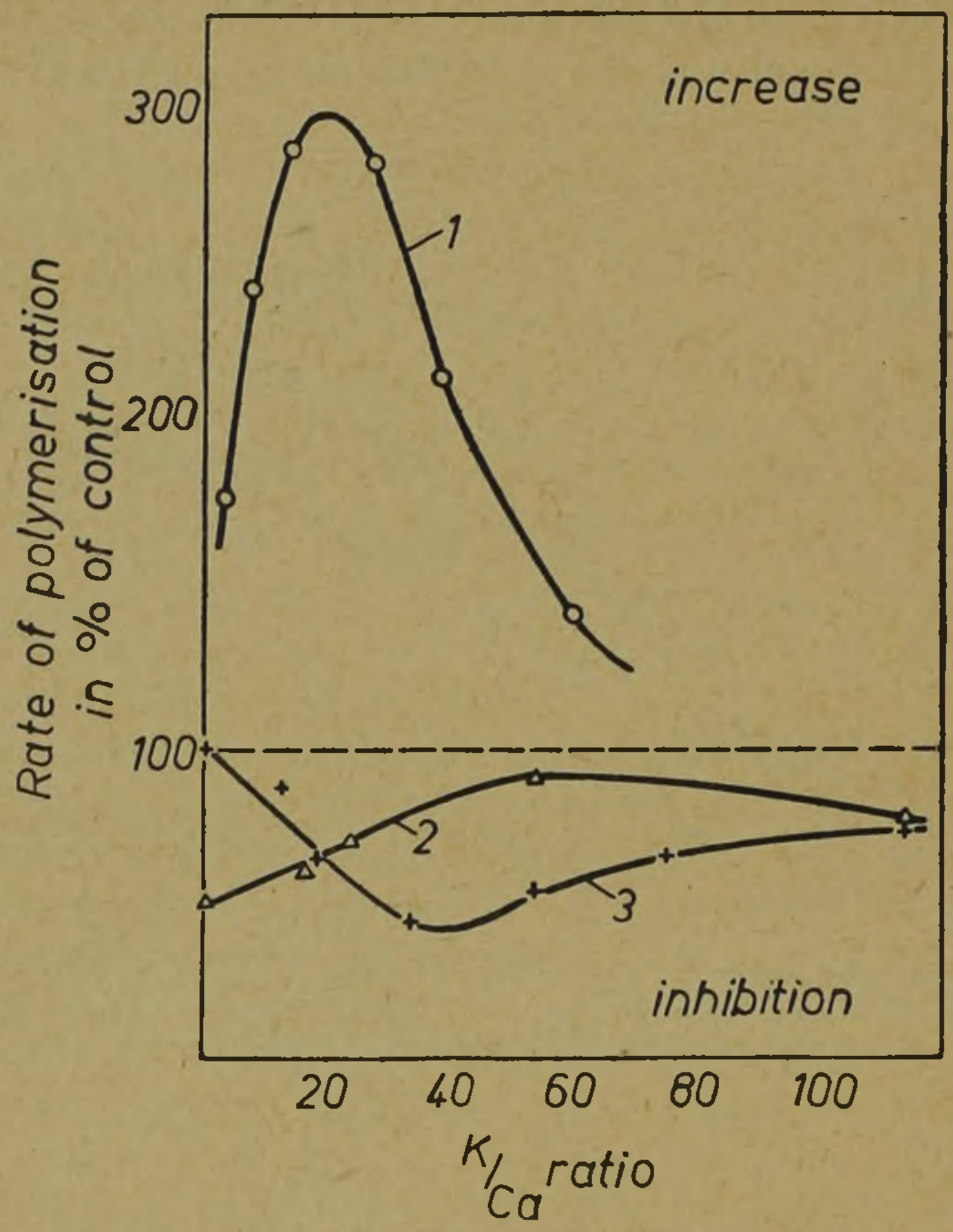

Fig. 3. Rate of polymerisation of actin in presence of drugs, expressed in % of the control rate (without the drug). Curve 1 : in presence of 0,1 mg adrenaline per ml. Curve 2 : in presence of 0,1 mg veratrine per ml. Curve 3 : in presence of 0,1 mg quinine per ml.

In order to evaluate these results, the relative rate of polymerisation (reciprocal value of minutes needed to reach 50% polymerisation) is plotted against the *K* : *Ca* ratio. The value obtained at the physiological ratio of 50, was taken as unity (Fig. 2.). It is seen, that at this point the rate of polymerisation is the highest and it declines when *K* or *Ca* is in excess of the physiological ratio.

The following drugs were tested for their action on the polymerisation of actin: acetylcholine, adrenaline, veratrine, quinine, strychnine. While acetylcholine has no effect at all, adrenaline enhances, veratrine, quinine and strychnine inhibit the polymerisation of actin.

The action of adrenaline, veratrine and quinine was studied in presence of varying amounts of *K* and *Ca*, on the pattern of the experi-

ments recorded in Fig. 1. All drugs were present in a concentration of 0,1 mg/ml. The rate of polymerisation (1/t 50%) in presence of the drug, was compared with the rate of polymerisation in absence of the drug, while the ionic milieu was exactly the same. Such measurements were made at different $K : Ca$ ratios. The results are shown in Fig. 3. The ordinate denotes the % rate of polymerisation, referred to the rate of

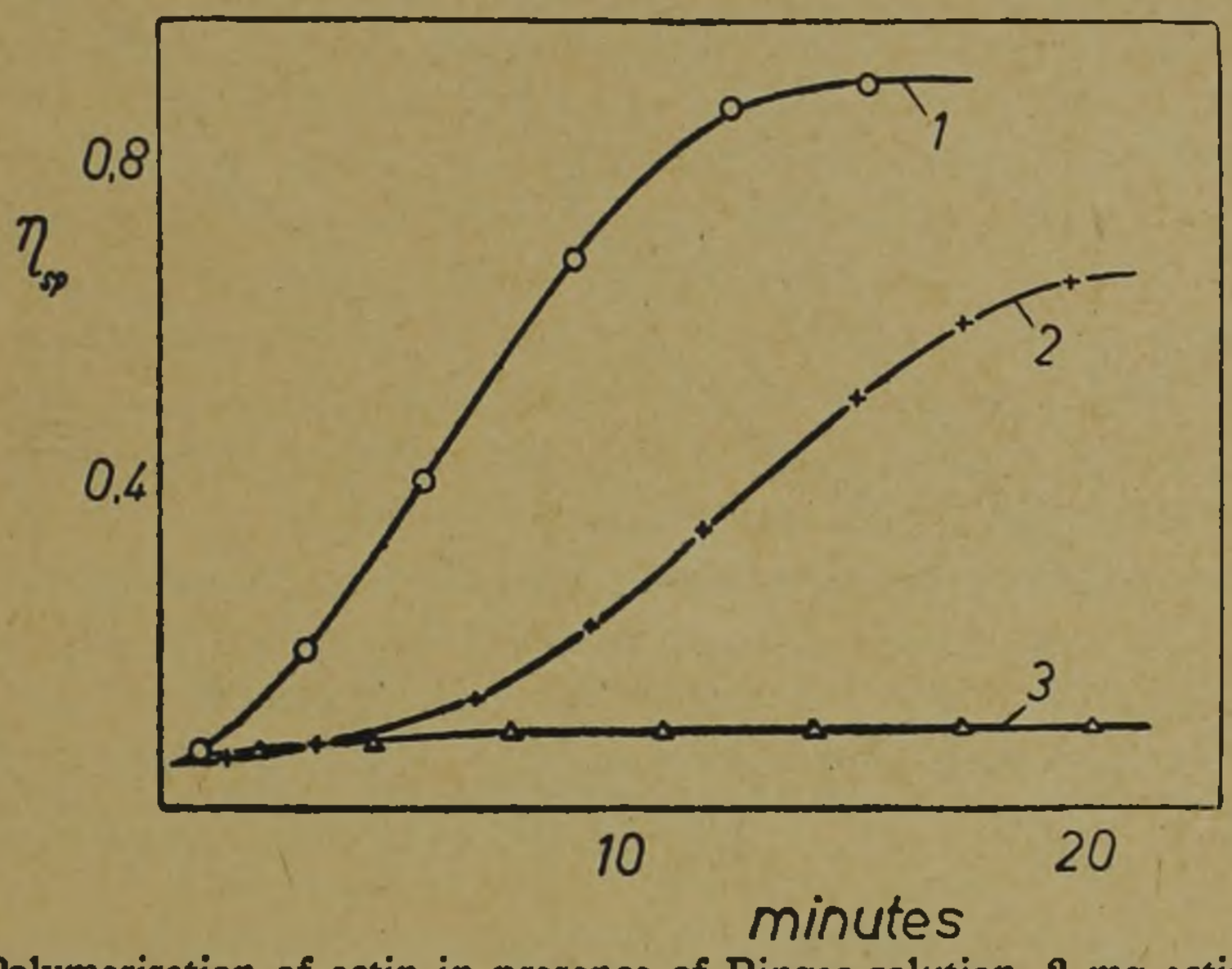

Fig. 4. Polymerisation of actin in presence of Ringer solution. 2 mg actin per ml. Curve 1 : No addition. Curve 2 : 0,1 mg/ml veratrine. Curve 3 : 1 mg/ml veratrine.

polymerisation measured in absence of the drug. It is important to note that the effect of veratrine is maximal, that of quinine is minimal at the physiological ratio of K and Ca ions, while that of adrenaline is optimal when less K is present.

The effect of adrenaline is only slightly increased by higher concentration, whereas 1 mg/ml veratrine and strychnine give almost complete inhibitions (Fig. 4. and 5.).

When compared with the above reactions — obtained at pH 7 — the action of adrenaline becomes less, that of veratrine becomes greater at more acid pH.

The effect of adrenaline is generally more pronounced when the polymerisation of actin does not proceed under optimal conditions. Thus the greatest effect is obtained at a $K : Ca$ ratio of 25, where the polymerisation of actin alone is not optimal (cf. Fig. 1.). In accordance with this observation, the inhibition caused by veratrine can be largely reversed by adrenaline. Fig. 6. records such an experiment. The inhibition

given by quinine is not so completely reversed by adrenaline, particularly at more acid *pH* values. It seems that the action of quinine is in part irreversible.

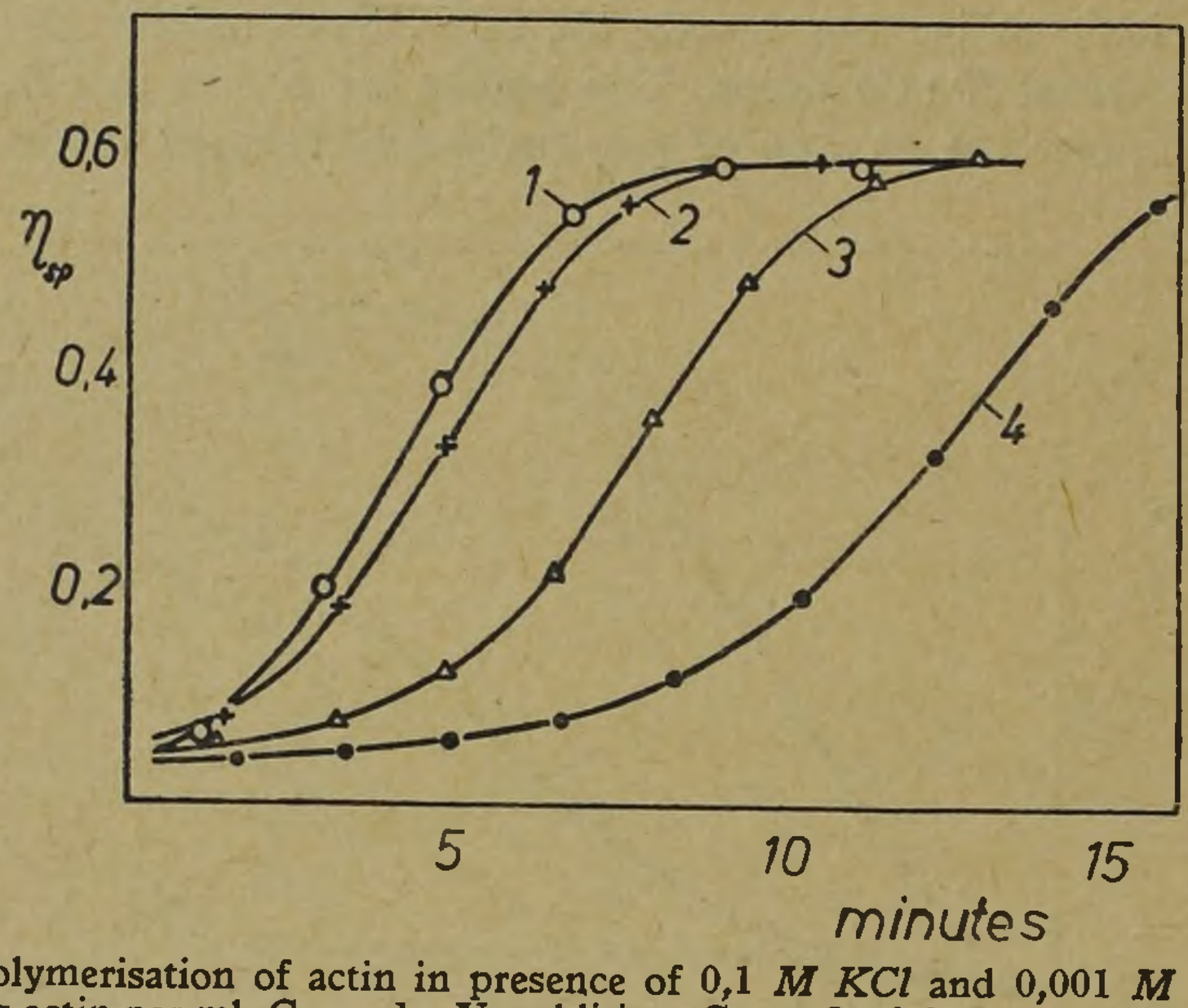

Fig. 5. Polymerisation of actin in presence of 0,1 *M KCl* and 0,001 *M* $MgSO_4$ at *pH* 7 2 mg actin per ml. Curve 1 : No addition. Curve 2 : 0,01 mg strychnine per ml. Curve 3 : 0,1 mg strychnine per ml. Curve 4 : 0,23 mg strychnine per ml.

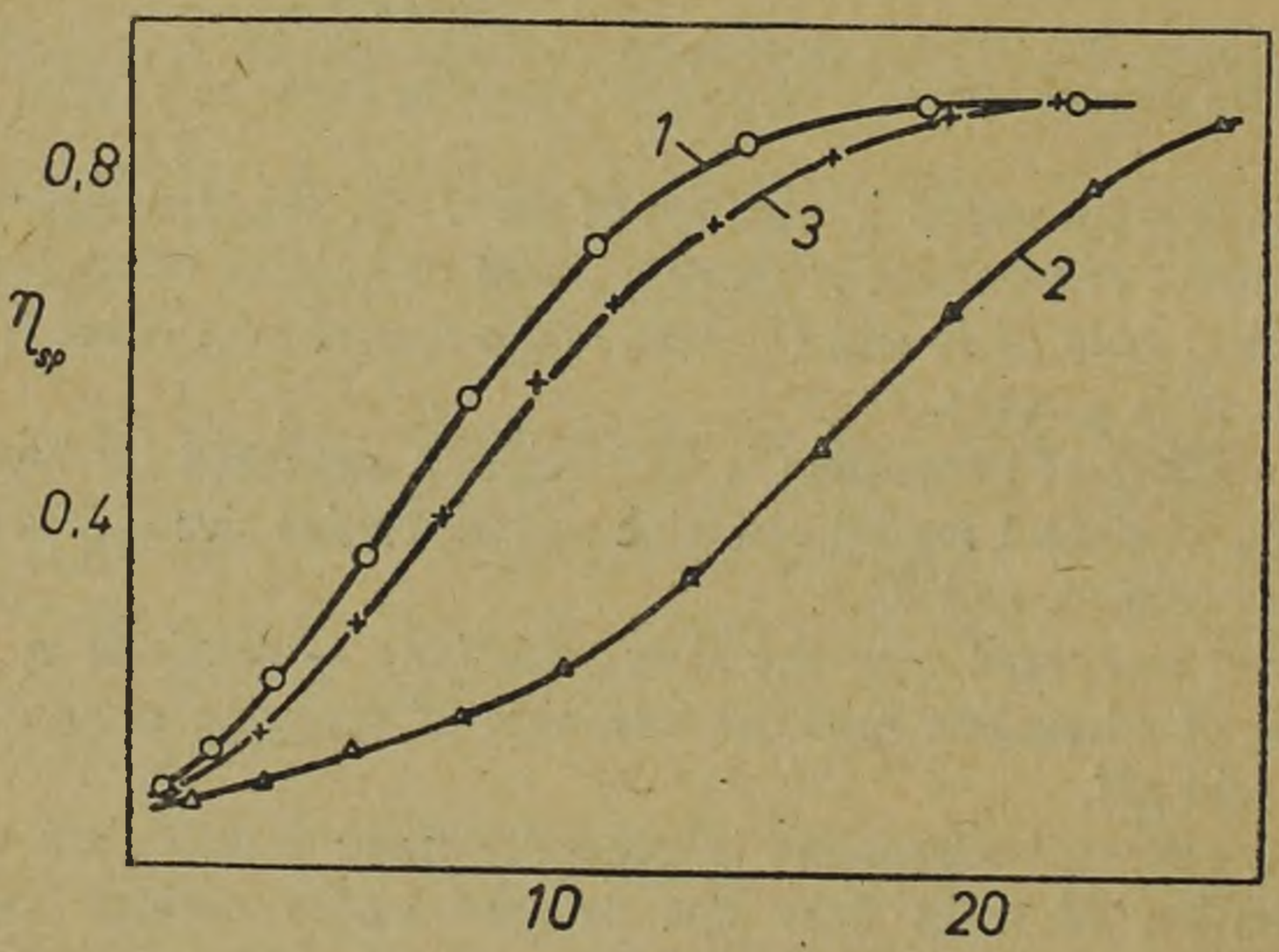

Fig. 6. Polymerisation of actin in presence of 0,014 *M KCl* and 0,001 *M* $CaCl_2$ and 3,3 mg ascorbic acid per ml. Actin concentration: 2 mg per ml. Curve 1 : No addition. Curve 2 : 0,1 mg veratrine per ml. Curve 3 : 0,1 mg veratrine and 0,1 mg adrenaline per ml.

DISCUSSION.

In a previous paper we have shown that the polymerisation of actin consists at least of two different reactions: the first is catalyzed by *Mg* (at higher temperatures also by *Ca*), the second needs monovalent ions only. The primary reaction leads to a change, accompanied by no increase of viscosity.

We have observed that various actin preparations have unpredictable rates of polymerisation when a standard salt mixture is added. This variability is not due to the difference in their respective *Ca* and *Mg* content. The following explanations may be offered: 1. actin is a mixture of two or more proteins, 2. the preparations are denatured to a variable extent 3. a coenzyme-like accessory substance is needed, the presence and concentration of which is accidental and variable.

Evidence obtained up to the present time indicates that actin consists of one protein component. However, the presence of small amounts of catalytically active proteins cannot be excluded. Unpublished work from this laboratory indicates that actin contains a prosthetic group, the addition of which, however, does not restore uniform and maximal velocity of polymerisation to defective actin preparations. Against the possibility of denaturation it can be argued, that the difference between actin preparations is not only in the rate of polymerisation but rather in their reaction to specific ion effects.

The most coherent picture at present is to suppose that the polymerisation of actin is the result of *several* catalytic processes. The catalytic protein may be actin itself, or — less likely — several proteins in small concentration are contaminating the protein of actin. The relative efficiency of these catalytic centers may be different from one preparation to the other. This will determine, which of the processes becomes the limiting factor. As different ions influence different processes, the specific ion effects will thus become variable.

The peculiar dependence of the action of adrenaline, veratrine and quinine on the $K : Ca$ ratio suggests that these drugs act on one or the other specific process. Thus, adrenaline seems to act on one process, which is not the limiting factor at physiological Ca/K rations. The change of ionic environment enhances the rate of one, and probably reduces the rate of the other reaction. Thus a change in ionic environment produces conditions in which the process, on which adrenaline has an enhancing influence, becomes the limiting factor.

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ÜBER DIE ADENYLSÄUREDESAMINASE.

MIT 4 ABBILDUNGEN IM TEXT.

VON V. SZ. HERMANN UND G. JOSEPOVITS.

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VORGELEGT VON PROF. DR. F. B. STRAUB, MITGLIED D. AKADEMIE.

(Eingegangen am 11. X. 1948.)

Schmidt (1) bereitete schon im Jahre 1928 einen Auszug aus Kaninchenmuskulatur, welcher spezifisch desaminierend auf die Adenylsäure wirkt. *Lohmann* und *Schuster* (2) fanden dann später, nach der Isolierung der Adenosintriphosphorsäure, dass die nach der Vorschrift *Schmidt*'s dargestellte Desaminase in verschiedenem Ausmasse auch vom ATP Ammoniak abspaltet, weshalb sie die Darstellung dieser Desaminase einer gewissen Modifikation unterwarfen. Das nach dieser modifizierten Methode gewonnene Extrakt übt seine desaminierende Wirkung ausschliesslich auf die Adenylsäure aus. *Summerson* und *Meister* (3) stellten dann fest, dass auch Myosin nach wiederholten Ausfällen desaminierend auf die Adenylsäure einwirkt, und *Ferdman* und *Nechiporenko* (4) wiesen nach, dass das Myosin eine wesentliche Menge Adenylsäuredesaminase enthält. Schliesslich gelang es dann *Kalckar* (5), teils aus wässerigem Muskelextrakt, teils aus nach der Vorschrift *Bailey*'s (6) hergestelltem Myosin eine derartige Desaminase zu isolieren.

Unsere in der letzten Zeit durchgeführten Untersuchungen zeigten nun, dass das nach *Szent-Györgyi* (7) dargestellte Actin-freie kristalline Myosin die Adenylsäure ebenfalls desaminiert und dass sich das kristalline Myosin in dieser Beziehung als ebenso wirksam, oder als noch aktiver erweist, als die bisher bekannt gewordenen Adenylsäuredesaminase-Präparate.

METHODIK.

Das zu unseren Versuchen verwendete Myosin stellten wir nach der Vorschrift *Szent-Györgyi*'s (7) dar. Durch eine Fleischmühle getriebene Kaninchenmuskulatur wurde 10 Minuten mit dem dreifachen Volumen einer Lösung von 0° Temperatur extrahiert, welche 0,15 Mol Kaliumphosphat-Puffer (*pH* 6,5) und 0,3 Mol Kaliumchlorid enthielt. Nach Verdünnung des Extraktes mit dem vierfachen Volumen dest. Wassers

von 22° wurde es durch ein Tuch filtriert. Das während 1—2 Stunden anhaltenden, ständigen Rührens ausfallende Actomyosin wurde dann abzentrifugiert, das in Lösung zurückgebliebene Myosin durch Zusetzen von $1^1/_2$ Volumen dest. Wassers ausgefällt und mit 0,02 Mol *KCl*-Lösung gewaschen. Dem so gewonnenen Myosin setzten wir nun solange 0,02 Mol K_2CO_3 zu, welches 0,01% Phenolphthalein enthielt, bis die Lösung ein *pH* von 8,3 erreichte (die Farbe der Lösung schlug in Rot um). Dem in Lösung übergegangenen Myosin wurden nunmehr pro g Myosin 4 ccm 2 Mol *KCl* zugesetzt und die Lösung schliesslich mit dest. Wasser verdünnt, wobei auf jeden ccm des angewendeten *KCl* 50 ccm Wasser kamen. Jetzt fiel das noch vorhandene Actomyosin aus und konnte abzentrifugiert werden. Aus der opaleszierenden Flüssigkeit wurde dann das Myosin durch Neutralisieren mit 1%-iger Essigsäure ausgefällt. Das so gewonnene Myosin wurde noch zweimal umkristallisiert, uzw. auf die Weise, dass es mit 0,5 Mol *KCl* gelöst und durch darauffolgende Verdünnung der Lösung mit dest. Wasser von neuem ausgefällt wurde.

Bei der Darstellung der Schmidt-Desaminase wurde die von *Lohmann* (2) gegebene Anweisung befolgt. Den Eiweissgehalt des Myosins, bezw. der Schmidt-Desaminase berechneten wir durch Bestimmen des Stickstoffgehaltes, den wir nach *Bailey* (6) mit der Zahl 6 multiplizierten.

Den Gesamtstickstoffgehalt der Adenylsäure (Adenosin-5-Phosphorsäure)[1] bestimmten wir nach vorangehender feuchter Veraschung mithilfe des Parnas—Wagner'schen Mikrokjeldahl-Apparates. Als ihre auf enzymatischem Wege abspaltbare Aminonitrogen-Menge nahmen wir $^1/_5$ der Gesamtstickstoffmenge an ($NH_2—N$ Wert). Das auf enzymatischem Wege abgespaltene $NH_2—N$ wurde ebenfalls mit dem Parnas—Wagner'schen Mikrokjeldahl-Apparat bestimmt, uzw. nach dem Verstreichen der entsprechenden Inkubations-Dauer in dem aliquoten Teil des mit Trichloressigsäure enteiweissten Filtrates. Bei unseren Versuchsserien bestimmten wir in allen Fällen auch den Wert für 0 Minuten Inkubation und brachten dann diesen Wert von den erhaltenen Resultaten in Abzug. Auf ähnliche Weise wurde Myosin unter entsprechenden Versuchsbedingungen auch ohne Zusetzen von Adenylsäure inkubiert, um feststellen zu können, ob es nicht auch unter diesen Umständen zu einem Freiwerden von Ammoniak kommt.

[1] Für die Überlassung der zu unseren Versuchen verwendeten Adenylsäure sprechen wir Herrn Professor *Dr. B. Tankó* (Debrecen) auch an dieser Stelle unseren herzlichsten Dank aus.

VERSUCHE.

Die desaminierende Eigenschaft des Myosins.

Konzentrations-Kurve (Abb. 1). Das Reaktionsgemisch enthält 1 ccm in 0,2 Mol *KCl* gelöstes kristallines Myosin (mit wechselndem Eiweissgehalt), 2 ccm 0,25 Mol Succinat-Puffer (*pH* 5,9) und 3 mg AP (120 γ NH_2-N), Gesamtvolumen 5 ccm. Inkubations-Temperatur 38°. Inkubations-Dauer 5 Minuten. Enteiweissung mit 1 ccm 20%-iger Trichloressigsäure. Die Menge des Myosins schwankte zwischen 0,025 mg und 0,5 mg, bezw. seine Konzentration betrug pro ccm 5—100 γ.

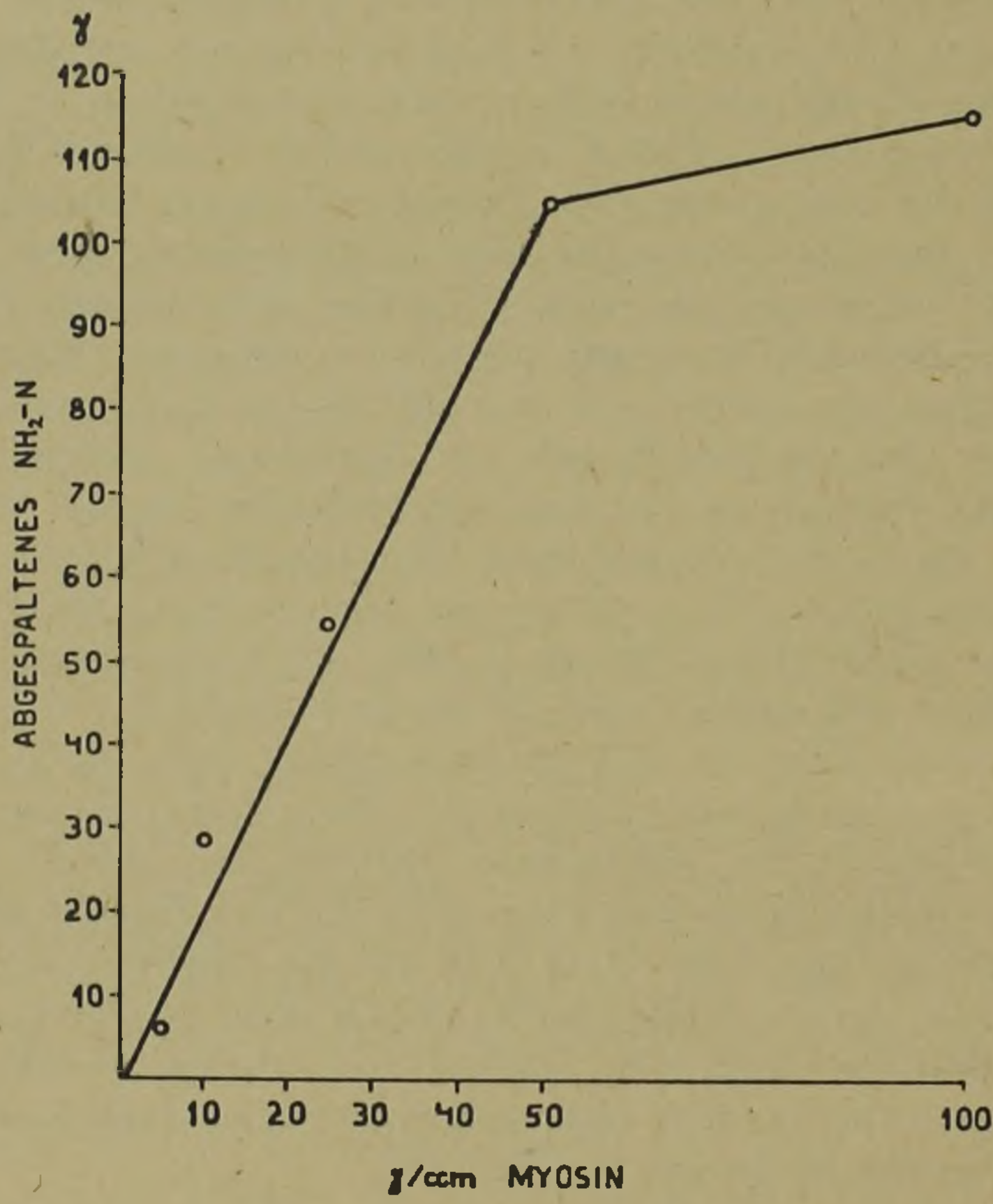

Abb. 1. Konzentrations-Kurve.

Wie aus Abb. 1 hervorgeht, ist die auf die Adenylsäure ausgeübte desaminierende Wirkung des Myosins seiner Konzentration proportioniert. Unter den angewendeten Versuchsbedingungen spalteten 50 γ/ccm Myosin in 5 Minuten die zugesetzte Adenylsäure fast vollständig.

Zeit-Kurve (Abb. 2.). Gesamtvolumen 10 ccm. Zusammensetzung des Gemisches bei Kurve 1 : 20 γ /ccm Myosin, 4 ccm 0,25 Mol Succinat-Puffer (*pH* 5,9) und 3 mg AP (120 γ NH_2—*N*), bei Kurve 2 : 40 γ /ccm Myosin, 4 ccm 0,25 Mol Succinat-Puffer (*pH* 5,9) und 6 mg AP (240 γ NH_2—*N*). Inkubations-Temperatur 27°. Enteiweissung mit 2 ccm 20%-iger Trichloressigsäure.

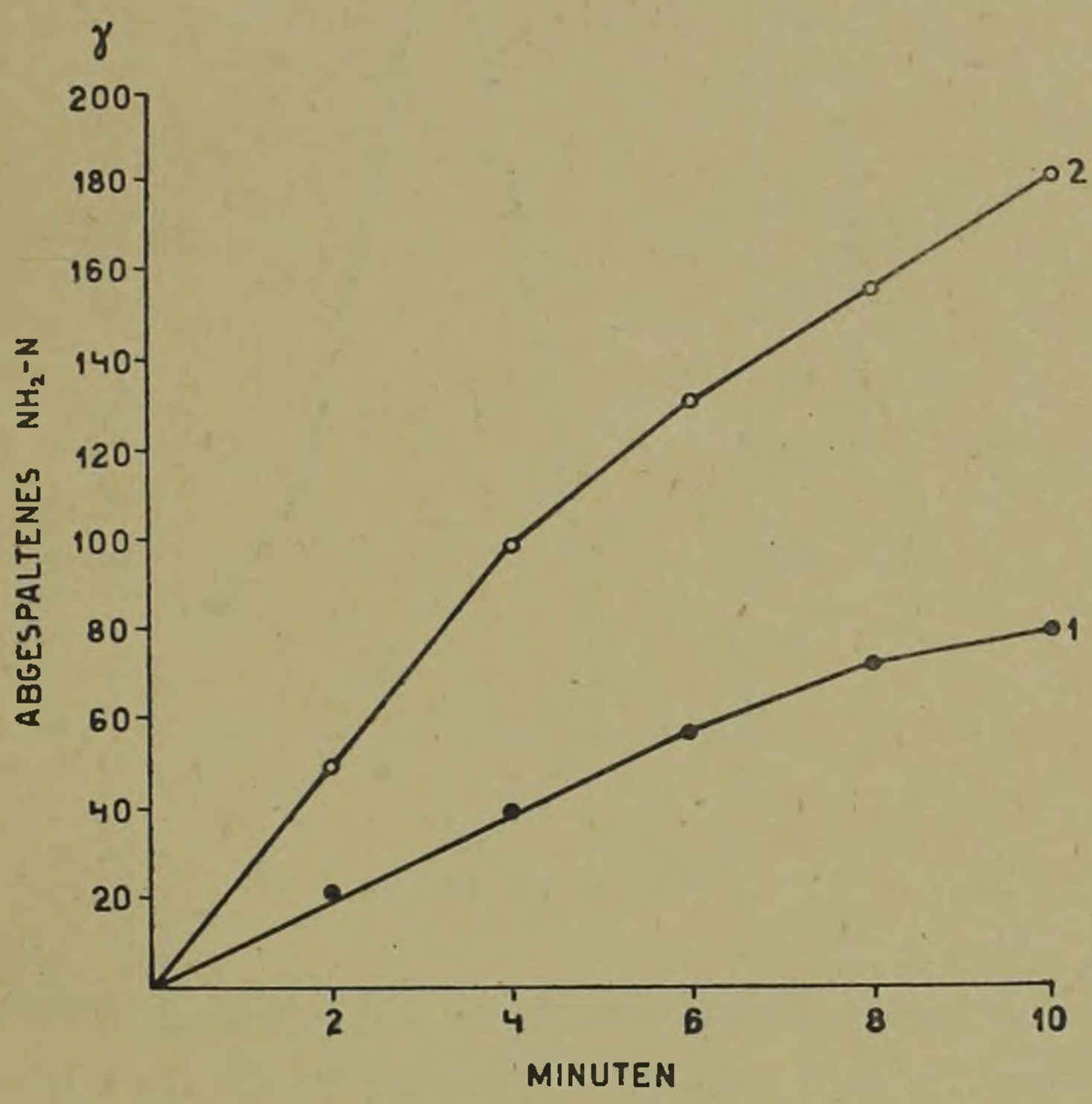

Abb. 2. Zeit-Kurve.

Der Grad der Desaminierung erscheint also auf Grund der Abb. 2 in beiden Fällen 4—6 Minuten hindurch als der verstrichenen Zeit proportioniert, sinkt aber später langsam ab.

pH-Kurve (Abb. 3). Gesamtvolumen 5 ccm. Das Gemisch enthält 0,1 Mol Succinat-, bezw. Phosphat-Puffer von entsprechendem *pH*, 30 γ/ccm Myosin und 3 mg AP (120 γ NH_2—*N*). Inkubations-Temperatur 38°. Inkubationsdauer 10 Minuten.

Schmidt (1) stellte ein scharfes *pH*-Optimum der Adenylsäuredesaminase bei einem *pH* von 5,7—6,1 fest. Das kristalline Myosin übt nach Abb. 3 seine desaminierende Wirkung auf die Adenylsäure optimal bei demselben *pH* aus, wie die Schmidt-Desaminase.

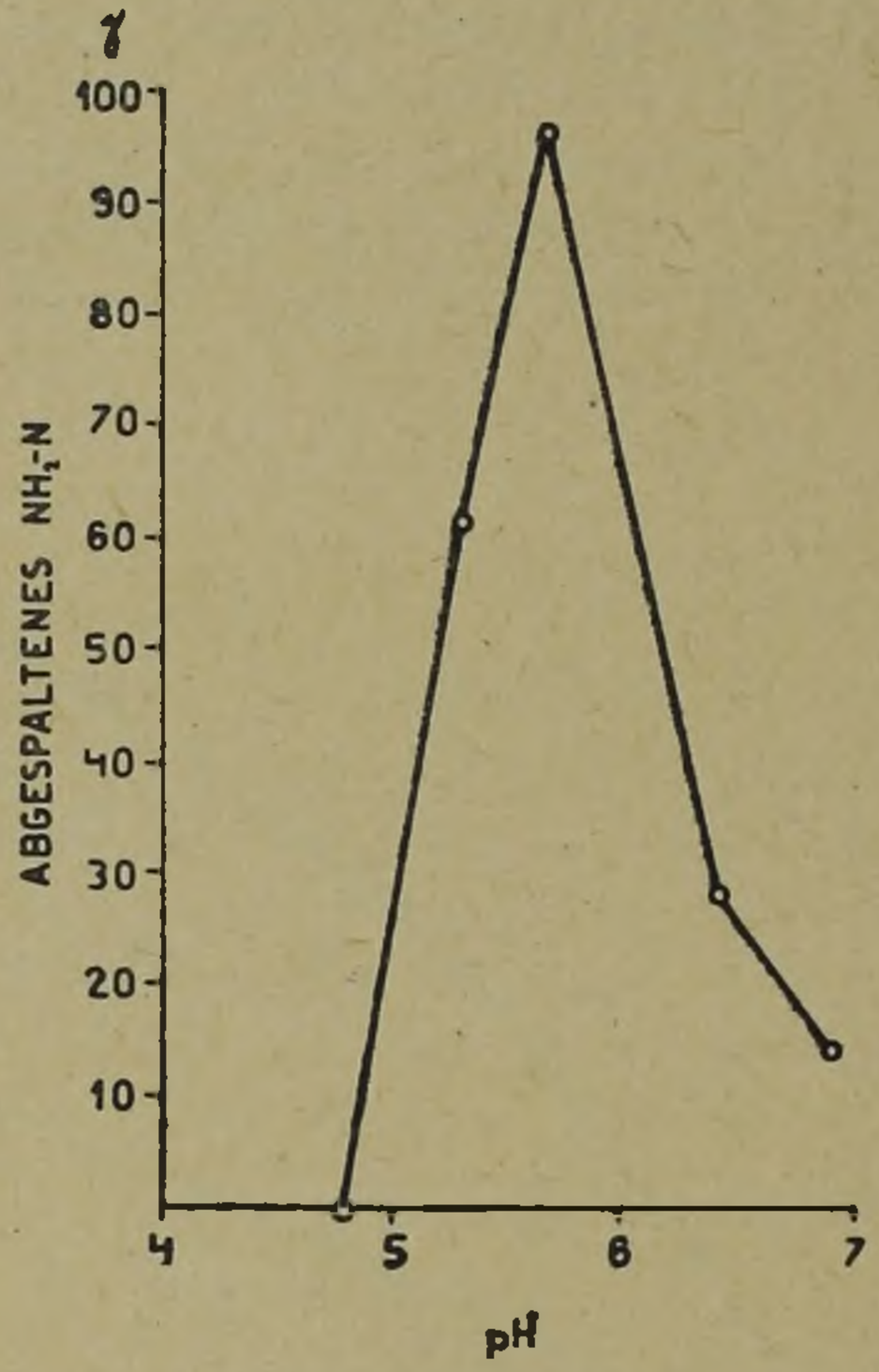

Abb. 3. pH-Kurve.

Vergleichende Untersuchungen mit Adenylsäuredesaminasen.

Schmidt (1) stellt seine Adenylsäuredesaminase aus Kaninchenmuskulatur durch Extrahieren mit 2%-igem $NaHCO_3$ dar. Wird die Muskulatur jedoch vor dem Extrahieren 4—5 mal mit 0,85%-igem *NaCl* ausgewaschen, so erhalten wir ein Präparat mit ähnlicher Aktivität, welches jedoch viel reiner ist. Der Stickstoffgehalt dieses Präparates beträgt nur $^1/_{10}$ des der vorigen Präparate, d. h. 0,025—0,04%. Nach Extrahieren mit $NaHCO_3$ erhält *Schmidt* eine dickflüssige, weisse Masse, die erst nach Herabsetzung der alkalischen Reaktion zentrifugierbar, bezw. filtrierbar wird. *Lohmann* (2) schreibt nun in der von ihm modifizierten Darstellungsmethode der Schmidt-Desaminase genau vor, wie oft, wie lange und in welchen Volumsmengen das Auswaschen mit 0,9%-igem *NaCl* zu erfolgen hat. Er extrahiert dann die Desaminase ebenfalls mit 2%-igem $NaHCO_3$,

doch ist diese nun bei genauem Einhalten seiner Vorschriften auch ohne Neutralisierung vom Muskelrückstand leicht zu trennen.

Bei unseren Versuchen stellten wir die Schmidt-Desaminase unter pünktlichem Einhalten der Vorschrift *Lohmann*'s her. Ihr Eiweissgehalt schwankte zwischen 1,5—3,5 mg/ccm. Abb. 4 zeigt nun den Einfluss der Veränderungen in der Konzentration auf den Desaminierungsgrad. Gesamtvolumen 5 ccm. Zusammensetzung des Gemisches: 10—100 γ/ccm Schmidt-Desaminase-Protein, 2 ccm 0,25 Mol Succinat-Puffer (*pH* 5,9) und 3 mg AP (120 γ NH_2—*N*). Inkubations-Temperatur 38°. Inkubations-Dauer 5 Minuten.

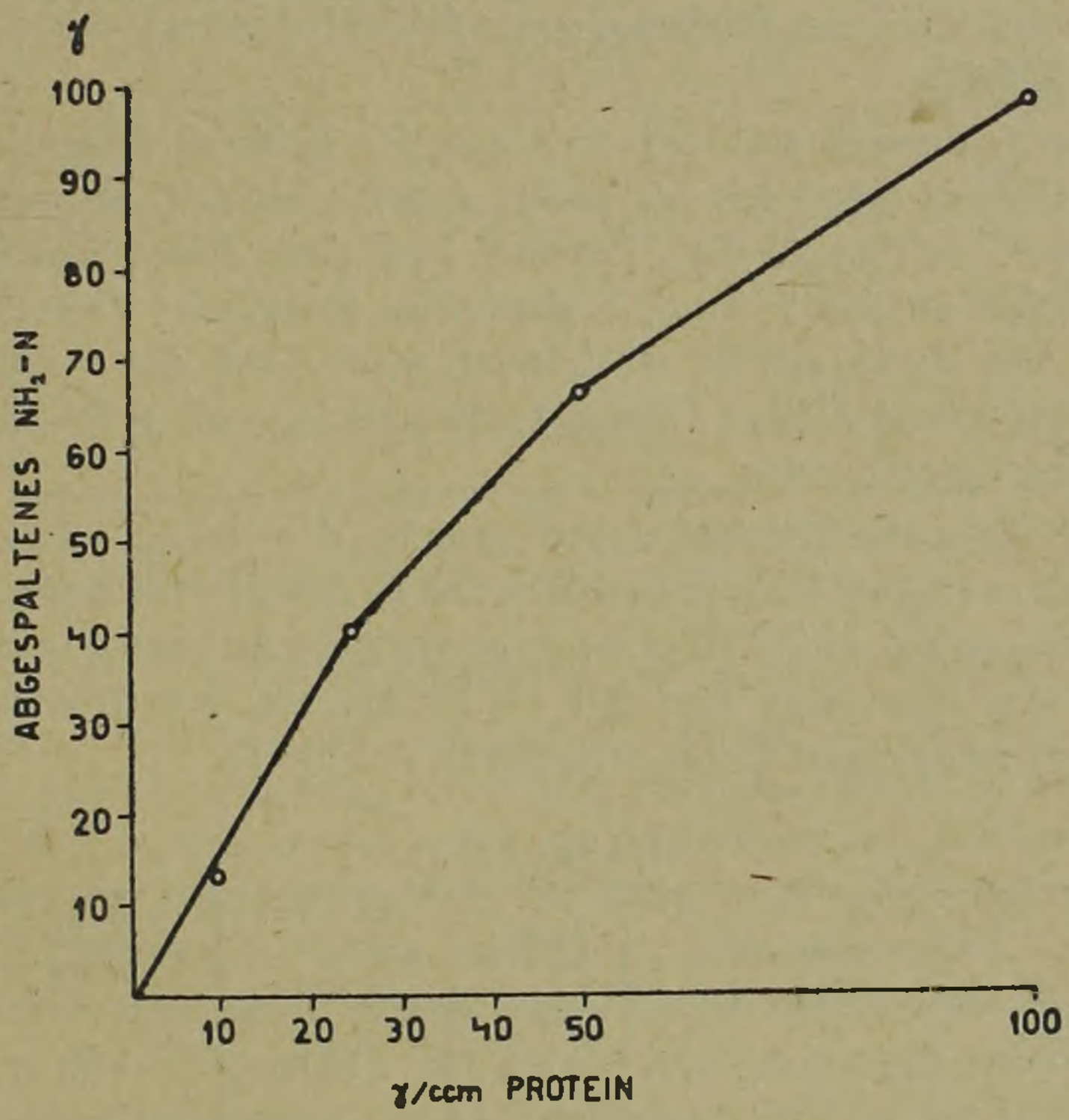

Abb. 4. Konzentrations-Kurve der Schmidt-Desaminase.

Wie aus Abb. 4 hervorgeht, zeigt die Schmidt-Desaminase bezüglich ihrer Aktivität eine sehr grosse Ähnlichkeit zu dem kristallinen Myosin (Abb. 1). Das Myosin steht nämlich in bezug auf seine auf Grund des Stickstoffgehaltes berechneten Eiweisses in nichts hinter der Aktivität der Schmidt-Desaminase zurück.

Die Schmidt-Desaminase zeigt, selbst nach der Vorschrift *Lohmann*'s hergestellt, in grösserer Konzentration häufig auch Adenosintriphosphatase-Wirkung. Ihre Aktivität als Adenosintriphosphatase kann aber mit

der des kristallinen Myosins in absolutem Sinne nicht verglichen werden, da die Darstellung der beiden Substanzen weitgehende Unterschiede aufweist. Auch das kristalline Myosin erfährt eine gewisse Herabsetzung seiner Aktivität, wenn es mit 2%-igem $NaHCO_3$ verdünnt, längere Zeit steht; so wird seine Aktivität z. B. nach 40 Stunden auf die Hälfte des ursprünglichen Wertes herabgesetzt.

Die Schmidt-Desaminase fällt bei *pH* 5,3 aus und ähnlich dem kristallinen Myosin auch bei Halbsättigung der Lösung mit Ammoniumsulfat. Die bei einem *pH* von 5,3 ausgefällte Schmidt-Desaminase kann nun ähnlich wie das kristalline Myosin nach Neutralisieren mit 0,02 Mol K_2CO_3 von neuem in 0,5 Mol *KCl* gelöst werden, wobei sie jedoch ihre Adenosintriphosphatase-Wirkung, sowie Adenylsäuredesaminase-Wirkung weiter beibehält.

Darauf, dass es sich bei dem aus der Schmidt-Desaminase ausgefällten Eiweiss eigentlich um nichts anderes handelt, als um Myosin, weist ferner auch noch der Umstand hin, dass dieses Eiweiss durch Actin aktiviert werden kann, d. h. dass seine spezifische Viskosität durch Zusetzen von Actin erhöht wird, aber wieder auf den Ausgangswert zurücksinkt, sobald diesem Lösungssystem auch noch Adenosintriphosphorsäure zugesetzt wird.

Die Adenosintriphosphatase-Aktivität des kristallinen Myosins wird, wenn es längere Zeit, z. B. 6 Stunden in Acetat-Puffer von *pH* 5,3 in Gegenwart von 0,5 Mol *KCl* steht, in gewaltigem Ausmasse herabgesetzt, während sich aber seine Aktivität als Desaminase bei denselben Konzentrationsbedingungen kaum verändert (Tabelle I).

TABELLE I.

Behandlungsweise	Myosin mg/ccm	vom ATP abgespaltenes *P* in γ	vom AP abgespaltenes NH_2—*N* in γ
Ohne Behandlung	1,0	120	114
6 Stunden bei *pH* 5,3...	1,0	11	95

Gesamtvolumen 5 ccm. Enthält 1 ccm in 0,5 Mol *KCl* gelöstes Myosin, 1 ccm 0,02 Mol Veronal-Puffer (*pH* 7,4) und 4,4 mg ATP mit 534 γ labilem *P*, bezw. 3 mg AP mit 120 γ NH_2—*N*. Inkubations-Temperatur 38°. Inkubations-Dauer 5 Minuten.

Bei einem Vergleich des kristallinen Myosins mit dem nach *Bailey* (6) hergestellten Myosin erwies sich die desaminierende Wirkung des kristallinen Myosins als wesentlich grösser. (Eingehende, diesbezügliche Untersuchungen stehen vor dem Abschluss.) Aus dem kristallinen Myosin

konnte bei 6-stündigem Dialysieren gegen 0,02 Mol Ammoniumacetat (*pH* 8) nach *Kalckar* (5), bezw. gegen 0,02 Mol $NaHCO_3$ (*pH* 8) keine Fraktion isoliert werden, welche sich als aktiver erwiesen hätte, als das ursprüngliche Myosin selbst. Nach dem Abzentrifugieren zeigte sich zwischen der Aktivität des in das Zentrifugat übergegangenen Myosins und der Aktivität der in dem über dem Zentrifugat stehenden Flüssigkeit zurückgebliebenen, gelösten Desaminase (Myosin) kein Unterschied (s. Tabelle II). Ähnlicherweise zeigte auch die Aktivität des schon zweimal umkristallisierten Myosins nach weiterem dreimaligem Umkristallisieren keine Veränderung.

TABELLE II.

	Inkubations-Dauer Minuten	Protein γ/ccm	Abgespaltenes NH_2—N γ
Zentrifugat	5	20	50
	10	20	109
Lösung	5	20	42
	10	20	117

Gesamtvolumen 10 ccm. Enthält 4 ccm 0,25 Mol Succinat-Puffer (*pH* 5,9) und 3 mg AP (120 γ NH_2—N). Inkubations-Temperatur 27°.

BESPRECHUNG DER ERGEBNISSE.

Die Schmidt-Desaminase enthält zweifellos Myosin. Ein vollgültiger, quantitativer Vergleich ist aber zwischen kristallinem Myosin und Schmidt-Desaminase infolge der verschiedenartigen Darstellungsweise nicht möglich. Das aus der Schmidt-Desaminase ausgefällte, desaminierend wirkende Eiweiss weist aber durch seine Lösbarkeit, Adenosintriphosphatase-Wirkung und durch die Veränderung seiner Viskosität bei Zusetzen von Actin und ATP darauf hin, dass die Schmidt-Desaminase als eine Myosin-Lösung zu betrachten ist, welche infolge ihrer glücklich erfassten Darstellungsweise das ATP zwar nicht dephosphoryliert, als Adenylsäuredesaminase jedoch aktiv wirkt (8). Das nach *Bailey* hergestellte, noch Actin enthaltende Myosin erweist sich als weniger aktiv als das kristalline Myosin. Aus dem kristallinen Myosin konnte mithilfe der von *Kalckar* beschriebenen Methode keine Desaminase isoliert werden, die sich als aktiver erwiesen hätte, als das Myosin selbst. Die Erklärung dieses Umstandes ist wahrscheinlich darin zu suchen, dass zwischen der Lösbarkeit des Actin-freien Myosins und der des Actomyosins wesentliche Unterschiede bestehen.

Das von uns hergestellte kristalline Myosin zeigt in 10 ccm Gesamtvolumen bei einer Zusammensetzung von 20 γ/ccm Myosin, 0,1 Mol (*pH* 5,9) Succinat-Puffer und 3 mg AP mit 120 γ NH_2—*N*, sowie bei einer Inkubations-Temperatur von 27° eine Aktivität, nach welcher 1 γ Myosin in 1 Minute 1—1,5 γ Adenylsäure spaltet.

ZUSAMMENFASSUNG.

Das nach *Szent-Györgyi* aus Kaninchenmuskulatur dargestellte kristalline Myosin erwies sich als Adenylsäuredesaminase von hoher Aktivität.

Es ist mit grosser Wahrscheinlichkeit anzunehmen, dass bei den bisher dargestellten Adenylsäuredesaminasen das in ihnen enthaltene Myosin für die enzymatischen Eigenschaften der Präparate verantwortlich gemacht werden muss.

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DIE ROLLE DES MYOSINS IM ENZYMATISCHEN ABBAU DER ADENOSINTRIPHOSPHORSÄURE.

MIT 6 ABBILDUNGEN IM TEXT.

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Engelhardt und *Ljubimowa* (1) wiesen als erste nach, dass sich im Muskel die Adenosintriphosphatase mit der Myosinfraktion des Muskels verbindet. Weiters stellten sie fest, dass das Myosin nur 1 Phosphat vom ATP abspaltet; soll aber auch das zweite Phosphat abgespalten werden, so muss dem Myosin auch noch wässeriger Muskelextrakt beigesetzt werden. Diese Untersuchungen *Engelhardt*'s und *Ljubimowa*'s wurden dann in der Folgezeit zum Gegenstand zahlreicher Untersuchungen anderer Forscher. So fand *Kalckar* (2, 3, 4) im wässerigen Muskelextrakt ein säurefestes Eiweiss, die sogen. Myokinase, deren Rolle in der Umwandlung von 2 Molekülen ADP in 1 Molekül ATP und 1 Molekül AP besteht. Setzen wir also dem Myosin Muskelextrakt zu, so erfolgt die Dephosphorylierung des ATP auf folgende Weise: Das Myosin spaltet vom ATP 1 Phosphat ab und das so entstandene ADP wird dann von der Myokinase des Muskelextraktes dismutiert, so dass das Myosin von neuem ATP dephosphoryliert; auf diese Weise können also vom ATP zwei PO_4 abgespalten werden.

Banga (5) erhielt mit dem nach *Szent-Györgyi* (6) dargestellten, Actin-freien, kristallinen Myosin ähnliche Ergebnisse. Auch das kristalline Myosin spaltet vom ATP 1 Phosphat ab, während das zweite Phosphat nur dann abgespalten werden kann, wenn dem Myosin auch Muskelextrakt zugesetzt wird. *Banga* (7) untersuchte ausser der Abspaltung des PO_4 auch noch die Verhältnisse bei der Desaminierung. Sie fand dabei, dass das ATP in Anwesenheit von Myosin allein nur dephosphoryliert wird, während in Gegenwart von Myosin + Muskelextrakt (Protein II) vom ATP zuerst 1 Phosphat abgespalten wird, dann aber parallel zu der Abspaltung des zweiten Phosphates auch die NH_2-Gruppe. Sie nahm weiters an, dass die Desaminierung des ADP auf die Weise erfolgt, dass

sich das eine Phosphat des ADP mit der NH_2-Gruppe verbindet — es wäre dies also das ADP_2, im Gegensatz zu dem über eine freie NH_2-Gruppe verfügenden ADP_1 —, so dass also die NH_2-Gruppe mit dem Phosphat gemeinsam abgespalten wird. Bei diesem Vorgang würde daher der Enzymkomplex Myosin + Protein II eine Rolle spielen.

Den Ausgangspunkt unserer im vorliegenden mitgeteilten Versuche bildeten folgende Fragen: Können Dephosphorylierung und Desaminierung des ADP voneinander getrennt werden? Welche Rolle kommt dabei dem Myosin zu und welche dem Muskelextrakt? Schliesslich, welche Enzyme spielen bei diesem Vorgang überhaupt eine Rolle?

METHODIK.

Myosin: Nach *Szent-Györgyi* (6) aus Kaninchenmuskulatur dargestelltes, Actin-freies, zweimal umkristallisiertes Myosin.

Muskelextrakt: Mit geringfügigen Abweichungen nach der Vorschrift *Kalckar*'s (3) hergestellt. Die durch eine Fleischmaschine getriebene Kaninchenmuskulatur wurde 10 Minuten mit dem $1^1/_2$-fachen Volumen dest. Wassers extrahiert, durch ein Tuch filtriert und dann 24—48 Stunden gegen dest. Wasser dialysiert. Nach Abzentrifugieren des entstandenen Niederschlages wurde die Flüssigkeit mit 0,04 Volumen n *HCl* angesäuert, 1 Minute lang aufgekocht und nach Abkühlen mit n *KOH* auf *pH* 6,2—6,3 eingestellt. Der neuerlich entstandene Niederschlag wurde ebenfalls abzentrifugiert. Das durch das Aufkochen mit Säure und darauffolgendem Neutralisieren entfernte Eiweiss stellt eine wesentliche Reinigung des Extraktes dar. Während nämlich das Dialysat unter anderem auch noch durch Magnesium aktivierbare, anorganische Pyrophosphatase enthält, ist von ihr in unserem Extrakt keine Spur mehr zu finden.

Schmidt-Desaminase: Nach *Lohmann* (8) dargestellt.

Substrate: ATP und ADP. Beide mit Kaliumoxalat aus sauren *Ca*-Salzen zu *K*-Salzen umgewandelt und mit n *KOH* auf *pH* 7 eingestellt. AP (Adenosin-5-Phosphorsäure)[1], ebenfalls mit n *KOH* auf *pH* 7 eingestellt.

Die leicht hydrolysierbare Phosphatmenge wurde nach 7 Minuten bei 100° in n *HCl* erfolgtem Hydrolysieren bestimmt. Die Phosophatbestimmungen erfolgten nach *Fiske* und *Subbarow*, modifiziert von *Lohmann* und *Jendrassik* (9), die Stickstoffbestimmungen unter Anwendung des *Parnas—Wagner*'schen Mikrokjeldahl-Apparates. Das Aminonitrogen nahmen wir mit $^1/_5$ der Gesamtstickstoffmenge an.

[1] Für die liebenswürdige Überlassung der zu unseren Versuchen verwendeten Adenylsäure sprechen wir Herrn Professor *Dr. B. Tankó* (Debrecen) auch an dieser Stelle unseren herzlichsten Dank aus.

VERSUCHE.

Alle Versuche erfolgten bei einem Gesamtvolumen von 5 ccm in Gegenwart von 0,1 Mol *KCl*. *pH* war mit Veronal-Acetat-Puffer auf 7,4 eingestellt. Inkubations-Temperatur 38°. Nach Verstreichen der Inkubations-Dauer wurde das Eiweiss mit 1 ccm 20%-iger Trichloressigsäure ausgefällt und nun erfolgten die Bestimmungen in aliquoten Teilen der abfiltrierten Flüssigkeit. Bei jeder Versuchsserie wurde auch der 0-Wert festgestellt. Ausserdem führten wir wiederholt Kontrollversuche durch, die zeigen sollten, ob es nicht unter den entsprechenden Versuchsbedingungen auch dann zu einem Freiwerden von Ammoniak, bezw. von anorganischen Phosphaten kommt, wenn das Enzym oder das Substrat allein inkubiert wird.

Enzymatische Spaltung des ATP in Gegenwart von Myosin und Muskelextrakt (Abb. 1).

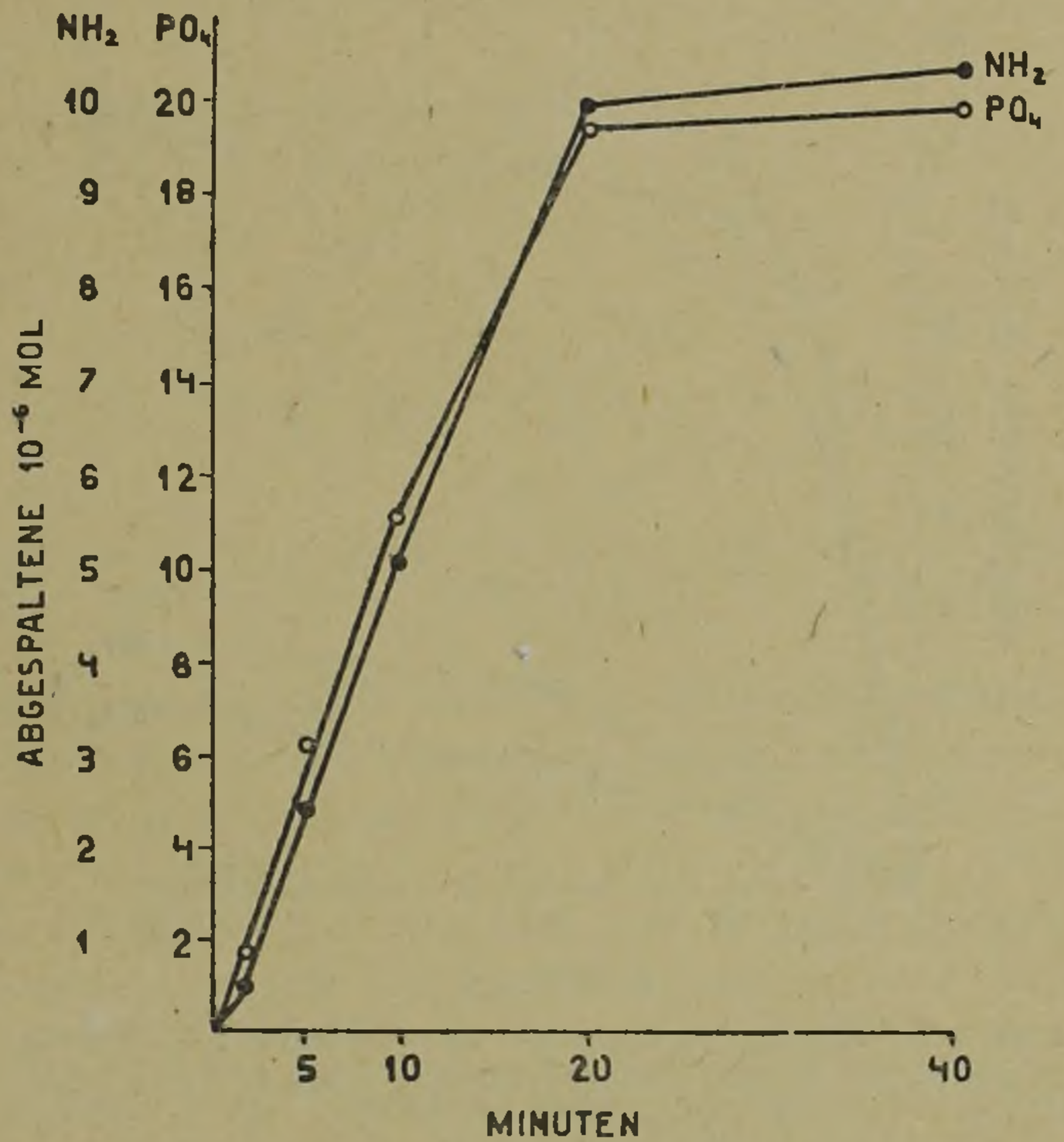

Abb. 1. PO_4- und NH_2-Abspaltung vom ATP durch Myosin + Muskelextrakt.

Das Reaktionsgemisch enthält 2 mg/ccm Myosin und 40 γ/ccm Muskelextrakt-Eiweiss. Labiles PO_4 des zugesezten ATP $= 20 \times 10^{-6}$ Mol, NH_2 des ATP $= 11 \times 10^{-6}$ Mol. Wie nun aus Abb. 1 hervorgeht,

werden unter der gleichzeitigen Einwirkung des Myosins und des Muskelextraktes zwei PO_4 vom ATP abgespalten, zugleich damit aber auch das NH_2. (Die Abspaltung des NH_2 übertrifft in ihrem Ausmasse sogar noch um etwas die des PO_4, was seine Erklärung darin findet, dass das ATP ein wenig durch Adenylsäure verunreinigt ist, wofür auch die Analyse des Präparates zeugt.)

Dephosphorylierung und Desaminierung des ADP durch Myosin allein, bezw. durch Myosin + Muskelextrakt zugleich (Abb. 2).

Reaktionsgemisch mit 2 mg/ccm Myosin, bezw. mit 2 mg/ccm Myosin + 40 γ/ccm Muskelextrakt-Eiweiss. Ist Myosin allein anwesend, so wird das ADP nur sehr langsam gespalten; parallel mit der Abspaltung des PO_4 erfolgt aber auch die des NH_2. Ist neben Myosin jedoch auch noch

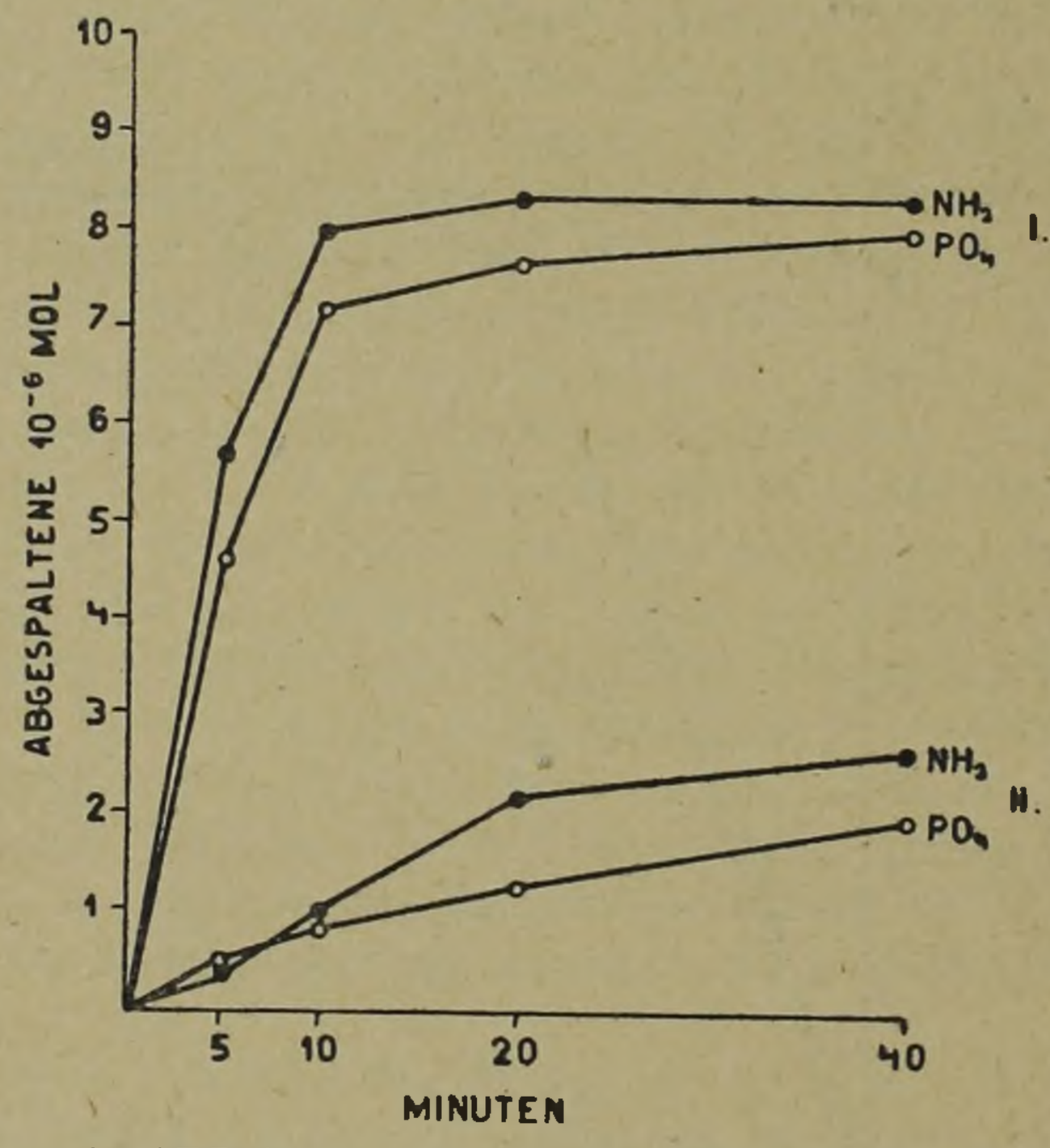

Abb. 2. Spaltung des ADP: in den Kurven I durch Myosin allein (2 mg/ccm), in den Kurven II durch Myosin (2 mg/ccm) + Muskel-Extrakt (40γ/ccm). Labiles PO_4 des ADP = $10{,}6 \times 10^{-6}$ Mol, NH_2 des ADP = $10{,}7 \times 10^{-6}$ Mol.

Muskelextrakt anwesend, so wird schon in den ersten 5 Minuten dei 9-fache Menge PO_4 abgespalten und parallel dazu das NH_2. Muskelextrakt allein spaltet weder vom ATP, noch vom ADP weder PO_4, noch NH_2 ab.

Trennung der Dephosphorylierung und der Desaminierung des ADP (Abb. 3).

Um entscheiden zu können, auf Grund welchen Mechanismus die Dephosphorylierung und die Desaminierung des ADP eigentlich vorsichgeht, versuchten wir diese beiden Vorgänge voneinander zu trennen.

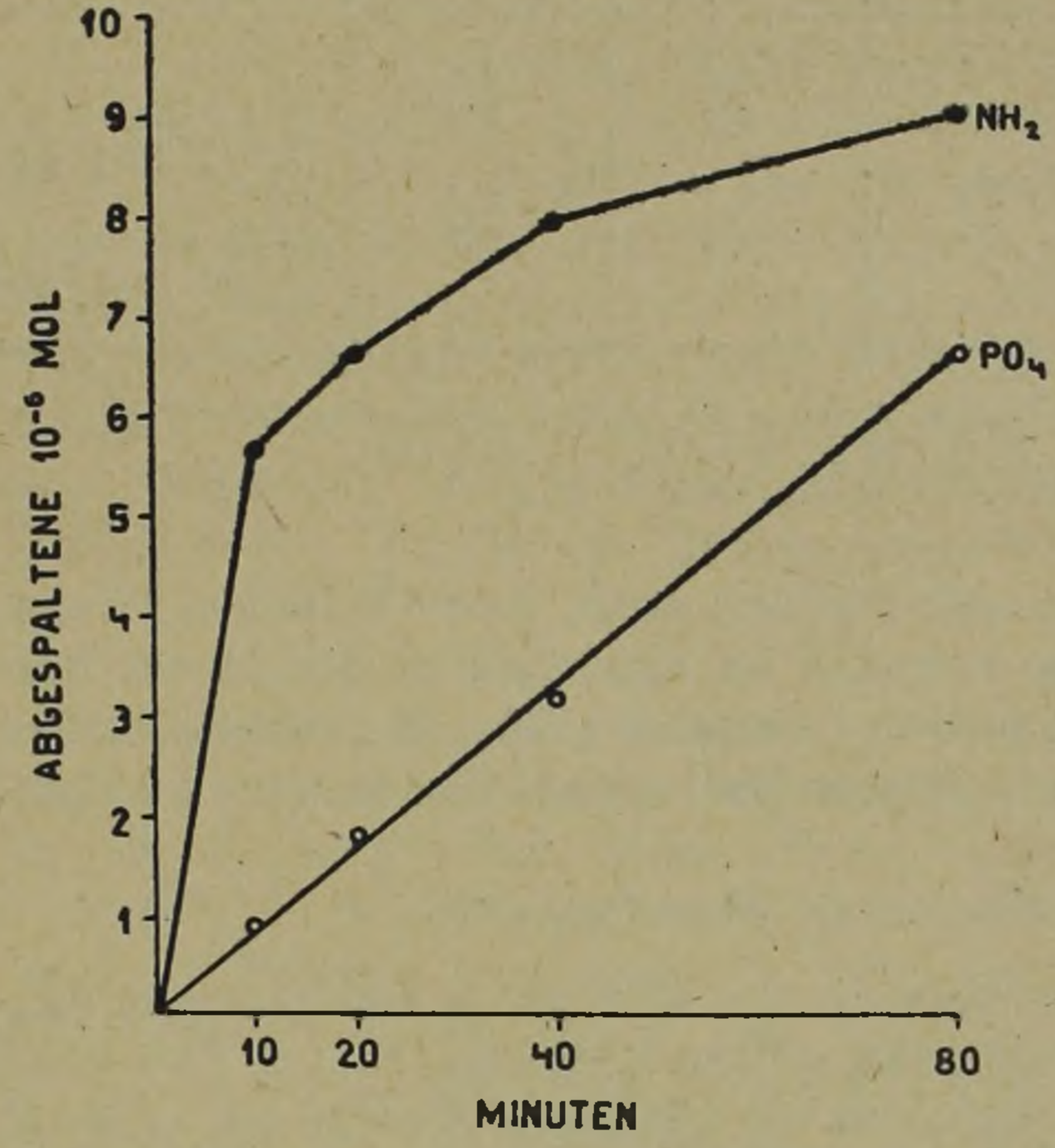

Abb. 3. Spaltung des ADP durch 0.3 mg/ccm Myosin + 0.2 mg/ccm Muskelextrakt-Eiweiss. Labiles PO_4 des ADP = 10.6×10^{-6} Mol, NH_2 des ADP = 10.7×10^{-6} Mol.

Das Reaktionsgemisch enthält 0,3 mg/ccm Myosin und 0,2 mg/ccm Muskelextrakt-Eiweiss. Die Abspaltung des NH_2 eilt, wie dies aus Abb. 3 deutlich zu entnehmen ist, der des PO_4 wesentlich voran, da in den ersten 10 Minuten ungefähr 6-mal so viel NH_2 abgespalten wird, wie PO_4. Später, nach längerer Inkubation wird dann dieser Unterschied allmählich wieder ausgeglichen (die beiden Kurven nähern sich einander). Die Abspaltung des NH_2 und die des PO_4 stellen also nicht einen durch ein einziges Enzym bedingten, simultanen Vorgang dar, sondern können voneinander getrennt werden. Dieser Versuch unterscheidet sich von dem in Abb. 2 abgebildeten Versuch darin, dass das Myosin in wesentlich geringerer Konzentration vorhanden ist (0,3 mg/ccm im Gegensatz zu 2 mg/ccm in Abb. 2), das Muskelextrakt-Protein aber in 5-facher Menge Anwendung findet (200 γ/ccm im Gegensatz zu 40 γ/ccm). Auf ähnliche Weise eilt aber die Abspaltung des NH_2 der des PO_4 auch dann voraus,

wenn nur 40 γ/ccm Muskelextrakt-Eiweiss im Reaktionsgemisch vorhanden sind (Tabelle 1).

TABELLE 1.

Inkubations-Dauer Minuten	Abgespaltenes PO_4 10^{-6} Mol	Abgespaltenes NH_2 10^{-6} Mol	Anmerkung
5	0,8	4,9	—
5	0,8	6,2	In Anwesenheit von 0,005 Mol $MgCl_2$
20	2,8	6,3	—
20	2,8	6,7	In Anwesenheit von 0,005 Mol $MgCl_2$

0,3 mg/ccm Myosin + 40 γ/ccm Muskelextrakt-Protein.
Labiles PO_4 des ADP = 10,6 × 10^{-6} Mol.
NH_2 des ADP = 10,7 × 10^{-6} Mol.

Die Grundbedingung dafür, dass die Desaminierung der Dephosphorylierung voraneilt, ist also nicht in der Menge des verwendeten Extraktes zu suchen. Ist nämlich genügend Extrakt anwesend, so muss nur das Myosin in entsprechend kleiner Konzentration vorhanden sein.

Die Bedeutung des Muskelextraktes.

Da Myosin allein das ADP nur in sehr geringem Ausmasse spaltet, liegt die Frage nahe, welche Bedeutung kommt dem Muskelextrakt zu? Deshalb untersuchten wir also zuerst den von uns verwendeten Muskelextrakt darauf hin, ob er Myokinase enthält, oder nicht. In diesen Versuchen wurde ADP mit Schmidt-Desaminase, bezw. mit Muskelextrakt allein inkubiert, dann aber mit Schmidt-Desaminase und Muskelextrakt gleichzeitig (Tabelle 2).

TABELLE 2.

Inkubations-Dauer Minuten	Schmidt-Desaminase	Schmidt-Desaminase + Extrakt	Schmidt-Desaminase	Schmidt-Desaminase + Extrakt
	abgespaltenes PO_4 10^{-6} Mol		abgespaltenes NH_2 10^{-6} Mol	
10	0	0	1,4	4,8
20	0	0	1,8	4,9

0,5 ccm Schmidt-Desaminase in einem Gesamtvolumen von 5 ccm.
40 γ/ccm Muskelextrakt-Protein.
Labiles PO_4 des ADP = 10,6 × 10^{-6} Mol.
NH_2 des ADP = 10,7 × 10^{-6} Mol.

Der Muskelextrakt allein übt, wie wir wiederholt feststellen konnten, weder auf ATP, noch auf ADP weder dephosphorylierend, noch desaminierend. Die Schmidt-Desaminase allein spaltet vom ADP kein PO_4 ab und auch NH_2 nur in sehr geringem Ausmasse. (Der Grund für diese Erscheinung ist in der schon von *Kalckar* beschriebenen Tatsache gelegen, nach welcher die Schmidt-Desaminase häufig auch etwas Myokinase enthält.) Schmidt-Desaminase und Muskelextrakt gemeinsam zugesetzt ergeben zwar keine PO_4-Abspaltung, aber dafür eine wesentliche Desaminierung. Mit Berücksichtigung des Umstandes, dass die Schmidt-Desaminase nur auf die Adenylsäure desaminierend wirkt, müssen wir also annehmen, dass in dem Gemisch unter dem Einfluss der im Muskelextrakt enthaltenen Myokinase Adenylsäure entsteht. Dass aber auch ATP gebildet wird, dafür spricht der folgende Versuch. Wenn Schmidt-Desaminase 10 Minuten mit ADP inkubiert wird, so erfolgt weder PO_4-, noch NH_2-Abspaltung. Wird nun ein Teil des trichloressigsauren Filtrates neutralisiert und ihm Myosin beigesetzt, so kommt es ebenfalls weder zu einer Abspaltung von PO_4, noch zu einer von NH_2. Werden aber Schmidt-Desaminase + Muskelextrakt mit ADP inkubiert, so kommt es nach 10 Minuten zwar nicht zur Abspaltung von PO_4, doch beträgt jetzt die Abspaltung des NH_2 $2{,}1 \times 10^{-6}$ Mol. Setzen wir nun einem Teil des trichloressigsauren Filtrates nach vorangehender Neutralisierung Myosin zu, so kann nach 10 Minuten Inkubation eine PO_4-Abspaltung von $2{,}6 \times 10^{-6}$ Mol beobachtet werden, bezw. eine NH_2-Abspaltung von $2{,}9 \times 10^{-6}$ Mol. Der Muskelextrakt bildet also aus dem ADP auch ATP.

Die Dephosphorylierung des ADP ist daher eine sich unter der Einwirkung der Adenosintriphosphatase abspielende Spaltung der in Anwesenheit von Muskelextrakt auf die Einwirkung der Myokinase hin entstandenen Adenosintriphosphorsäure.

Die Bedeutung des Myosins.

Eine weitere Frage ist nun die, in welchem Stadium erfolgt eigentlich die Desaminierung? Weder Myosin, noch Muskelextrakt sind für sich allein imstande ATP, bezw. ADP zu desaminieren. Das Myosin erweist sich jedoch als eine stark aktive Adenylsäuredesaminase (10). Vergleichen wir also unter den gegebenen Versuchsbedingungen die Aktivität des Myosins als Adenosintriphoshatase, bezw. als Adenylsäuredesaminase, so finden wir, dass das Myosin als Adenylsäuredesaminase eine bedeutend grössere Aktivität aufweist wie als Adenosintriphosphatase (Abb. 4).

Unter Veränderung der Myosinkonzentration (von 10 γ/ccm bis 500 γ/ccm) verwendeten wir in der einen Versuchsreihe als Substrat Adenylsäure, in der anderen die äquimolekulare Menge ATP. Nach einer

Inkubations-Dauer von 5 Minuten ist es klar zu erkennen, dass das Myosin in einer Konzentration, in welcher es das ATP nur in geringem Ausmasse, seiner Konzentration entsprechend dephosphoryliert, die Adenylsäure schon fast vollständig desaminiert.

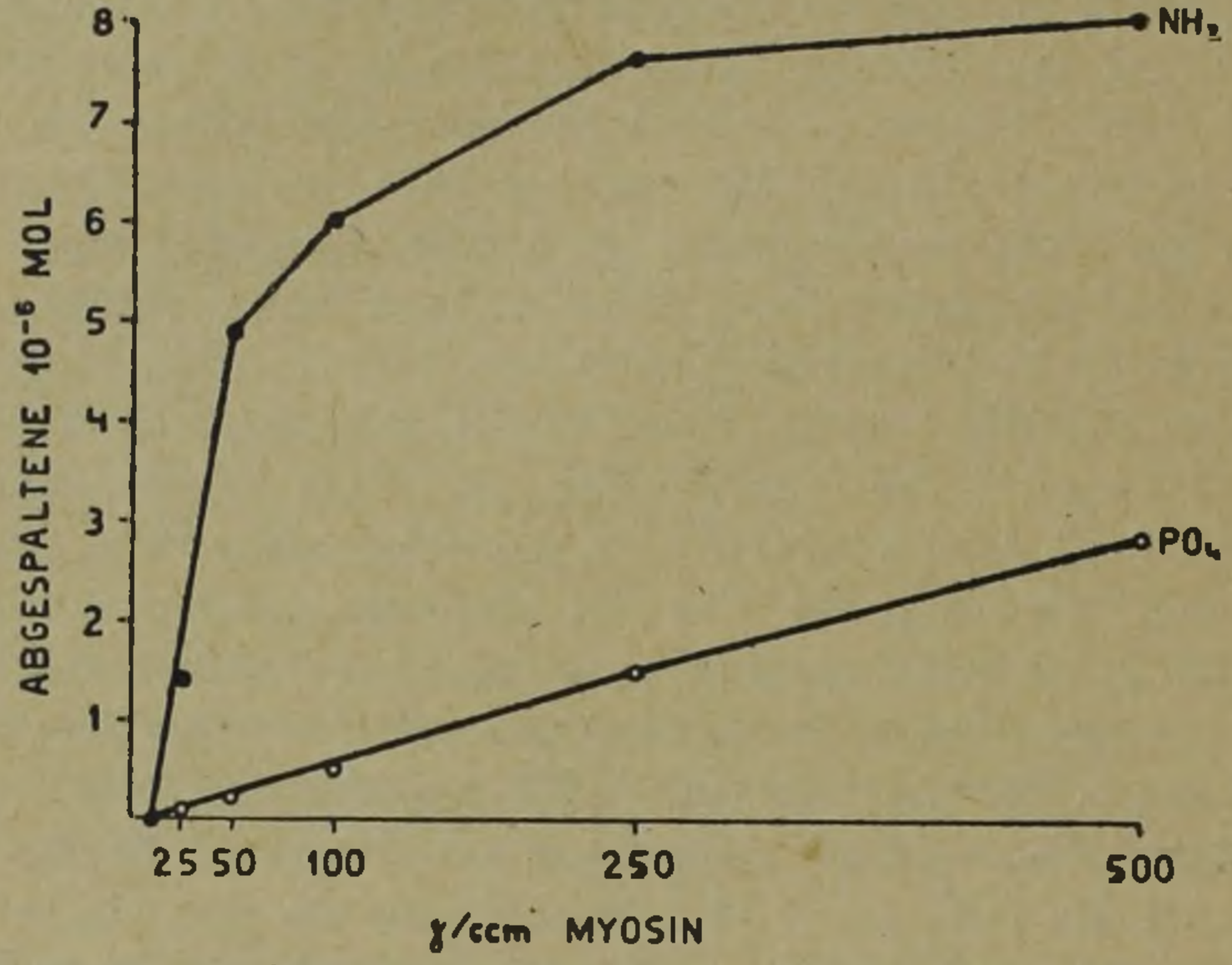

Abb. 4. Die Abhängigkeit der Dephosphorylierung des ATP, bezw. der Desaminierung des AP von der Myosinkonzentration. Labiles $PO_4/2$ des ATP $= 8{,}6 \times 10^{-6}$ Mol, NH_2 des AP $= 8{,}6 \times 10^{-6}$ Mol.

Die Tatsache, dass es die Konzentration des Myosins ist, welche das Verhältnis zwischen Dephosphorylierung und Desaminierung regelt, wird auch durch folgenden Versuch bestätigt (Abb. 5).

Im Reaktionsgemisch sind 0,3 mg/ccm Myosin und 0,2 mg/ccm Extrakt-Protein vorhanden, ausserdem aber auch noch 0,005 Mol $CaCl_2$. Es ist allgemein bekannt, dass das Kalzium die Adenosintriphosphatase sehr stark aktiviert. Trotzdem nun in diesem Versuch die Zusammensetzung des Reaktionsgemisches, abgesehen von der Anwesenheit des Kalziums, vollkommen mit den Verhältnissen des in Abb. 3 besprochenen Versuches übereinstimmt, erscheint die Abspaltung des NH_2 in den ersten 10 Minuten kaum doppelt so gross wie die des PO_4, während sie in den Versuchen ohne Kalzium den 6-fachen Wert ergeben hatte. Das mit *Ca* aktivierte Myosin wirkt also so, als ob es einfach in grösserer Konzentration anwesend wäre.

Wenn die Myokinase des Muskelextraktes das ADP in ATP und AP verwandelt, bilden ATP und AP gleichzeitig das Substrat für das Myosin. Dass nun das Myosin die Adenylsäure selbst dann desaminiert, wenn gleichzeitig auch ATP in äquimolekularer Menge zugegen ist, zeigt Abb. 6.

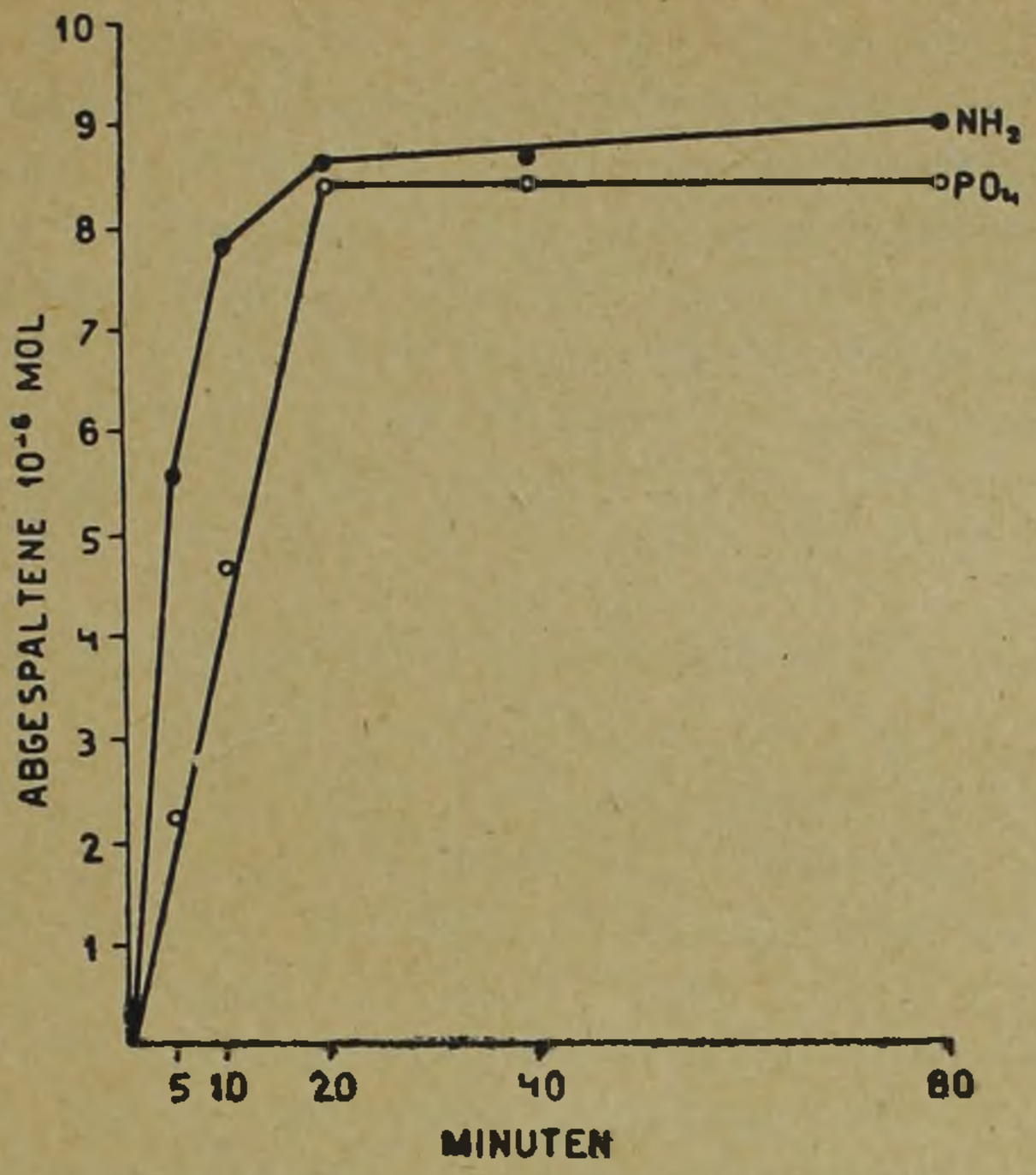

Abb. 5. Spaltung des ADP durch 0,3 mg/ccm Myosin und 0,2 mg/ccm Muskelextrakt-Eiweiss in Anwesenheit von 0,005 Mol $CaCl_2$. Labiles PO_4 des ADP = = 10,6 × 10^{-6} Mol, NH_2 des ADP = 10,7 × 10^{-6} Mol.

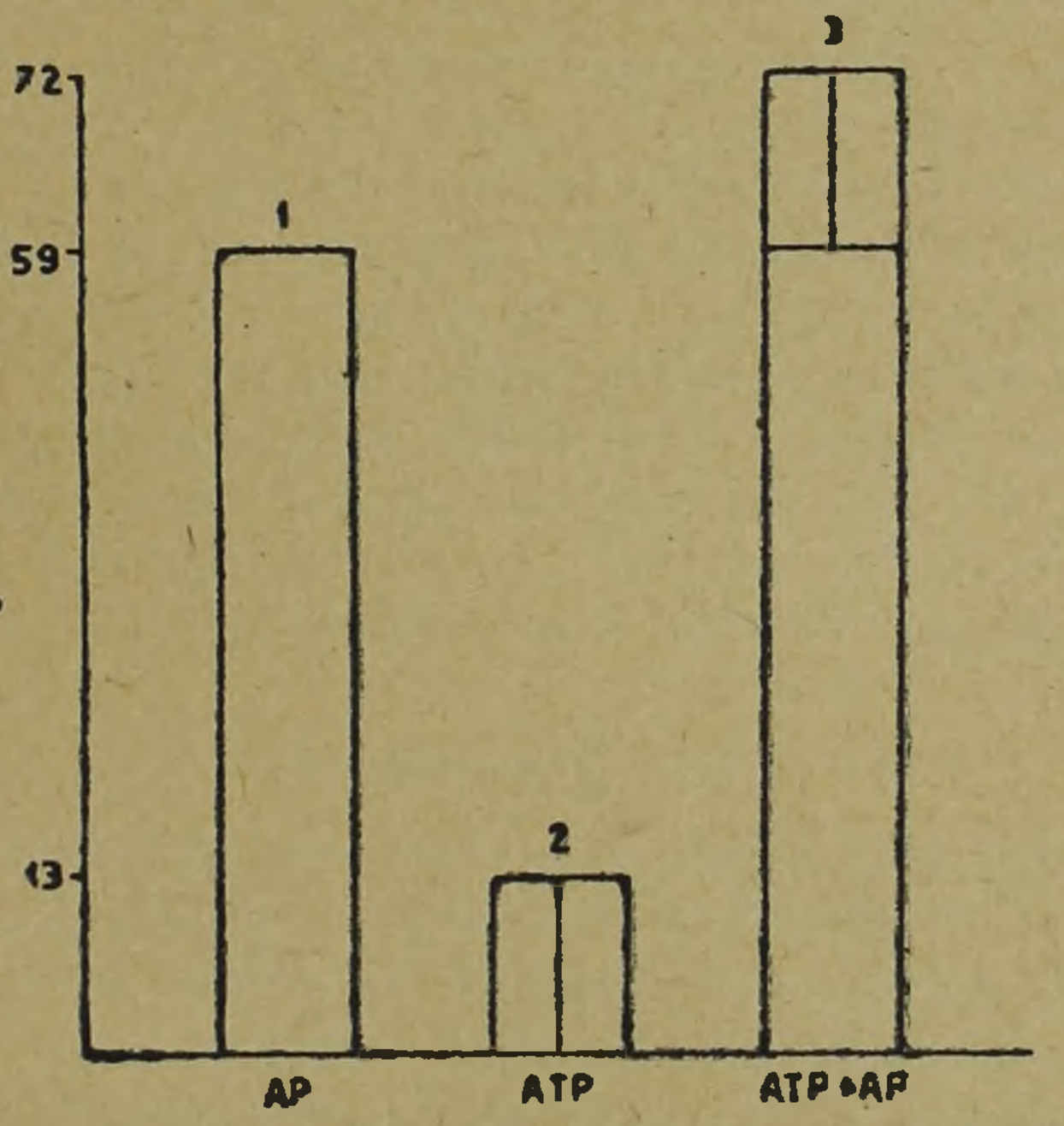

Abb. 6. Desaminierung des AP allein (1), sowie gemeinsam mit äquimolekularem ATP (3). 0,8 mg/ccm Myosin (Inkubations-Dauer 5 Minuten).

Wird Myosin mit 1,5 mg AP (60 γ NH_2—N) allein inkubiert, so werden 59 γ Aminonitrogen abgespalten, wird es mit der äquimolekularen Menge ATP allein inkubiert, so beträgt die Abspaltung des Aminonitrogens 13 γ. (Der Grund dafür liegt in der Verunreinigung des ATP mit AP.) Inkubieren wir nun Myosin mit beiden Substanzen gleichzeitig, so erhalten wir für die jetzt auftretende Abspaltung einen Wert von 72 γ Aminonitrogen. Das Myosin desaminiert also das ihm zur Verfügung stehende AP auch dann, wenn ATP und AP in äquimolekularen Mengen gleichzeitig in dem Gemisch vorhanden sind.

BESPRECHUNG DER ERGEBNISSE.

Auf die gleichzeitige Einwirkung von Myosin und Muskelextrakt hin wird das ADP dephosphoryliert und desaminiert, d. h. es entsteht letzten Endes Inosinsäure. Der Mechanismus dieses Vorganges kann folgendermassen veranschaulicht werden. Die im Muskelextrakt vorhandene Myokinase wandelt das ADP zu ATP und AP um. Das Myosin dephosphoryliert dann das ATP und desaminiert das AP. Dephosphorylierung und Desaminierung können voneinander getrennt werden, wenn in Anwesenheit hinreichender Myokinase das Myosin in einer Konzentration Anwendung findet, in welcher es unter den gegebenen Verhältnissen als Adenosintriphosphatase weniger aktiv ist, als in seiner Eigenschaft als Adenylsäuredesaminase. Die Spaltung des ADP erfolgt daher nicht durch eine eigene Adenosindiphosphatase, bezw. ADP-Desaminase, sondern nach der Dismutation des ADP durch die schon bekannte Adenosintriphosphatase und Adenylsäuredesaminase.

Ist das Substrat nicht ADP, sondern ATP, so verläuft der ganze Vorgang auf genau demselben Wege, nur wird das ATP zuerst vom Myosin zu ADP umgewandelt. Sobald aber ein Teil des ATP dephosphoryliert ist, dismutiert die Myokinase sofort das entstandene ADP und dieser Vorgang setzt sich dann solange fort, bis das gesamte ATP zu Inosinsäure umgewandelt ist. In Reaktionsgemischen entsprechender Zusammensetzung verlaufen die beiden Vorgänge annähernd parallel zueinander.

ZUSAMMENFASSUNG.

Die Adenosintriphosphorsäure wird unter gleichzeitiger Einwirkung von Myosin und Muskelextrakt dephosphoryliert und desaminiert, wobei als Endprodukt des ganzen Vorganges Inosinsäure entsteht.

Während des Abbaues funktioniert das Myosin nicht nur als Adenosintriphosphatase, sondern auch als Adenylsäuredesaminase. Die Rolle des Muskelextraktes besteht in der Dismutation des ADP durch die in

ihm enthaltene Myokinase. Eine eigene Adenosindiphosphatase und eine eigene ADP-Desaminase treten während des Abbaues nicht auf.

Das gegenseitige Verhältnis zwischen der scheinbar am ADP erfolgenden Dephosphorylierung und Desaminierung kann durch Veränderungen der Myosinkonzentration geregelt werden.

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THE INTERACTION BETWEEN ACTOMYOSIN AND POLYPHOSPHATES.

WITH 4 FIGURES IN TEXT.

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(RECEIVED FOR PUBLICATION 15. 10. 1948.)

INTRODUCTION.

It has been the fundamental observation of recent developments in the chemistry of muscle contraction that adenosine triphosphate (ATP) causes profound changes in the physicochemical properties of actomyosin. Thus a solution of actomyosin in high salt concentration becomes less viscous, a gel of actomyosin in low salt concentration shows extreme shrinking on addition of ATP. The former effect is attributed to the dissociation of actin and myosin, whereas there exists no coherent and proven theory for the latter phenomenon. The two effects are, however, most likely related.

As the beginning of the understanding of the said phenomena, we have tried to determine the conditions which influence the interaction of actomyosin and ATP, i. e. the formation of the actomyosin-ATP complex. It is known that pyrophosphate (1) produces a similar action on dissolved actomyosin, as ATP. We have found that inorganic triphosphate[1] and calgon too decrease the viscosity of an actomyosin solution. The effect of pyrophosphate was observed only at a lower temperature, but not at room temperature. It occured to us, that these data could be correlated assuming that the polyphosphates form dissociable complexes with actomyosin. In the present paper we describe the determination of the dissociation constants of the actomyosin-ATP, actomyosin-pyrophosphate, actomyosin-triphosphate and actomyosin-calgon complexes, together with some data on the change of these constants with temperature, *pH* and salt concentration.

EXPERIMENTAL.

The observations were made on dissolved actomyosin and the viscosity effects of the above mentioned compounds were determined.

[1]Prepared according to *F. Schwartz*, Z. anorg. Chem. *9*, 249 (1895).

Ostwald type viscosimeters were used, from which the outflow of about 1 ml fluid lasted 30—40 seconds. When not otherwise stated, the measurements were performed at 0 C°.

An actomyosin solution was made up of myosin and actin so that it contained the desired concentration of *KCl* and 0,02 *M* phosphate or 0,03 *M* veronal-acetate buffer of *pH* 7. The concentration of *Mg* and *Ca* ions varied from 0—0,001 *M*. 2,5 ml of such a solution were placed in the viscosimeter. Having determined the viscosity of the solution, 0,1 ml of the suitabely diluted reactant (e. g. ATP) was added, mixed and the time noted. Viscosity was then repeatedly determined as the function of the time from mixing.

The concentration of myosin was usually 0,8 mg/ml and that of actin 0,3 mg/ml.

RESULTS.

When a small amount of ATP is added to a solution of actomyosin in 0,5 *M KCl*, the viscosity of the solution decreases, but rises again in time, until the original viscosity is reached. This is illustrated in Fig. 1. The measurements were performed in an ice bath at 0 C°, so that the secondary rise in viscosity — which is due to the adenosine triphosphatase activity of myosin — is slowed down. But even so, the rise in viscosity can be followed only if the time of outflow from the viscometer is short, e. g. within a minute. This is achieved by using viscosimeters of small capacity, with some sacrifice of exactness.

If the viscosity values, obtained after the addition of ATP are plotted against the time, a nearly straight line is obtained, as shown in Fig. 1. Extrapolation to time 0 gives the viscosity value which might be supposed to have prevailed at the moment of mixing of actomyosin and ATP.

If an excess of ATP is added, the viscosity becomes lowered nearly to the viscosity value of the myosin present in the actomyosin mixture. This value does not change until the hydrolysis of the ATP is nearly complete, when it suddenly begins to rise in the same way as it does — instantaneously — on addition of a small amount of ATP.

The decrease in viscosity of an actomyosin solution on addition of ATP is due to the dissociation of the actomyosin complex, free myosin and actin are formed. It is reasonable to suppose that those molecules of actomyosin, which are bound to ATP are dissociated completely and those which are not bound to ATP are not changed at all. Thus, if the extrapolated change in viscosity is only half as great on addition of a small amount of ATP as on addition of an excess of ATP, then it might be

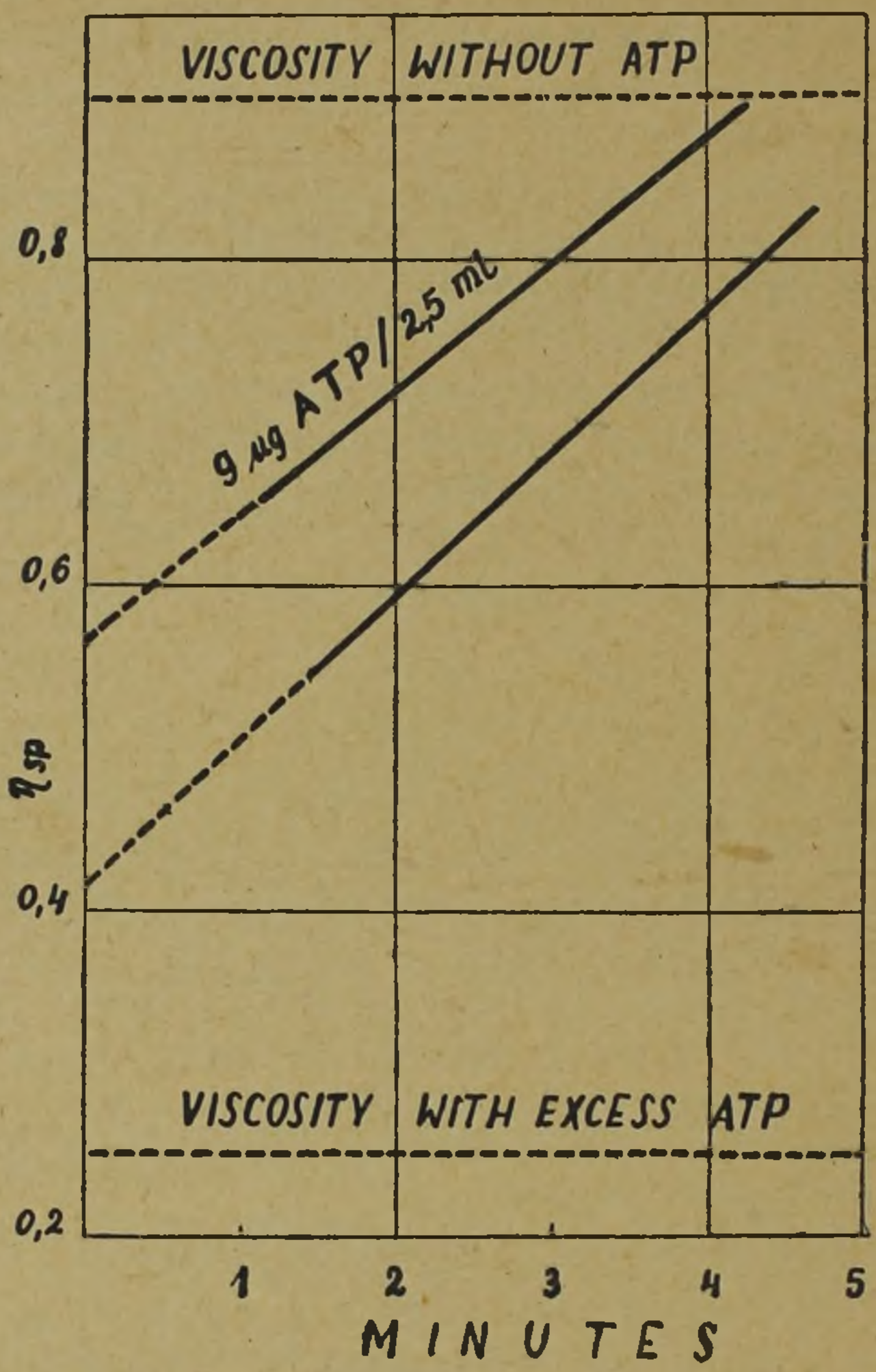

Fig. 1. Effect of small amounts of ATP on the viscosity of actomyosin solution.

supposed that half of the actomyosin present is combined with ATP and the rest is uncombined.[1]

Fig. 2. shows the correlation between the concentration of ATP and the % viscosity effect. The latter symbol is defined as:

$$\% \text{ viscosity effect} = \frac{\text{decrease of } \eta_{sp} \text{ on addition of ATP}}{\text{decrease of } \eta_{sp} \text{ on addition of excess ATP}} 100$$

[1] This involves the assumption that in presence of free myosin, actomyosin gives a linear viscosity increment, an assumption which cannot experimentally be verified, neither is it expected to be true. The error thus introduced is manifested when we try to fit the experimental data with the dissociation curve, calculated from the derived dissociation constant. This error, however, does not materially influence the following conclusions.

The dissociation constant of the actomyosin-ATP complex is defined:

$$K = \frac{\text{(free ATP)} \times \text{(free actomyosin)}}{\text{(actomyosin-ATP complex)}}$$

It is seen from Fig. 2. that $4{,}10^{-6}$ mol/liter ATP produces a 50% viscosity effect. It follows from the above considerations that in this case 50% of the actomyosin is bound to ATP and the rest is free. Therefore in this case the dissociation constant is:

$$K = \text{(free ATP)}$$

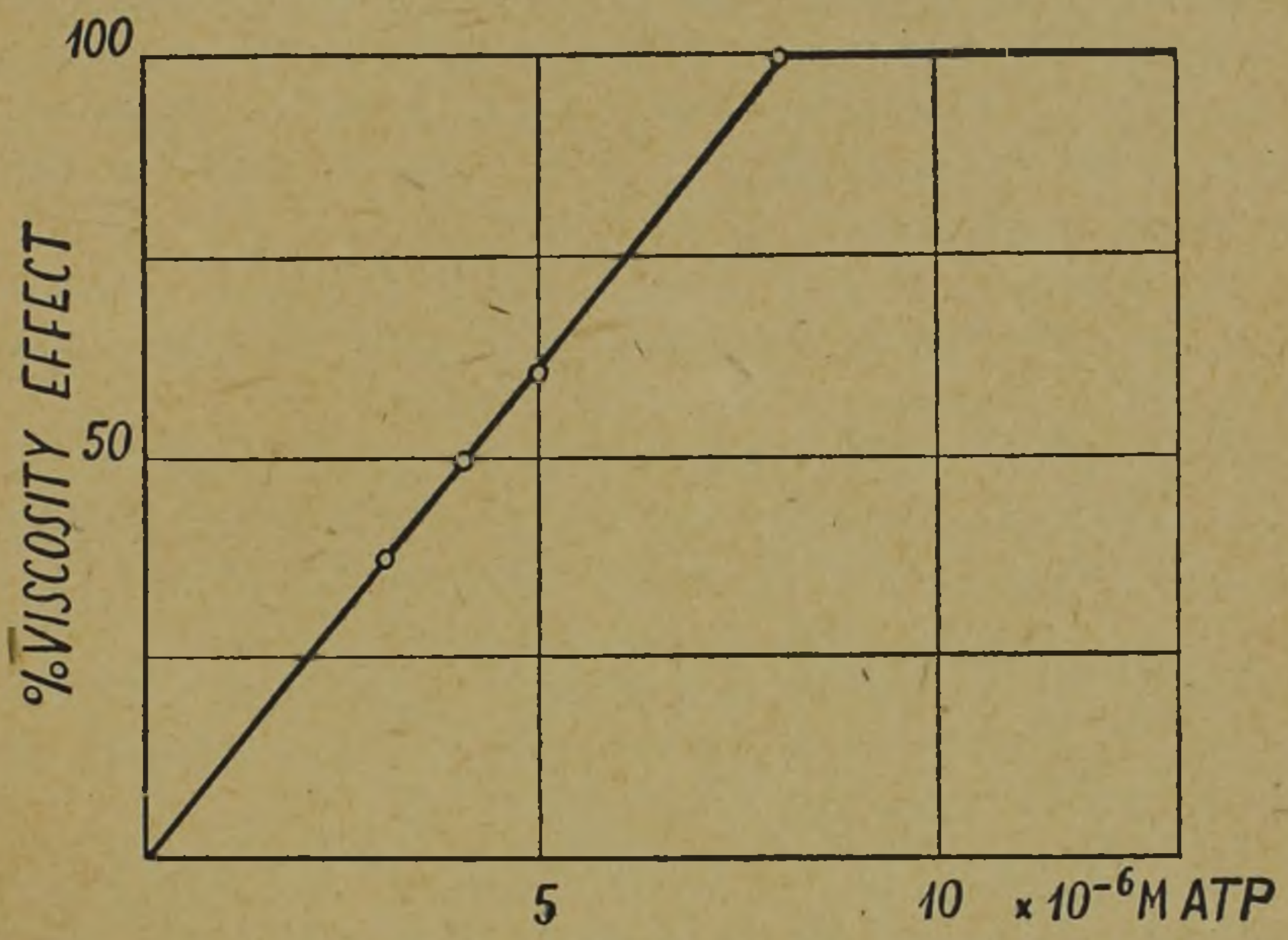

Fig. 2. Viscosity effect in relation with the concentration of ATP added. On the ordinate, viscosity effects extrapolated to time are 0 plotted against the molar concentration of ATP.

To find the value of free ATP, the molar concentration of combined ATP must be subtracted from the total concentration of total ATP added. The concentration of combined ATP is naturally equal to the concentration of combined actomyosin, i. e. $^1/_2$ of the total actomyosin present. In order to obtain the numerical value of the dissociation constant, we have to know the equivalent reacting weight of actomyosin. As it is the myosin portion of the actomyosin, which reacts with ATP, the concentration and equivalent reacting weight of myosin can be used instead of the data of actomyosin. As the molecular weight of myosin is not yet definitely established, we have tried to fit the data by assuming several multiples

of 35.000 to be the equivalent reacting weight of myosin. It turned out that there is no solution for the equation of the dissociation constant, if the equivalent reacting weight of myosin is taken to be less than 140.000. It follows therefore that one gramm molecule of ATP is able to react with 140.000 g of myosin. Actomyosin is therefore split into myosin and actin, when one gramm molecule ATP is bound to 140.000 g of myosin. Whether actomyosin is able to bind more ATP than this amount — as indicated by experiments of quite another type (2) — cannot be decided from our results.

With the same technique we have determined the actomyosin-ATP complex formation at lower ionic strength (0,3 *M KCl*) and at higher temperatures. The high rate of ATP hydrolysis under these conditions renders the results somewhat uncertain, but it appears that the dissociation constant of the actomyosin-ATP complex is not influenced by either of these factors to any appreciable extent.

The average value of the actomyosin-ATP dissociation constant was found to be $2{,}10^{-6}$ (in presence of 0,001 *M Mg* ions), if the equivalent reacting weight of myosin is taken to be 140.000.

Owing to the relative constancy of the value of the dissociation constant through ionic strength and temperature variations, it is possible to calculate the situation in the muscle. Here, the concentration of ATP is $5{,}10^{-3}$, that of myosin (M. W. 140.000) is $0{,}57 \cdot 10^{-3}$. It is found that practically all myosin is bound to ATP, but only 10% of the ATP is bound to myosin. This conclusion is not materially altered by assuming another reasonable equivalent reacting weight for myosin.

The difficulties in the determination of the actomyosin-ATP dissociation constant are due to the rapid hydrolysis of ATP. As pyrophosphate, inorganic triphosphate and calgon are not split by actomyosin, the determination of the dissociation constants in these cases is much easier. Once the viscosity is lowered on addition of these compounds, it does not change any more. We have therefore studied the actomyosin-pyrophosphate complex formation in greater detail, in order to obtain data, which are, no doubt of value for the understanding of the formation of the actomyosin-ATP complex.

The values of the dissociation constants are summarised in Table I, when the actomyosin was dissolved in 0,5 *M KCl*, at *pH* 7, in presence and in absence of *Mg* ions. Temperature 0 C°. In case of inorganic polyphosphates, the compound was added to the actomyosin solution and the viscosity determined after 30 minutes, in order to obtain real equilibrium values.

TABLE I.

Dissociation constants of actomyosin complexes $\times 10^6$.

Complex	Without Mg	In presence of 0,001 *M* $MgSO_4$
Actomyosin — ATP	3,25	2,45
Actomyosin — triphosphate	23,7	3,7
Actomyosin — pyrophosphate	31,7	14,7
Actomyosin — calgon	no measurable complex formation	350

It is seen that the complex formation is the strongest in case of ATP. Next comes inorganic triphosphate, pyrophosphate and finally calgon. All these compounds have in common the grouping:

$$O = P - O - P = O$$
$$\quad OH \qquad\quad OH$$

which seems to be therefore the active group producing the viscosity effect.

Table I. shows that the dissociation constant of all complexes is much lower, when 0,001 *M Mg* ions are added to the actomyosin solution. Higher concentrations of the divalent ions were not studied, as they begin to precipitate actomyosin.

The temperature dependence of the dissociation constant of actomyosin-pyrophosphate was investigated in relation to earlier observations (1) that pyrophosphate does not affect the viscosity of actomyosin at room temperature. The data summarised in Table II. give a clear picture of the situation.

TABLE II.

Dissociation constant of actomyosin-pyrophosphate.

Temperature C°	Concentration of *KCl*	*pH*	Dissociation constant . 10^4	
			without *Mg*	with 0,001 *M Mg*
0	0,5	7	31,7	14,7
15	0,5	7	1200	160
25	0,5	7	—	350
0	0,5	7	—	13
0	0,3	7	—	38
0	0,5	7	—	12
0	0,5	6	—	13
0	0,5	8	—	18

The effect of *Mg* and *Ca* ions on the actomyosin-polyphosphate complex formation was studied in some detail as these ions have marked

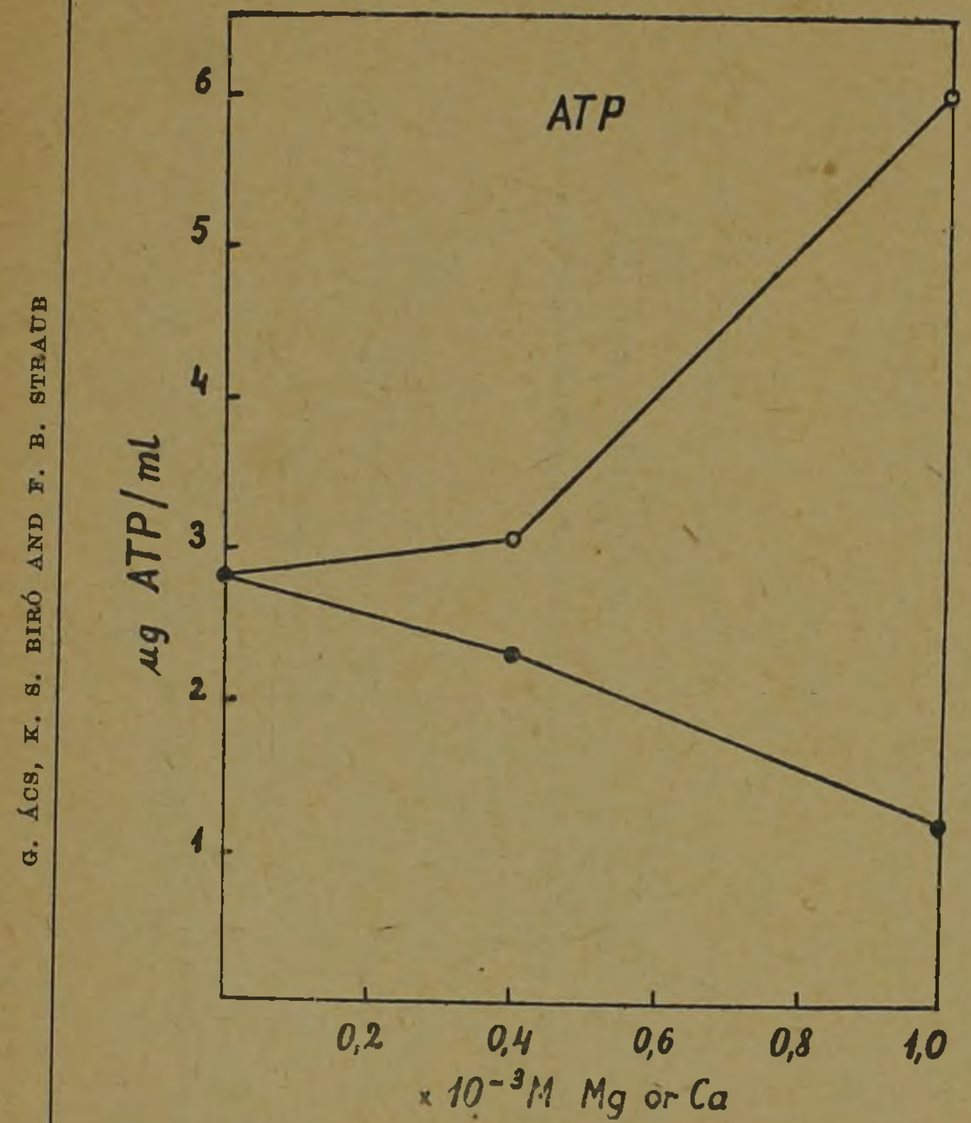

Fig. 3. Effect of divalent ions on the binding of ATP.

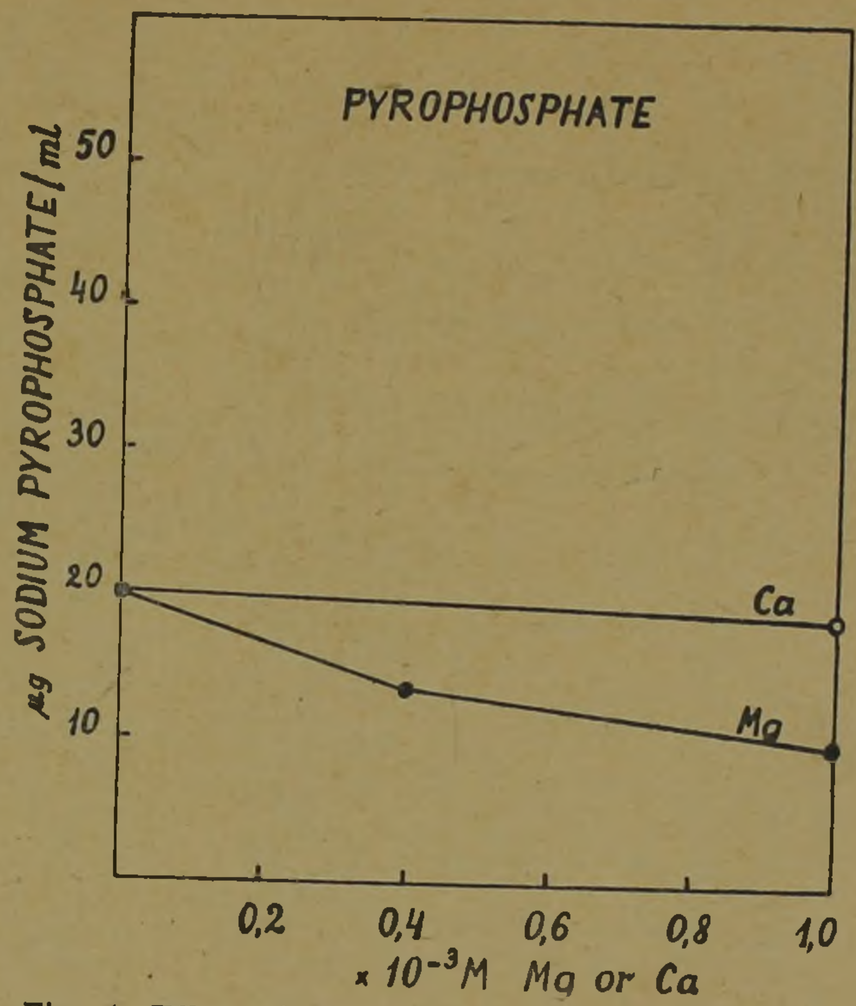

Fig. 4. Effect of divalent ions on the binding of pyrophosphate.

effects on the enzymatic activity of myosin and on the contraction of actomyosin threads (2). We have determined the concentration of ATP and pyrophosphate, needed to obtain a 50% viscosity effect, at varying levels of *Mg* and *Ca* concentrations. These results are shown in Fig. 3. and 4. These curves show that in case of pyrophosphate *Ca* seems to have no effect at all, whereas *Mg* decreases the dissociation constant, i. e. it gives a better binding of pyrophosphate. In case of ATP, the effect of *Mg* is similar, whereas *Ca* greatly inhibits the binding of ATP, it increases the value of the dissociation constant. The dissociation constant of the actomyosin-calgon complex is reduced equally well by both *Mg* and *Ca* ions. These data are however more difficult to evaluate, owing to secondary effects, as nearly 100 times more calgon has to be added to actomyosin to obtain similar viscosity effects as with ATP and pyrophosphate.

DISCUSSION.

The above experimental data may be explained in the following way. The polyphosphates, which are able to split actomyosin into myosin and actin, do so only in form of their *Mg* complexes. *Mg* is bound to the protein and ATP and similar compounds are bound to this *Mg* atom. Actomyosin, as prepared in our experiments, seems to contain all the necessary *Mg*, as a maximal viscosity effect can be obtained with ATP, pyrophosphate and inorganic triphosphate without any addition of *Mg* ions. This *Mg* is however loosely bound, it becomes firmly attached only when an excess of *Mg* is present in the solution.

As *Ca* and *Mg* give similar complexes with the polyphosphates, it is expected that the addition of *Ca* reduces the available polyphosphate. *Ca* is supposed to be unable to be bound to the proper place on the surface of the protein. This is actually found to be the case with ATP.

Another factor however, must be taken into consideration. The polyphosphates and the protein both carry negative charges at physiological *pH*. When a *Mg* complex is formed, the charge is reduced and this facilitates the association of protein and polyphosphate. *Ca* and *Mg* should be equally effective in this respect. If the polyphosphate — having bound let us say one *Ca* — has still available groups to react with the *Mg* attached to the protein, then *Ca* — by reducing the charge of the polyphosphate anion — has actually facilitated the protein polyphosphate complex formation. This is the case of calgon, the molecule of which contains the largest number of negatively dissociated groups. If, however, the binding of one divalent ion makes it impossible for the compound to react with *Mg*, then an excess of *Ca* ions will actually inhibit the formation of the actomyosin-polyphosphate complex. This seems to be the case with ATP. Pyrophos-

phate behaves in an intermediary way, the twofold effects of *Ca* cancelling each other.

The same reasoning explains the relative efficiency of the polyphosphates in the formation of actomyosin-polyphosphate complexes. At the same time it explains why inorganic triphosphate is relatively inefficient in absence of *Mg*. (Cf. last column of Table I.)

SUMMARY.

Actomyosin reacts with the *Mg* complex of adenosine triphosphate, pyrophosphate, inorganic triphosphate and calgon, to form dissociable complexes. The formation of these complexes results in the splitting of actomyosin into myosin and actin.

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TEMPERATURE REGULATION OF THE DENERVATED LIMB.

WITH 5 FIGURES IN TEXT.

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(RECEIVED FOR PUBLICATION 20. 10. 48.)

The old assumption, which attached a desicive importance to the hypothalamus in the regulation of the body temperature, seemed disproved, when *Popoff* (18) and one year later *Thauer* (22) found that after section of the spinal cord at the neck, the animals (dogs, rabbits) regained their capability for the regulation of the body temperature so much, that after one or two weeks they are capable to maintain their body temperature at room temperature. *Thauer* and *Peters* (23) found moreover that after cutting all nerve paths by transection of the brainstem, their animals too regained after a time their temperature regulation.

Though *Ranson* et *al.* (19) undoubtly proved the leading role of the hypothalamus, by heating circumscribed points of the regio supraoptica, and by causing different lesions, the way in which the regulation of the body temperature is maintained after transsection of the spinal cord still remained unexplained. That these animals have a real chemical temperature regulation was established by *Issekutz jr.* (8). He found, that dogs 5—6 days after section of their spinal cord at the sixth cervical segment, showed an increase of their metabolic rate of about 70—80 percents when they were cooled. *Hermann, Jourdan, Morin* and *Vial* (7) and *Henri, Morin, Vial* (6) after transection of the cervical cord removed the thoracic and lumbal part of it, exstirpated the ganglia stellata, cut both vagi and even these animals partly regained after a time their capability of temperature regulation.

From these experiments it could be concluded, that in the temperature regulation of such animals hormones having metabolism raising effect, play a leading role. With these results the experiments of *Freund* and *Janssen* (4) were not compatible. These authors found, that the denervated gastrocnemius of the cat, after periarterial sympathectomy, looses its ability of temperature regulation and reacts to cooling with a decrease of its metabolic rate. Considering however that they used the method of Barcroft

and Verzár to determine the bloodflow, they were not able to make more than five or six determinations on one limb. Meanwhile they were compelled to give the animals salt infusions to compensate its loss of blood. So it seemed necessary to recapitulate these experiments with modern methods.

METHODS.

Some days after transection of the spinal cord the dogs were narcotised with 2—3 mg/kg morphine s. c. The vena femoralis was prepared and tied up 2—3 cms below the influx of the vena profunda femoris. Proximally from the tying up in the direction of the vena iliaca a "T" canula was fastened into the vein, which was covered with a solution of 10% gelatine, and dried, to prevent clotting *(Jancsó)* (12). The long stem of the canula was connected with a glass tube 50 cm long and 8 mm wide

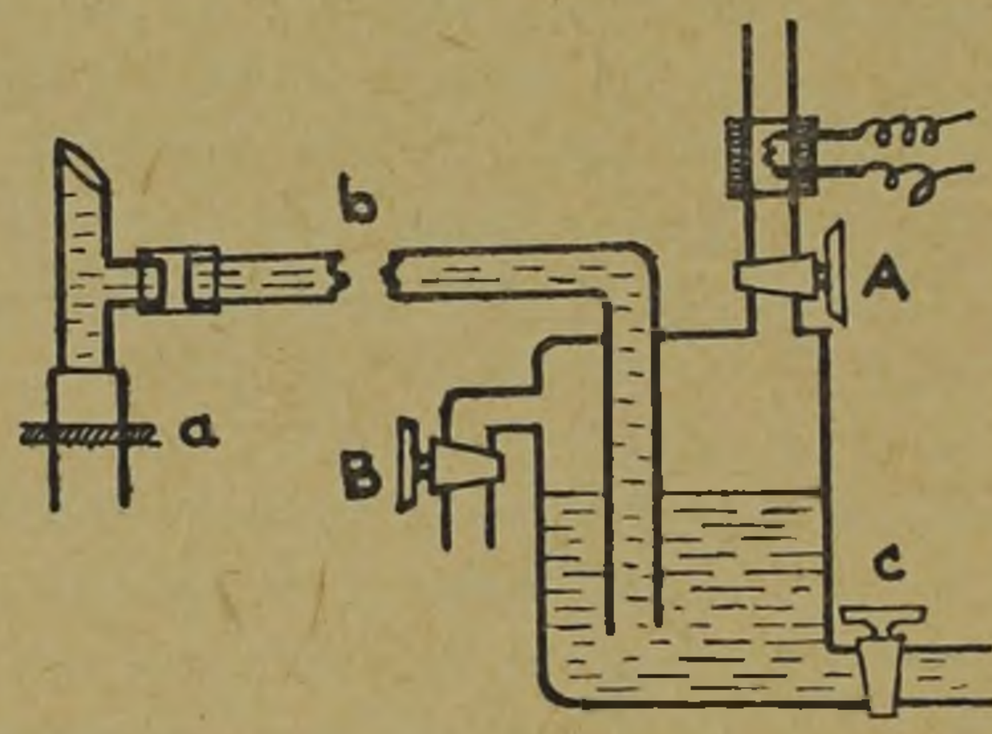

Fig. 1. Explanation see in text.

(fig. 1b.). This was filled with 0,9% solution of *NaCl*, containing 8 I. U. heparine/m. l. (Liquemin Roche). This long tube ended in a cylindrical glass vessel (appr. 300 m. l.), below a surface of heparinized salt solution. The glass vessel comminucated with a cylinder furnished with the cock "B". A third tube supplied with the cock "A", lead to a hot wire anemometer *(Anrep* 1.*)*, which was connected into a Wheatstone bridge. The third stem of the canula was furnished with a clip "a", and served for the elimination of air bubbles.

Proximally from the influx of the vena profunda, at the level of the ligamentum inguinale, an easily managable springy clamp was put on the vena femoralis. All branches of the vena femoralis which came from above or from the genitals were tied up.

Opening the cock "A" and shuting the clamp, the blood coming from the muscles of the leg of the vena profunda, flowed into the long glass pipe. The velocity of the inflow is promptly recorded by the galvanometer

of the anemometer. Meanwhile in the cylindrical vessel the surface of the liquid rises a little, but this as we ascertained in many controll experiments, does not influence the velocity of the inflow. After the determination of the deflection of the galvanometer at a ground glass, standing appr. 2 meters far, the clamp was re-opened, the cock "A" shut and through the cock "B" by means of the compressed air the invaded blood was forced back into the vein. The whole determination and the forcing back of the blood last only 20—25 seconds, and so the blood has no time to coagulate. The advantages of this method are its great exactness (appr. 1—2%), and prevention of blood coagulation, without much heparine getting into the animal. It becomes possible to make long experiments lasting 10—12 hours, under physiological circumstances.

The determination of the arterio-venous oxygen difference was made by means of our previously devised method. At the desired time arterial blood (from the common carotid artery) and venous blood (by pinching the rubber tube connecting the "T" cannula with the tube "b") was taken, and hemolysed. Using absorption cells of one mm thickness, the difference between the extinctions was determined in Havemann's photoelectrical colorimeter. From this the arterio-venous oxygen difference was obtained. *(B. Issekutz jr., G. Hetényi jr.* and *I. Feuer.* 9.).

These two methods enabled the determination of the oxygen-consumption of the leg with an approximate accuracy of 5% and frequently (20—30 times) in an experiment if required.

RESULTS.

A) *Heat regulation of the hind leg with full innervation.*

First of all it was necessary to measure the heat regulating ability of the muscles belonging to the vena profunda femoris. For this purpose experiments were performed on dogs narcotised superficially with morphine and ethylurethane. Such an experiment is exhibited in Fig. 2.

A dog of six kg weight was narcotised with 0,6 g/kg ethylurethane and 3 mg/kg morphine, both administered subcutaneously. The oxygen consumption of the left hind leg was determined on three or four occasions. If the oxygen uptake remained constant, cooling was begun, by ice-lumps put on the chest and neck of the dog. This caused a strong shivering, both the blood flow and the utilisation of the blood increased heavily causing a mighty increase of the metabolic rate of about 300%. Denervation (without periarterial sympathectomy) — performed at this time — caused after a transitional increase, a diminution of the blood flow, and a sudden decrease of the arterio-venous oxygen difference. 75 minutes after the

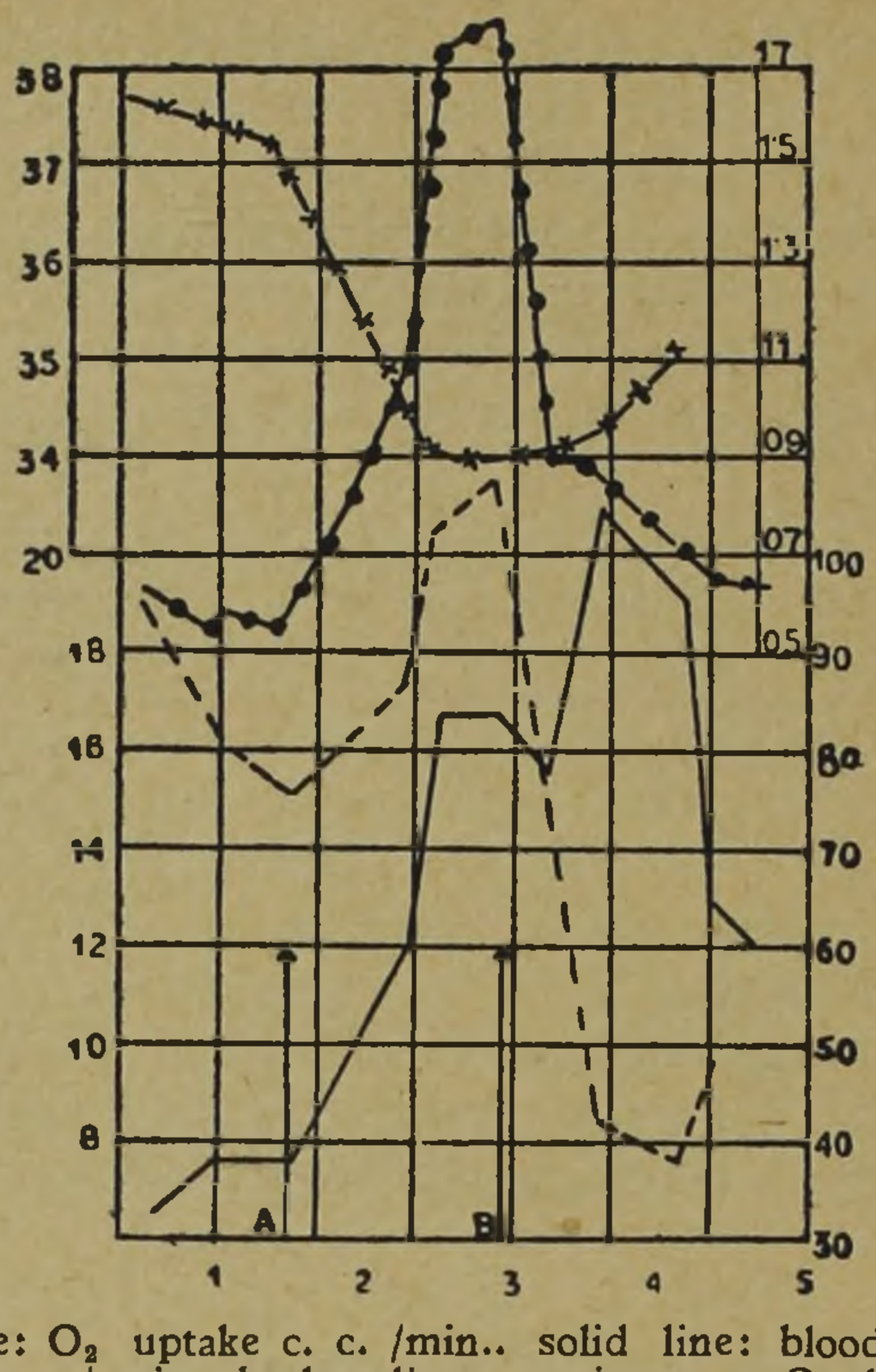

Fig. 2. Heavy line: O_2 uptake c. c. /min.. solid line: blood flow in the vena profunda feromis c. c./ min., broken line: arteriovenous O_2 difference m. m. /c. c., broken line with crosses: rectal temperature C°. At „A" cooling was begun, at „B": denervation. Left hind leg of a dog of 6 kg weight narcotised with, 0,6 g/ kg ethylurethane and 3 mg/kg morphine.

denervation the oxygen consumption of the leg was only 10% above the basal metabolic rate. Considering however that the rectal temperature was 1,5 C° below the starting point, it could be said, that the leg possessed some regulation, though due to the narcosis it was impaired. To the cause of this phenomenon we shall come back below.

B) *Regulation of body temperature after section of the cervical spinal cord.*

Experiments were carried out on dogs after section of the spinal cord at the sixth cervical segment. They received 1 mg/kg morphine s. c. The beginning of the active temperature regulation — caused by cooling the chest and neck — was indicated by the shivering of the muscles of the neck.

Fig. 3. shows the course of one of these experiments. The oxygen-consumption of the leg of a 14 kg. dog was 1,7 c. c./min., its rectal temperature 36,6 C°. After about half an hour's cooling the oxygen consumption

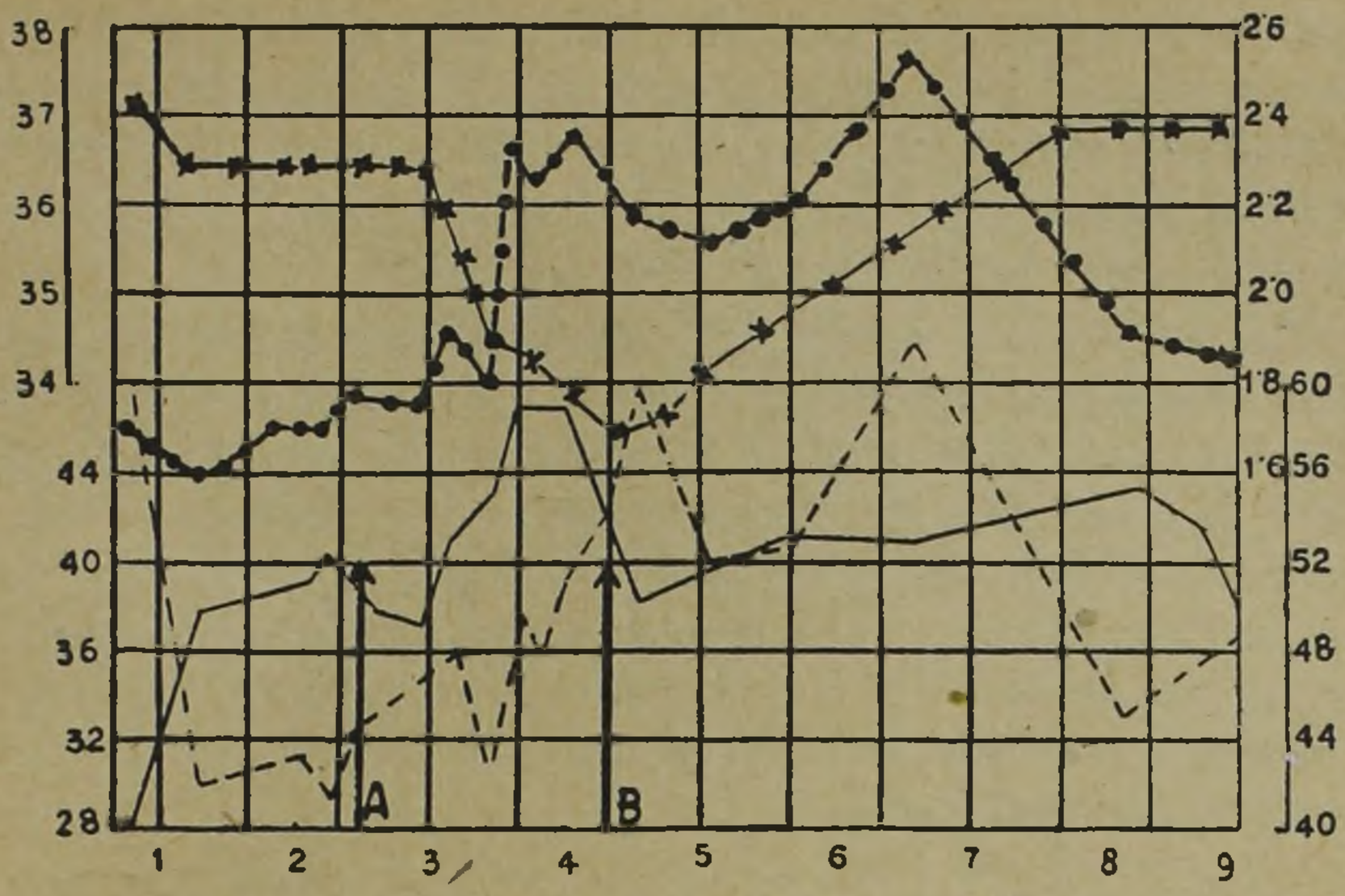

Fig. 3. The same symbols are used as in Fig. 2. At „A" cooling was begun, at „B" it was ended. Left hind leg of a dog of 14 kg, the cervical spinal cord of wich was transected.

begins to rise, and whilst the rectal temperature decreased by 2,2 C°, the oxygen consumption of the leg increased by 38%. Cooling the animal further, the oxygen consumption decreases a little, but stopping the cooling, the rectal temperature begins to rise, and so does the oxygen consumption too (+ 50%), showing that the chemical heat regulation was hindered by the strong refrigeration. At the time the rectal temperature regains its original value, the metabolic rate of the leg drops again to its starting point.

As it can be seen from the curves, the increase of the metabolic rate is primarily due to the increased utilisation of oxygen from blood. The curve of the blood flow, after an initial fall, rises with the beginning of the cooling and, then, possibly due to the strong refrigeration, falls again. Finally it remains constant at a value of 40—43 c. c./min., and at the time the body temperature reaches its original value, the curve of the blood flow too regains its starting point. (38 c. c./min.).

This experiment proves, that after section of the spinal cord, there exists a real chemical temperature regulation in the limb, confirming thus the earlier experiments of one of us made with Rein's Thermostromuhr. (*Issekutz jr.* 10.). It must be noted that the temperature regulation of such animals can only be shown in animals with an undisturbed circulation. A reliable sign of this is a value of the arterio-venous oxygen difference not exceeding 80 c. mm./c. c. Every experiment in which we obtained a higher

value showed a total lack of regulation. There was no shivering on the neck, the temperature of the animal fell rapidly, and the metabolic rate of the limb, following van t'Hoff's law, decreased. This may be the reason, that *Freund* and *Janssen* (4), who made their experiments the day following the operation, did not find any trace of regulation.

The limb of the animal with transected cervical spinal cord has however its vegetative innervation. The effect of this innervation on muscle metabolism was demonstrated by *Mansfeld* and *Lukács* (13), who showed that the so called chemical tone of muscles is maintained in this way. Though according to the investigations of *Issekutz jr.*, the spinal centers do not play any role in the heat regulation of the extremity (cooling of the extremity rises its metabolism even after the lumbar part of the spinal cord was crushed), it seemed after all necessary to continue the experiments on totally denervated limbs.

C) *Temperature regulation after total denervation.*

The spinal cord of dogs (of 10—14 kg weight) was transected at the tenth thoracic segment. Four or five days after this operation the left hind leg was entirely denervated, by cutting the nervi ischiadicus, femoralis, obturatorius, and by additional sympathectomy, removing the adventitia of the femoral artery and rubbing the denuded part with 5% carbolic acid solution. This seemed important, because *Freund* and *Janssen* (4) regarded the periarterial sympathetic fibres as an important factor in maintaining temperature regulation.

Such experiments were made on six dogs with identical results. A characteristic example is shown in Fig. 4. Before the experiment the dog received 3 mg/kg morphine subcutaneously. The normal oxygen consumption of its hind leg was 1,35 c. mm/min. Soon after beginning of the cooling the upper part of the body began to shiver, and at the same time the blood flow in the vena profunda femoris got faster; obviously because the heart of the shivering animal worked, as well known, with a larger output, driving more blood through the dilated vessels of the denervated leg. As a result the arteriovenous oxygen difference decreased, and for some time there was no considerable increase in the oxygen consumption of the leg. After about 45 minutes when the temperature of the rectum had fallen 2,3 C°, the arterio-venous oxygen difference suddenly began to increase. The average rise of the metabolic rate was about + 40%, with the highest value of + 66%. It is a remarkable phenomenon, observable in other experiments too, that, if the refrigeration was not too severe, the curves of the rectal temperature and of the oxygen consumption of the leg

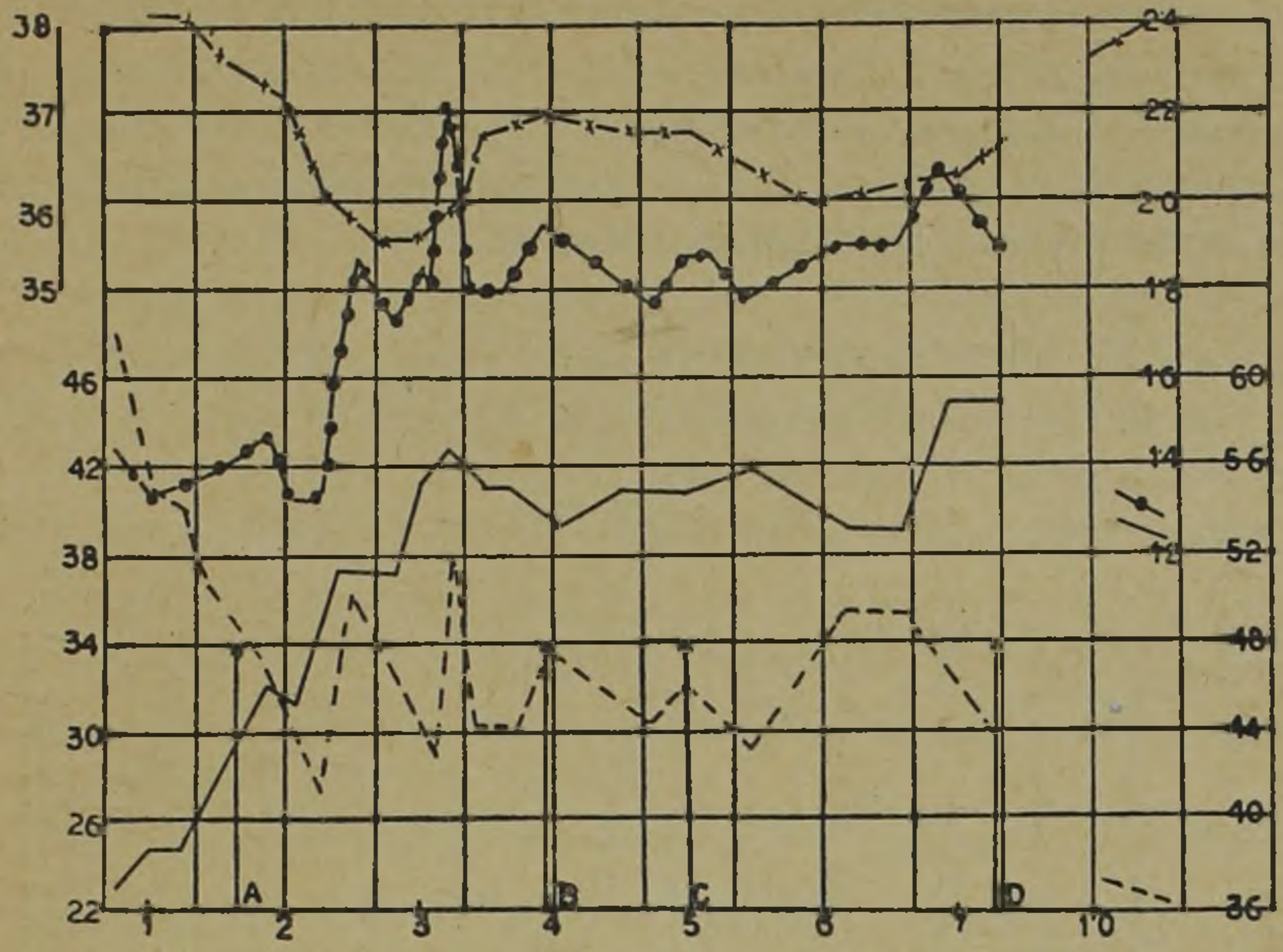

Fig. 4. The same symbols are used as in previous figures. At „A" cooling was begun, at „.B" and „C" further cooling. Left hind leg of a dog of 10 kg weigt, the thoracic spinal cord of wich was transected at the X. segment, and the sciatic, femoral and obturatorial ncrves were severed.

run in opposite direction only the later is delayed about $1-{}^1/_2$ hours. The increase of the temperature is followed by a decrease of the metabolic rate of the leg. By increasing the cooling the further diminution of the rectal temperature causes a new maximum of the metabolic rate. After a moderate refrigeration lasting about six hours the dog was warmed again. Three hours later, when the temperature of the rectum nearly reached its starting point we found the oxygen consumption of the leg again at its normal level.

TABLE I.

Effect of cooling on the O_2 consumption on the denervated limb.

Date Number of exp.	Before cooling		During cooling		Change of O_2 uptake	Remarks
	Rectal temp.	O_2 cc/min	Rectal temp.	O_2 cc/min		
1. IV. 28.	37,5	0,45	33,9	0,63	+40%	Transection at XI. Th. seg. + denervation.
2. V. 6.	39,0	0,32	37,5	0,50	+59%	
3. V. 3.	38,0	0,38	35,5	0,50	+32%	
4. VI. 12.....	38,2	1,60	37,3	2,20	+50%	
5. VI. 19.	38,2	1,32	35,5	2,20	+66%	
6. III. 27.	36,8	0,58	34,3	0,88	+51%	

Mean of changes of O_2 consumption = 49,6%.

In all the six experiments we observed a considerable rise (32—66%) of the metabolic rate of the denervated leg, in spite of the decrease of the body temperature, which, according to van t'Hoff's law diminishes the metabolism. So it can be said, that *in spite of the total denervation there existed a chemical heat regulation in the tested limb.*

D) *Effect of narcosis on temperature regulation.*

The temperature regulation of the denervated leg could only be showed on animals which were not narcotised. Fig. 5. shows, that the slightest narcosis is sufficient to abolish the rise of the metabolic rate due

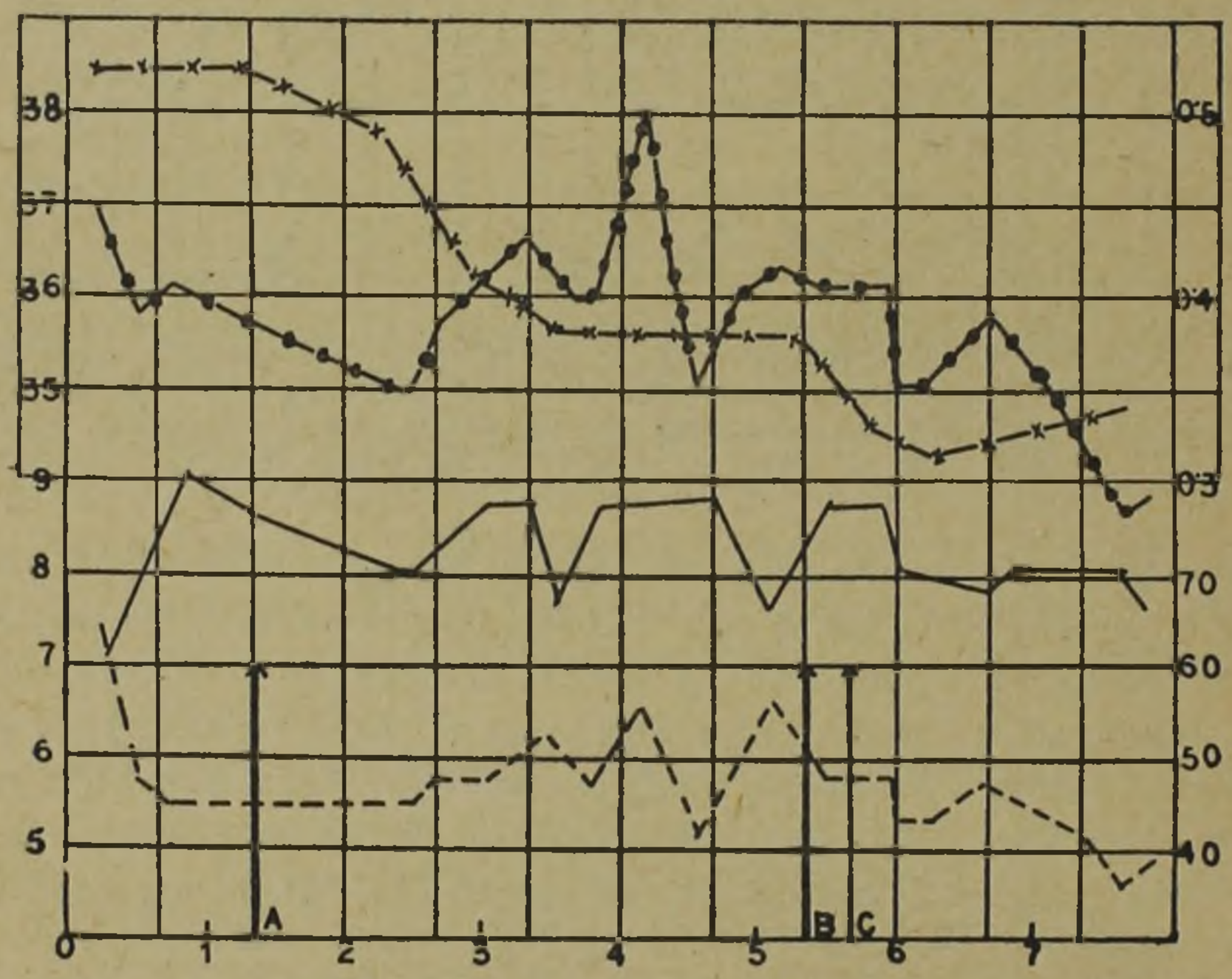

Fig. 5. The same simbols are used as in previous figures. At „A" cooling was begun, at „B" and „C" 1—1 mg/kg morphine was admimistered. Totally denervated left hind limb of a dog of 12 kg., narcotised with 0,5 g/kg ethylurethane and 3 mg/ kg morphine.

to refrigeration. Four days after section of the spinal cord at the tenth dorsal segment, the following experiment was carried out: 0,5 g/kg ethylurethane and 3 mg/kg morphine was administered s. c., and a leg denervated. After determining the basal metabolic rate of the leg, cooling was begun. The oxygen consumption of the leg rose only a little above the starting level, the average rise was only 7,7%. As the rectal temperature decreased by 3 C°, without the corresponding decrease of the oxygen consumption, we can say, that the temperature of the leg still was to some

degree regulated. Thereafter 2 × 1 mg/kg morphine were entirely sufficient to abolish the impaired regulation. The rectal temperature fell to 34,3 C°, and the oxygen consumption of the leg decreased 23%. As is to be seen from the figure, this decrease of the metabolic rate is not the result of the decrease of the blood flow, but of a diminished utilisation of the blood. It is especially remarkable, that the upper part of the body of such an animal still shivers, consequently the temperature regulation of the denervated leg can more easily be diminished by narcotics, than shivering.

DISCUSSION.

Popoff (18) and *Thauer* (22) concluded from their experiments that after elimination of the temperature regulating center, the animals are still able to maintain their body temperature, because it is now regulated by their peripheral nervous system.

Our experiments show, that the metabolic rate of the refrigerated leg increases not only after section of the spinal cord, but also after total denervation. This increase can be abolished more easily than the shivering of the intact part of the body. From this it can be concluded, that this increase of the metabolic rate is due to an excitation of the temperature regulating center, which exerts its effect through the blood stream.

Under physiological circumstances, in intact animal the chemical temperature regulation, which is independent of nervous pathways plays only a subordinate role. While the oxygen consumption of the shivering muscle rises with 300—500%, the denervated limb raises its metabolism only with 30—50%, that means, that the humoral heat regulation represents only about 10% of the total regulating capacity. It is at all events enough to explain the increase of the metabolic rate of 70—80% on dogs whose spinal cord was transected at the sixth cervical segment. This increase is partly due to the shivering of the muscles of the head and neck, partly to the increased metabolic rate of the paralysed part of the body.

In what way the humoral chemical heat regulation comes into being is a question long discussed. It was *Montuori* (15), who first pointed out the possibility of such a regulation; the far reaching investigations, which begun in 1913, of *Mansfeld* and coworkers proved, that the endocrine glands, especially the thyroid and the pituitary play the leading role. (Lit.: *G. Mansfeld*, Die Hormone der Schilddrüse und ihre Wirkungen. Basel. 1943 and *G. Mansfeld*, Thyroid hormones and their actions. *Frederick Muller* Ltd. London, 1949.) (14). The role of the thyroid is also emphasized in the experiments of *Issekutz jr.* (8) He demonstrated, that thyreoectomy greatly impairs the temperature regulation against cooling

of dogs, the cervical spinal cord of which was earlier transected. Thyroxin rendered in these experiments the temperature regulation normal again.

On the other hand several data refer to the important role of the adrenal medulla. Cooling diminishes the adrenalin content of the suprarenal *(Crowden)* (3), but raises the plasma level of it. This rises metabolism. *(Hartmann* and *Hartmann* (5), *Cannon et al.* (2), *Morin et al.* (16), *Schaeffer* and *Thibault)* (21). *Issekutz* and *Harangozó—Oroszy* (11) and *Ring* (20) showed, that the metabolic rate of thyroxine treated animals is more raised by adrenalin, than that after thyreoectomy. *Ring* established further that the effect of adrenalin is increased in animals, which were hold for a long time in cold environment. As this phenomenon is abolished after thyreoectomy, Ring concludes that the synergism of thyroxin and adrenalin may play a considerable role.

Naturally many other factors may have a role. Thus it is possible that from the intact part of the body on account of the strongly increased metabolic rate, and the shivering, some substances are getting into the blood, which directly increase the metabolic rate of the denervated muscle. (E. g. mobilisation of glucose lactic acid or sympathin e. t. c.)

SUMMARY:

1. A new method for the determination of the blood flow is described. It is possible to measure during 10—12 hours the oxygen consumption of the muscles of the hind leg of dogs under wholly physiological circumstances.

2. After section of the spinal cord, the hind legs possess a certain temperature regulation. They react to refrigeration with an increase of their metabolic rate of about 50%.

3. The denervated limb of dogs, whose thoracic spinal cord was transsected, showed an increase of the metabolic rate with 30—50%, when the upper part of the body was cooled.

4. This remaining temperature regulation can be easily abolished by narcotics. From this we concluded that it is established by the excitation of the temperature regulating center.

5. The humoral temperature regulation represents only about 10% of the total capacity of the chemical temperature regulation.

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MICROCALORIMETRIC ANALYSIS OF THE MODE OF ACTION OF PENICILLIN.

WITH 10 FIGURES IN TEXT.

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The quantitative control of the life activity of bacteria became more desirable since the invention of the antibiotics. The multiplication of bacteria is controlled very often by the method of turbidimetry. The failure of this method is, that in this way the number of living and dead bacterial cells can not be differentiated. The sedimentation of the bacteria proved to be a disturbing factor, too. The direct counting of the cells and the determination of the number of living cells by the help of culture-plates is too tiresome and not a sufficiently exact method for continuous observation. Another frequently applied method is the measurement of bacterial respiration by the microrespiratory apparatus of *Warburg*. On this way we can, however, observe only changes in that part of metabolism, which is connected with O_2-consumption and CO_2-liberation.

On the contrary we can obtain a good composite picture from the activity of living bacterial cells by determinating the heat production during the multiplication of bacteria. Essential to this is, that the heat production may be sufficiently high and the instrument used may be sufficiently sensitive for small quantities of heat energy. Certainly we have to take into account the heat of dilution and neutralisation as disturbing factors and we have to consider the possible interaction of exothermic and endothermic processes in the bacterial metabolism.

Generally the bacterial growth takes place in the presence of an adequate source of energy. The energy, withdrawn from the source is transformed partially into daughter cells representing the "Internal Work of Growth" and partially into other fractions, representing the "External Work of Growth" and the "Work of Maintenance" *(N. C. Wetzel)*.

$$\left[\begin{array}{c}\text{Energy of the}\\ \text{Source}\end{array}\right] \rightarrow \left[\begin{array}{c}\text{Internal Work}\\ \text{of Growth}\end{array}\right] + \left[\begin{array}{c}\text{External Work}\\ \text{of Growth}\end{array}\right] + \left[\begin{array}{c}\text{Work of}\\ \text{Maintenance}\end{array}\right]$$

The heat-production during bacterial metabolism (fermentation, "spontaneous inflammation" of decaying substances) is an old observation.

M. Rubner (1904) was the first who measured directly the heat production of bacteria. *Fr. Tangl* (Budapest, 1903) applied the method of chemical calorimetry for the study of live-activity of *B. anthracis, B. subtilis* and *B. suipestifer.*

O. Meyerhof. (1912), like *Rubner,* combined the indirect chemical and the direct physical calorimetry and came to the conclusion, that not all the heat produced by the bacteria originates from oxidativ processes.

C. Körössy (Budapest, 1913) constructed an ethermicrocalorimeter for his experiments. He measured the heat-production of a denitrifying soilmicroorganism and he assumed the existence of bacteria with endothermic metabolism.

Shearer (1921) presented data to demonstrate that bacteria are producing less heat, if they have at their disposal free amino-acids in the medium, compared to the case, when proteins or complex protein derivatives are the source of energy.

With very accurate method *St. Bayne—Jones* (1929) took up again the question. He modified for the special purposes of bacteriology the differential microcalorimeter of *A. V. Hill.* From his experiments he drew the conclusion, that young bacterial cells *(E. coli* and *Staphylococcus aureus)* produce in the first 3 hours of growth relatively much heat and with the changes of heat-production characteristic morphological changes and life-phases are associated. The composite picture of heat-production and growth-curve has revealed critical periods, which are of fundamental importance for the bacterial metabolism. By using the formula of *Buchanan* this author calculated the amount of heat produced by a single bacterium.

The differential microcalorimeter I have used in the present experiments was constructed by *Prof. Szalay* and myself (1943) for biological purposes and it was prepared in the mechanical workshop of the Department of Physics on our University (Figure Nr. 1). This microcalorimeter like that of *L. Meitner* and *W. Orthmann* (Z. Physik, *60,* 143, 1930) consists of two accurately equal and symmetrical nickeled red-copper tubes. In both copper-tubes glass-tubes of equal size and with two departments in each are suitingly fitted. The lower department is hermetically closed with a glassrod polished at its end, which-when uncorked-serves to mix the reacting substances separated in the lower and upper departments and to occasionally stir the fluid during the experiment.

In one glass-tube was performed the reaction under investigation, in the second one the control-experiment with 25 cc. total quantity of

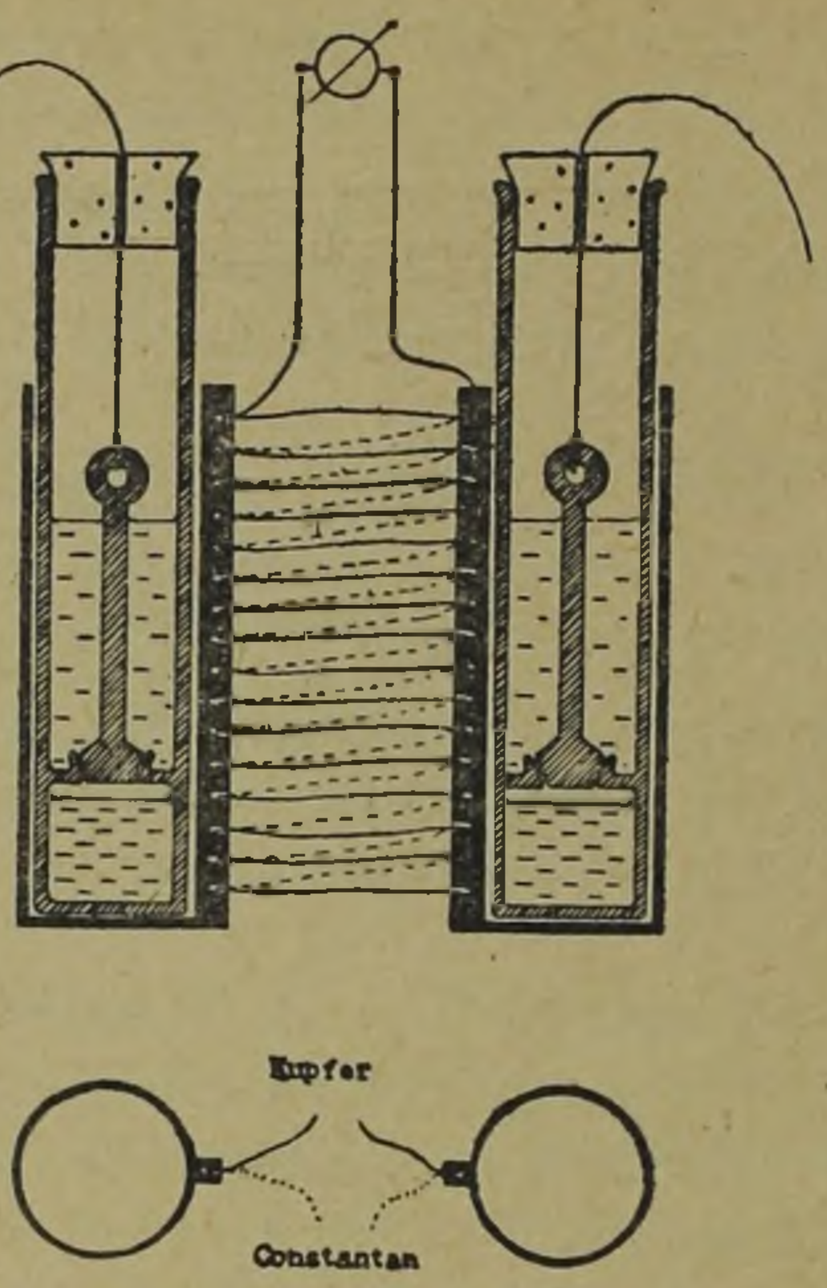

Fig. 1.

fluid in each. The copper-tubes are connected with 16 thermocouples in a state of good heat-conductivity and electrically isolated. The terminals of the thermo-column are connected with the poles of a Multiflexgalvanometer of the type of *Dr. Br. Lange* (Berlin), which instrument has a great volt-sensitivity and a low internal resistance and makes possible the autographic registration. The newtonian curve of cooling and then the sensitivity to artificial heating with a heating coil have been determined (Figure Nr. 2). A deflection of the galvanometer-index with 98 mm was equal to 11,3 cal. (Q = c. i. v. t; c = 0,239 q; i = 0,1275 Amp; v = 2,05; t = 180 sec.; Q = 11,3 cal.) One millimeter deflection of the index was equal to 0,115 cal.

I have measured the heat production of the *Staphylococcus aureus* (Oxford H.) strain in order to avoid complications by producing heat of dilution in the most simple medium of a 2% dextrose-solution (500 mg in 25 ccm physiol salt-solution.) In the lower department of one from the two glass-tubes I placed 500 mg. dextrose, the fermentation of which, after preliminary trials, was sufficiently intensiv in order to give rise to a measurable quantity of heat. In the first experiments I placed there dextrose alone, later on also penicillin in varying (1—6 mg) quantity. In the upper department of the glass tube the suspension of staphylococci was placed. In the second glass-tube the same amount (25 ccm) of physiol.

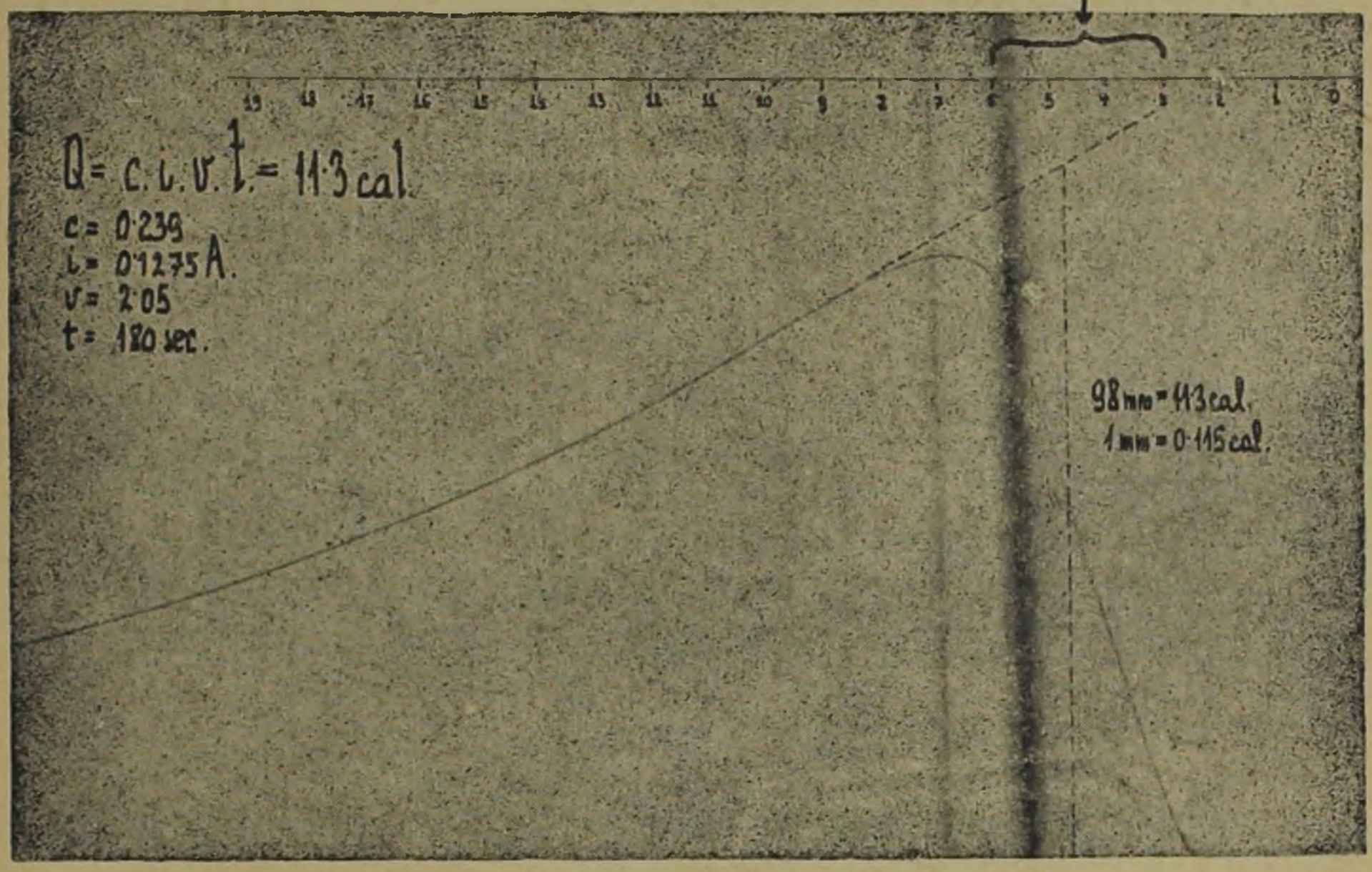

Fig. 2.

salt solution was given, as much as the bacterium suspension contained in the first tube.

The calorimeter with the filled glass tubes was put first in a *Dewar-flask* and then altogether in a 37 C°. incubator. The Dewar-flask was uncorked at the beginning until thermoequilibrium ensued (generally in 5—7 hours), what could be controled by the galvanometer. At this point the Dewar-flask was closed and after an additional $^1/_2$—$^3/_4$ hour the experiment was started. In both calorimeter glass tubes an accurately polished glass-stopper did separate the contents of the lower and upper departments. At the upper ends of these glasstoppers a silk-thread was tied. These threads were conducted through the cork of the Dewar-flask and through an upper opening of the incubator. By simultaneous pulling these two threads the contents of the lower and upper departments in both tubes were mixed and they served also later on for stiring the mixtures. In the lower part dextrose and penicillin was given. In the upper part of the tubes I placed the bacterium suspension completed to 25 ccm. with physiol. salt solution. At first I prepared a homogeneous suspension from 3 fresh agarslant cultures of Staphylococcus. One half of it was used for

filling the tubes, the other half was given into anevaporating dish, then evaporated and dried until constant weight. The dry weight of the applied staphylococcus suspension varied between 0,264—0,515 g. The time of observation lasted between 20—23 hours. The amount of heat, produced during this time, was demonstrated by the curve registered. The space between this curve and the base-line was planimetrically integrated in order to have a comparable value for the calories and hours expressed in mm^2.

In the first experiments I studied the heat production of the staphylococci in dextrose solution without adding penicillin. The heat production started in the majority of the cases almost immediatly after the mixing, in some cases, however, after a lag-phase of 1—2 hours. In the presence of 600 mg. dextrose in the second hour 7,475 cal. in the 19th hour 9,20 cal., in the 23 d hour 8,625 cal. could be measured. Planimetrically: + 15,112 mm^2 (Exper. Nr. 1, Figure Nr. 3.). From 500 mg. dextrose

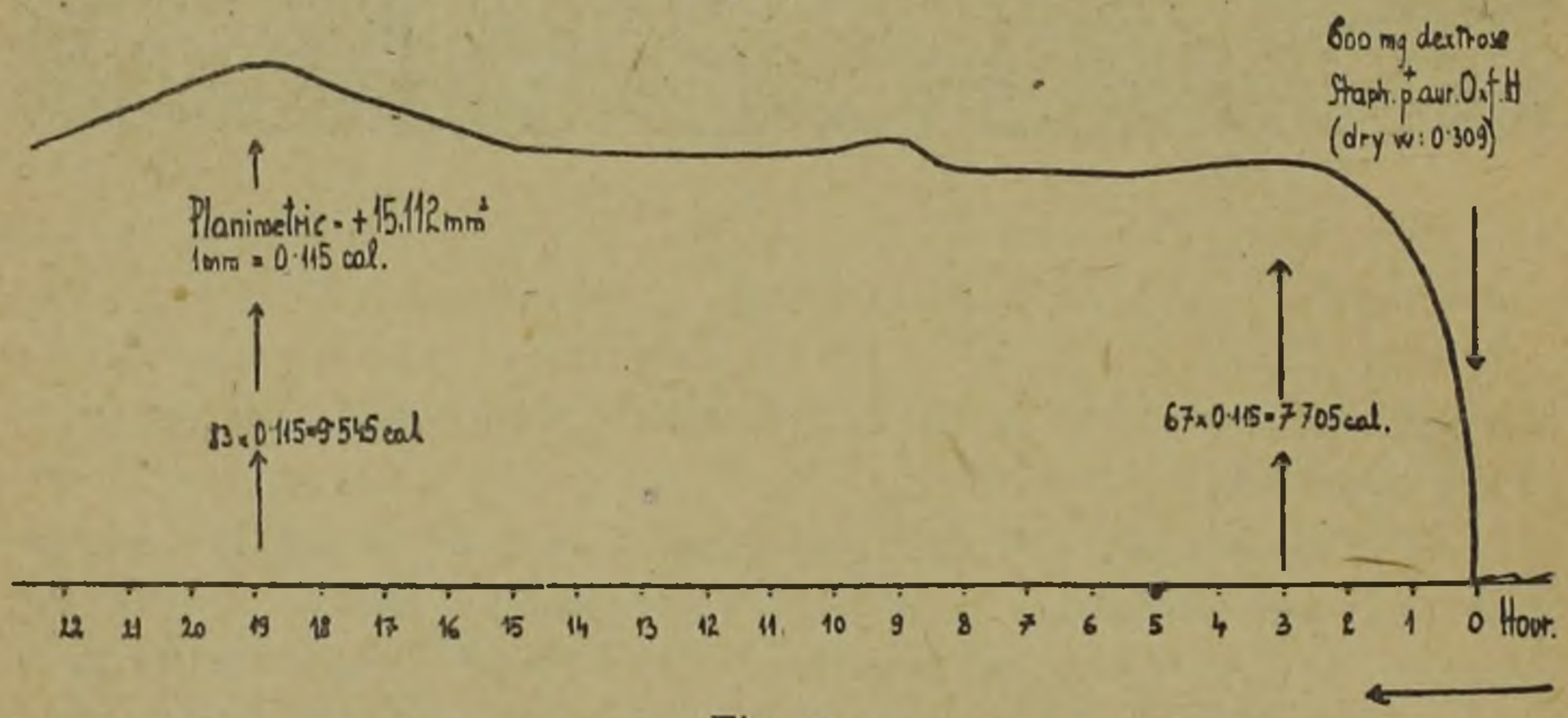

Fig. 3.

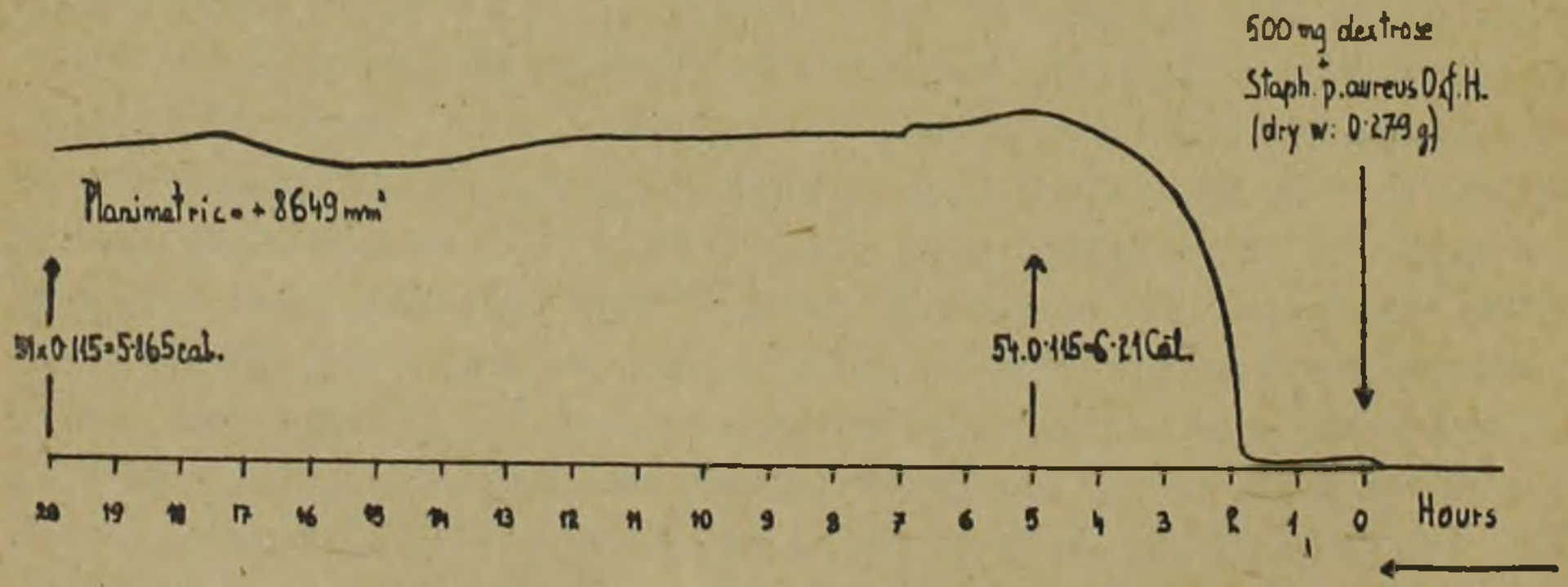

Fig. 4.

after a lag-phase of two hours during 20 hours somewhat less heat was produced, than in the previous experiment. Planimetrically: + 8649 mm² (Experiment Nr. 2. Figure Nr. 4.).

Upon the action of 1 mg penicillin the heat production in the presence of the same amount of dextrose somewhat decreased, planimetrically: + 6921 mm² (Experiment Nr. 3. Figure Nr. 7). In a similar subsequent experiment in the presence of 1 mg penicillin the heat-production was even as high as without penicillin, planimetrically: + 9873 mm². (Experiment Nr. 4. Figure Nr. 5.)

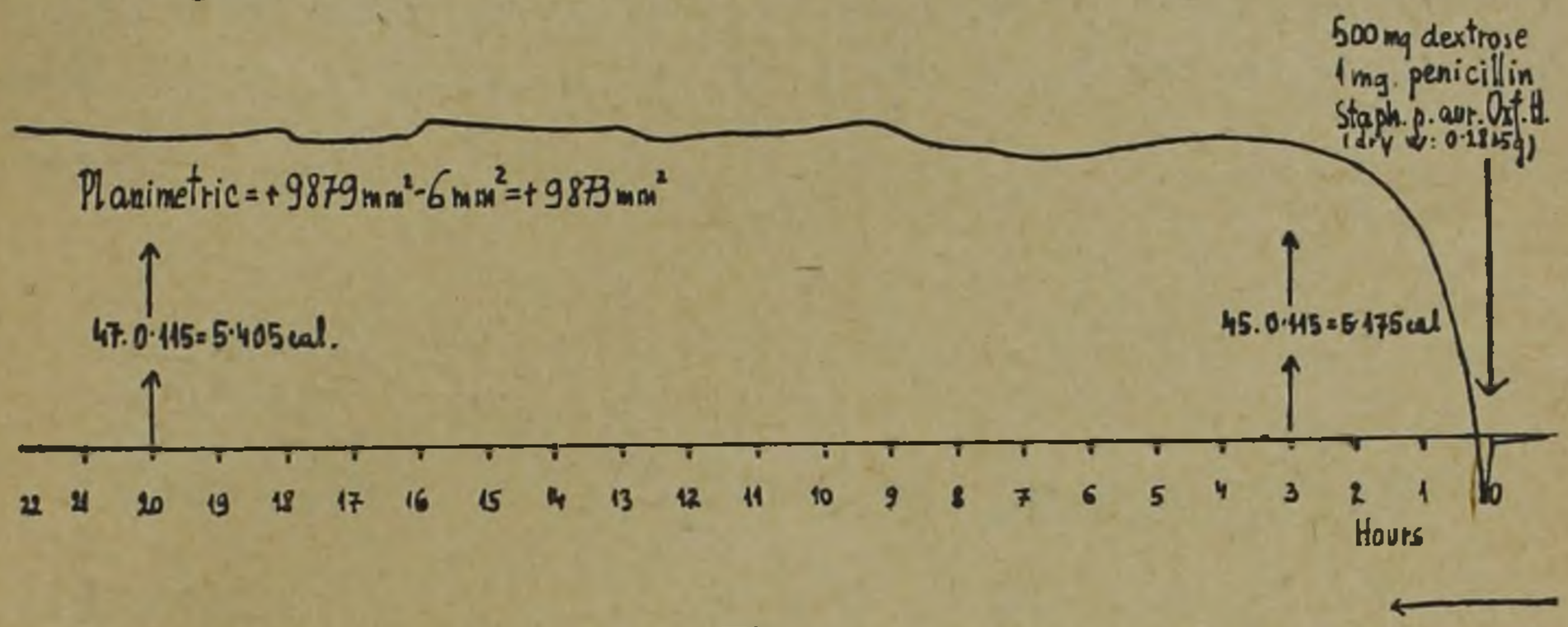

Fig. 5.

2 mg of penicillin in the presence of a comparatively greater inoculum (dry weight: 0,515 g) under otherwise similar conditions (500 mg dextrose) slightly yet perceptibly diminished the heat-production. Planimetrically: + 5872 mm². (Experiment Nr. 5. Figure Nr. 6.)

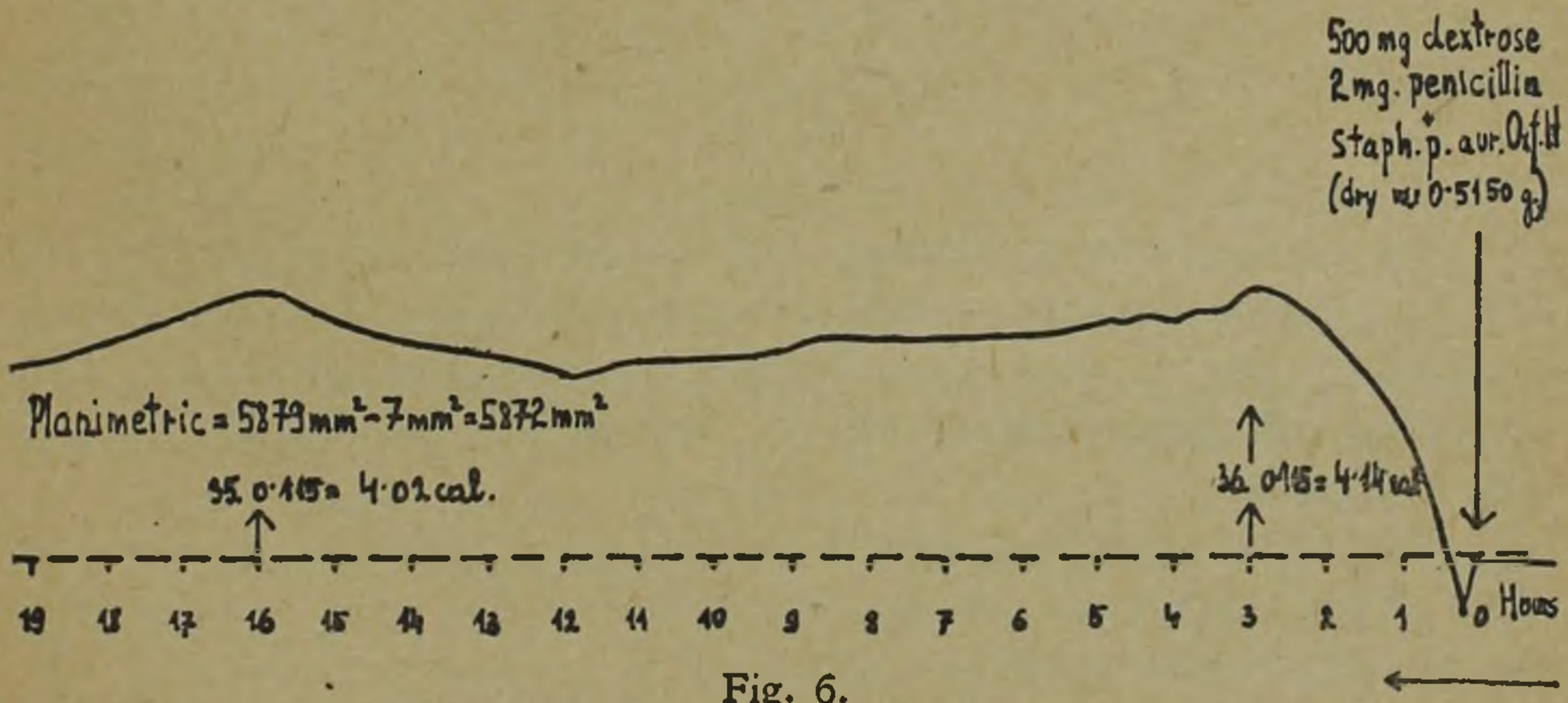

Fig. 6.

The action of 3 mg penicillin was not more expressed then that of 2 mg. penicillin. The heat production corresponded to + 5855 mm². (Experiment Nr. 6. Figure Nr. 7.)

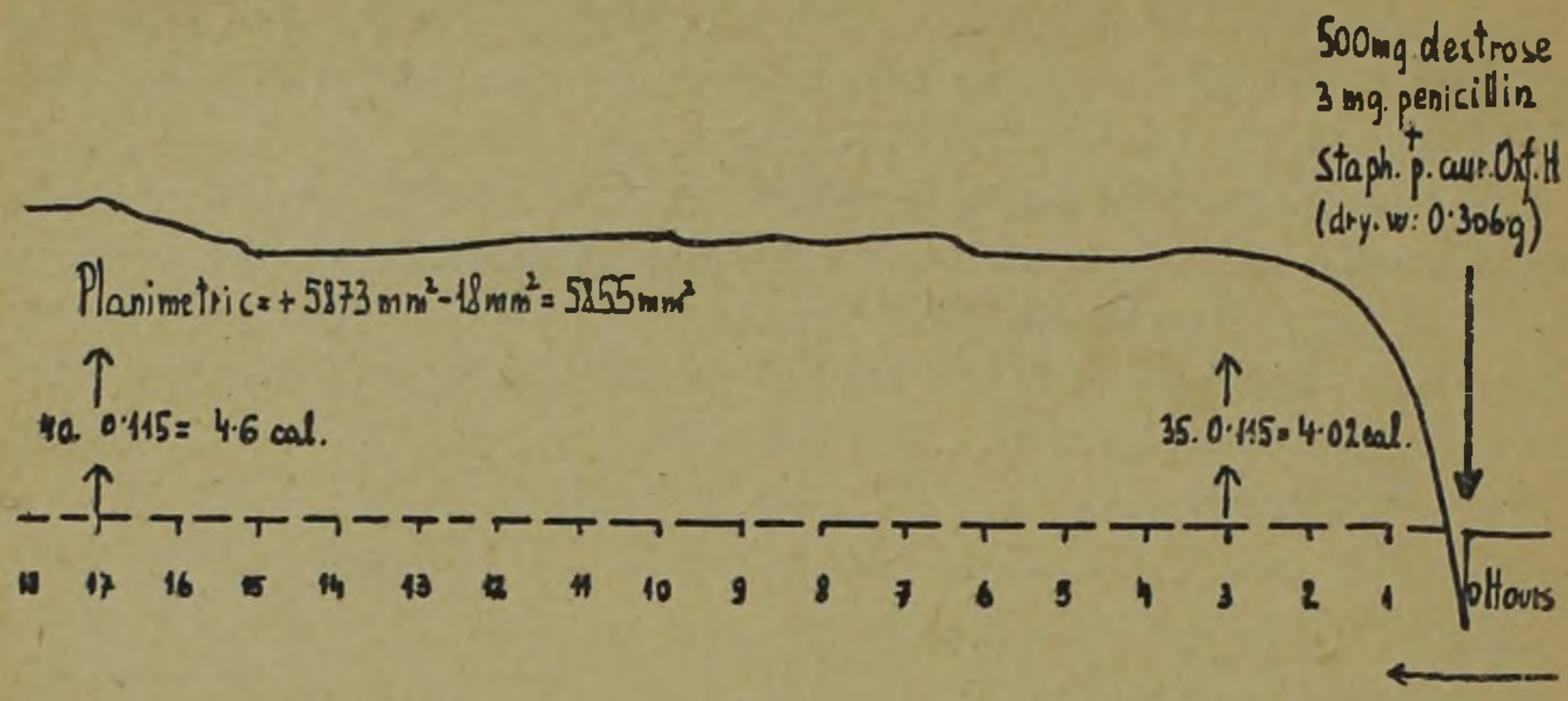

Fig. 7.

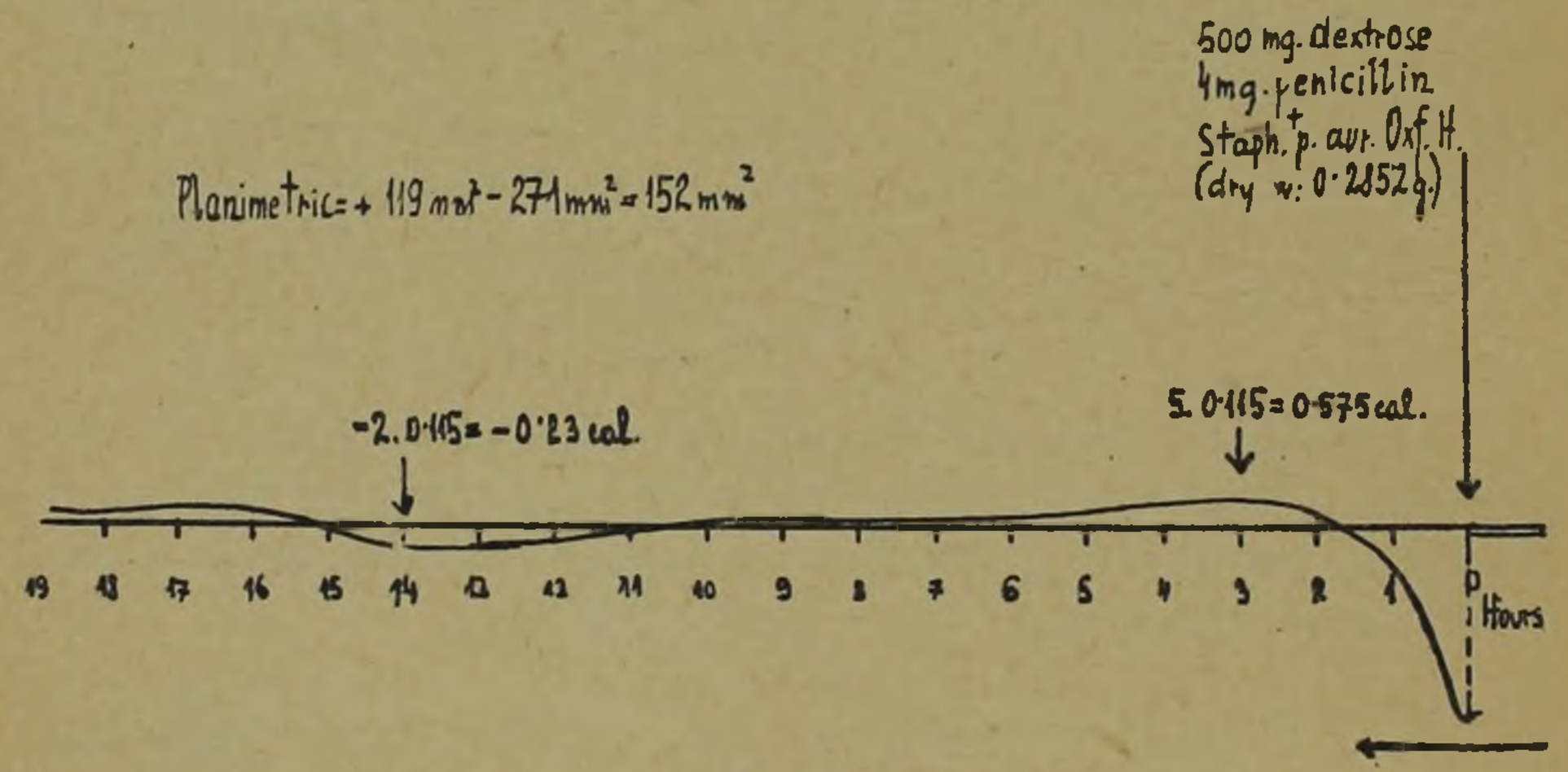

Fig. 8.

4 mg penicillin in the presence of the same amount of dextrose and of about the same quantity of staphylococci has presented a sudden change, a remarkable drop in the heat production, which became almost completly suspended. Planimetrically: only + 152 mm². (Experiment Nr. 7. Figure Nr. 8.)

The repetition of this experiment with 4 mg penicillin gave the same result. The endothermic processes prevailed and there was a considerable loss of heat. Planimetrically: — 952 mm².

5 mg penicillin suspended almost completly the heat production, too. Planimetrically: + 1035 mm².

The loss of heat was even more stressed in the presence of 6 mg penicillin. Planimerically: — 1423 mm². (Experiment Nr. 10. Figure Nr 9.)

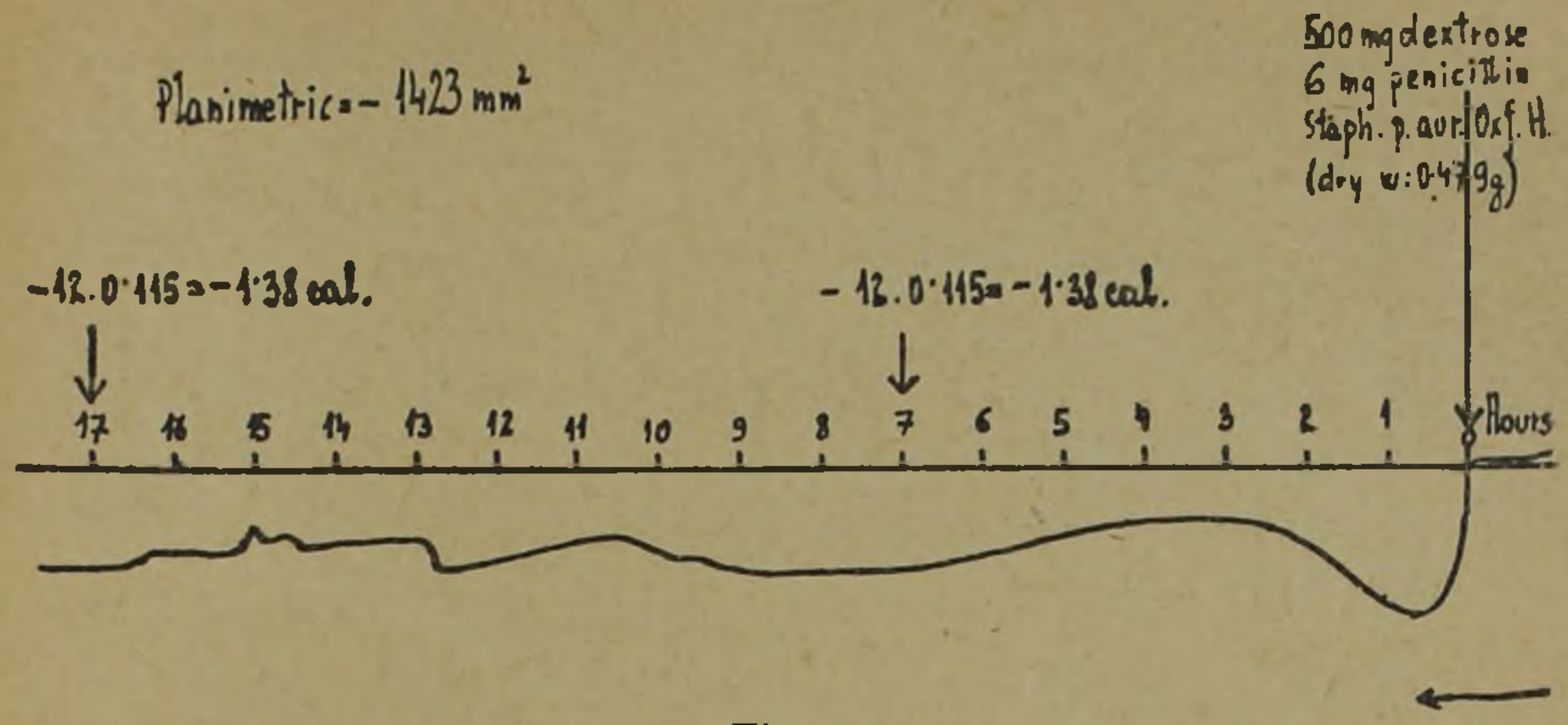

Fig. 9.

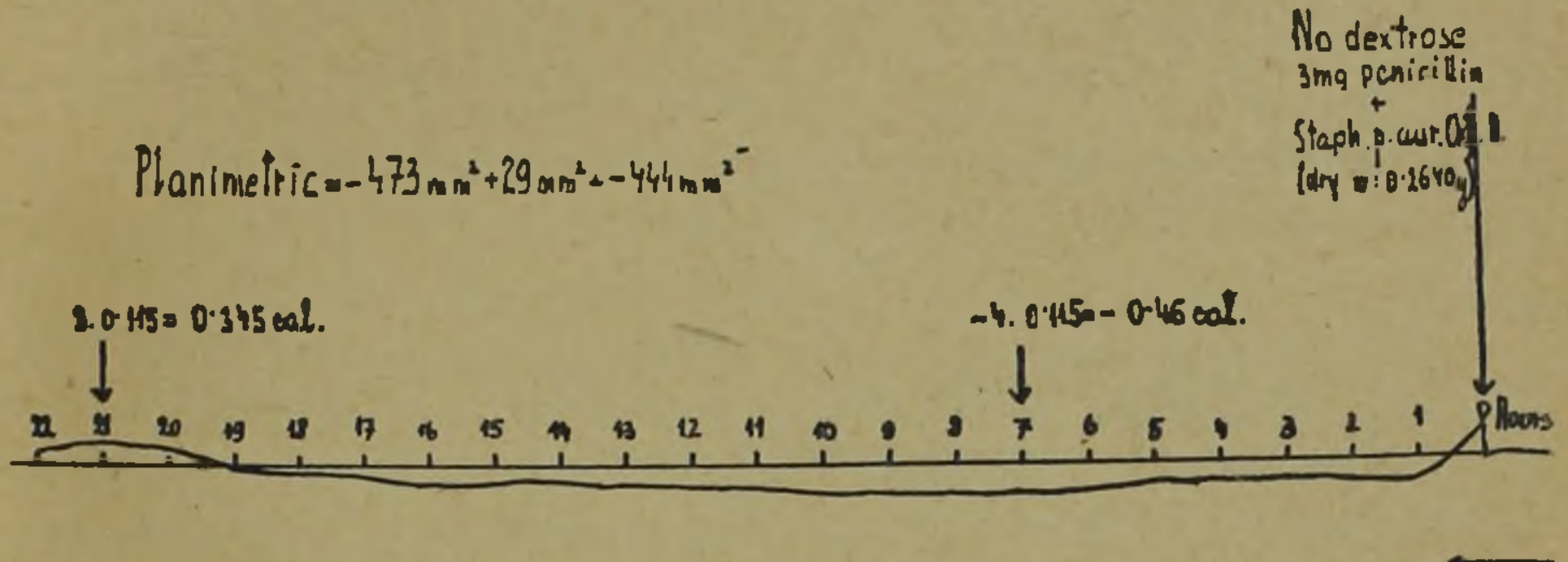

Fig. 10.

If dextrose is not present in the medium, penicillin (3 mg) alone produces also a loss of heat. Planimetrically: — 444 mm². (Experiment Nr. 11. Figure Nr. 10.)

If we regard the relation between the quantity of penicillin applied and the dry-weight of staphylococcus suspension used, we can seen that, as far as the application of penicillin amounts from 1 to 3 mg the heat production is very considerable, it is almost undisturbed. When the quantity of penicillin is elevated from 3 to 4 mg under otherwise similar conditions, the relativ quantity of heat production dropped suddenly from + 5855 to + 152 mm². The relation of penicillin to staphylococci was, at this point, higher than 10×10^{-3}. The endothermic processes became more expressed, if the quantity of penicillin was raised to 6 mg. (Table Nr. 1.)

TABLE 1.

Exper Nr	Dextrose mg	Penicillin mg	Dry weight of staphylococci mg	*Penicillin* / Staphylococci	Heat production estimated planimetrically in mm^2
3.	500	1	301	0,0030	+ 6291
4.	„	1	284	0,0034	+ 9873
5.	„	2	515	0,0038	+ 5872
6.	„	3	306	0,0098	+ 5855
7.	„	4	285	0,0140	+ 152
8.	„	4	399	0,0110	— 952
9.	„	5	413	0,0120	+ 1035
10.	„	6	479	0,0130	— 1423

DISCUSSION.

According to this it seems that the action of penicillin can be complete only, if a certain critical relation is established between the amount of penicillin and that of bacteria. This critical relation appears suddenly, at the very moment, when for each individual bacterial cell is a certain amount of penicillin free to exert an action. We may infer from this observation, that in the field of practical application it is very important, that the organism should be at the very beginning inundated with a sufficient amount of penicillin. We are safe to do it, because, in contrary to the chemotherapeutics, the penicillin does not exert any toxic effect on higher animals and men.

We have seen also, that if the action of penicillin is a complete one, in that case the process becomes endothermic. We have come to see an endothermic process also in that case, when only penicillin was applied to the staphylococcus suspension and no dextrose was added. According to the laws of bioenergetics biosyntheses are known as endothermic processes *(J. Lefévre* and *C. Oppenheimer)*. If the action of penicillin on the bacteria represents an endothermic process, we can infer from this, that the penicillin molecules or the two aminoacids (dimethylcystein and serin), components of the penicillin are incorporated, assimilated, at least transiently, by the bacteria into their own protein substances. These aminoacids, however, do not belong to the so called "natural" *l*-aminoacids, in the contrary they are *d*-aminoacids

and they represent the "not natural" series of aminoacids. For this reason theire assimilation can not be a definitive one. The transient nestling of thes *d*-aminoacids into the proteinstructure of the penicillin-sensitive bacteria disturbs the life-activity of the latter. Giant cells or long thread-like bacilli are formed, which are not capable of living. That may be the explanation for the bacteriostatic action of penicillin. Bacteria like these are soon to disintegrate. During this autolytic process the very same "unnatural" *d*-aminoacids can be liberated and are prepared for the repetition of the very same process. It is perhaps due to this possibility, that with comparatively small amounts of penicillin many sensitiv bacteria can be influenced.

Besides, the measurement of the heat production of the bacteria can be applied also for a quantitativ evaluation of new penicillin-preparats and other antibiotics.

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THE ACTIVE PRINCIPLE OF MUSCLE EXTRACTS INCREASING THE PERFORMANCE OF THE HYPODYNAMIC FROG'S HEART: ADENOSINETRIPHOSPHATE.

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(RECEIVED FOR PUBLICATION 18. 11. 48.)

INTRODUCTION.

In a previous communication (1) we have described the action of adenosinetriphosphate (ATP) on the quinine treated or *Ca*-deficient frog heart. The present paper deals with the analysis of a similar effect produced by boiled muscle extracts. These investigations have revealed: 1. that the ATP content of muscle extracts accounts quantitatively for the effect observed with muscle extracts; 2. that adenosinediphosphate (ADP) acts qualitatively and quantitatively like ATP; 3. that ATP (or ADP) is a regular constituent of actin solutions and 4. that ATP is never completely broken down in the minced tissues.

METHODS.

The contractions of the frogs isolated heart were recorded on a slow moving kymographion. The heart suspended on a Straub-canula was rendered hypodynamic by treatment with a quinine-Ringer solution, as previously described in detail (1). The effect of the muscle extracts was studied by adding the suitably diluted extract together with the same amount of quinine as was used to reduce the amplitude of the heart to 20—30% of the normal. Quantitative data were obtained by determining the greatest dilution which was still able to restore the normal amplitude of the quinine treated heart in spite of the continued presence of quinine to the normal level of amplitude, within a few minutes. In the case of ATP, 0,5 μg/ml is the greatest dilution giving still maximal response.

RESULTS.

1. *The effect of ADP on the quinine treated heart.* ADP was prepared enzymatically by the action of myokinase-free myosin on ATP.

Determination of labile *P* after 15 minutes incubation of 1 mg ATP with 10 mg myosin at room temperature has proved that all of the ATP was transformed. This point was further checked by studying the effect of the decomposed ATP solution on the viscosity of an actomyosin in 0,5 *M KCl*. As ADP does not decrease the viscosity of myokinase free actomyosin, whereas extremely small amounts of ATP do so (2), the presence of very minute amounts of ATP can be detected by this method. The preparation of ADP, used in our experiments gave no reaction with actomyosin, thus it was completely free of ATP.

In the frog heart test, ADP was just as effective in restoring the normal amplitude of the quinine treated heart as ATP. 0,5 μg ADP restored the normal amplitude of the hypodynamic heart.

When 1 mg ATP or 1 mg ADP were incubated with 10 mg myosin in 0,1 *M KCl*, and 0,001 *M* $MgSO_4$ at *pH* 7 in *presence of myokinase*, the solutions lost all activity after a period of 15 minutes at room temperature. The mixtures were deproteinized by the addition of trichloroacetic acid and the neutralized filtrates were added in suitable dilution (1 : 100 — 1 : 1000). All the activity could be observed when deproteinization was carried out before incubation, whereas no activity was found after 15 minutes incubation. As ATP and ADP are hydrolysed to adenylic acid in presence of myosin and myokinase, the above findings agree well with former observations (1) that adenylic acid is inactive in the frog heart test.

Effect of actin solutions. The discovery of the heart effect of ATP originated with our observation in 1945 that a dilute solution of actin increases the amplitude of the (*Ca*-deficient) hypodynamic frog heart. Actin contains 0,2% *Ca*, calculated on the basis of its protein content (3). We have tried to determine by the frog heart method of *Hastings et al* (4) the amount of ionised *Ca* present in actin solutions. We observed that actin solutions restored the normal amplitude of the *Ca*-deficient frog heart far above that expected from their total *Ca* content. We have next established the fact that ignition but not boiling destroys the factor responsible for this *Ca* like effect. A boiled actin solution (4 mg protein per ml) was effective in a dilution of 1 : 100 in restoring the normal amplitude of the heart when the *Ca* content of the Ringer solution used was only 15—20% of the normal. Such a dilution contained only 0,08 μg *Ca*/ml yet it was able to restore the normal amplitude of the heart washed with a Ringer solution containing 16 μg *Ca*/ml. In absence of the boiled actin solution, the addition of 25—50 μg *Ca*/ml would have been necessary to obtain a similar effect as with the diluted actin solution.

We have fractionated the active substance from actin solutions. ATP was isolated in good yield, when its original amount was calculated from the effect obtained in the frog heart test. The ATP content of actin was found to be about 1% calculated on the basis of the protein content. In another study it will be described that this ATP is the prosthetic group of actin.

Effect of muscle extracts. We have found that a boiled watery muscle extract has a similar action on the frog heart, as a boiled actin solution (1). Rabbit muscle was minced immediately after excision and then dropped into 2 volumes of boiling distilled water. The mixture was brought to boil and then cooled and filtered. The resulting clear yellow solution was able to restore the normal amplitude of the quinine treated isolated frog heart, when applied in a 1000—1500 fold dilution. This agrees well with the possibility that the active substance of the muscle extract is ATP. The ATP content of rabbit muscle is of the order of 2,5—3,0 mg/g wet tissue. In the boiled muscle juice therefore, 1 mg ATP/ml is expected.

One observation, however, did not agree with the known properties of ATP. When rabbit muscle, kidney, liver or heart tissue was minced and left to stand at room temperature for various length of time and aliquots extracted with boiling water as described above, the activity of the resulting solutions showed a decrease of activity in the frog heart test, as shown in Fig. 1. Similar results were obtained with horse muscle and bullock's heart tissue. In all these different organs 10-40 % of the

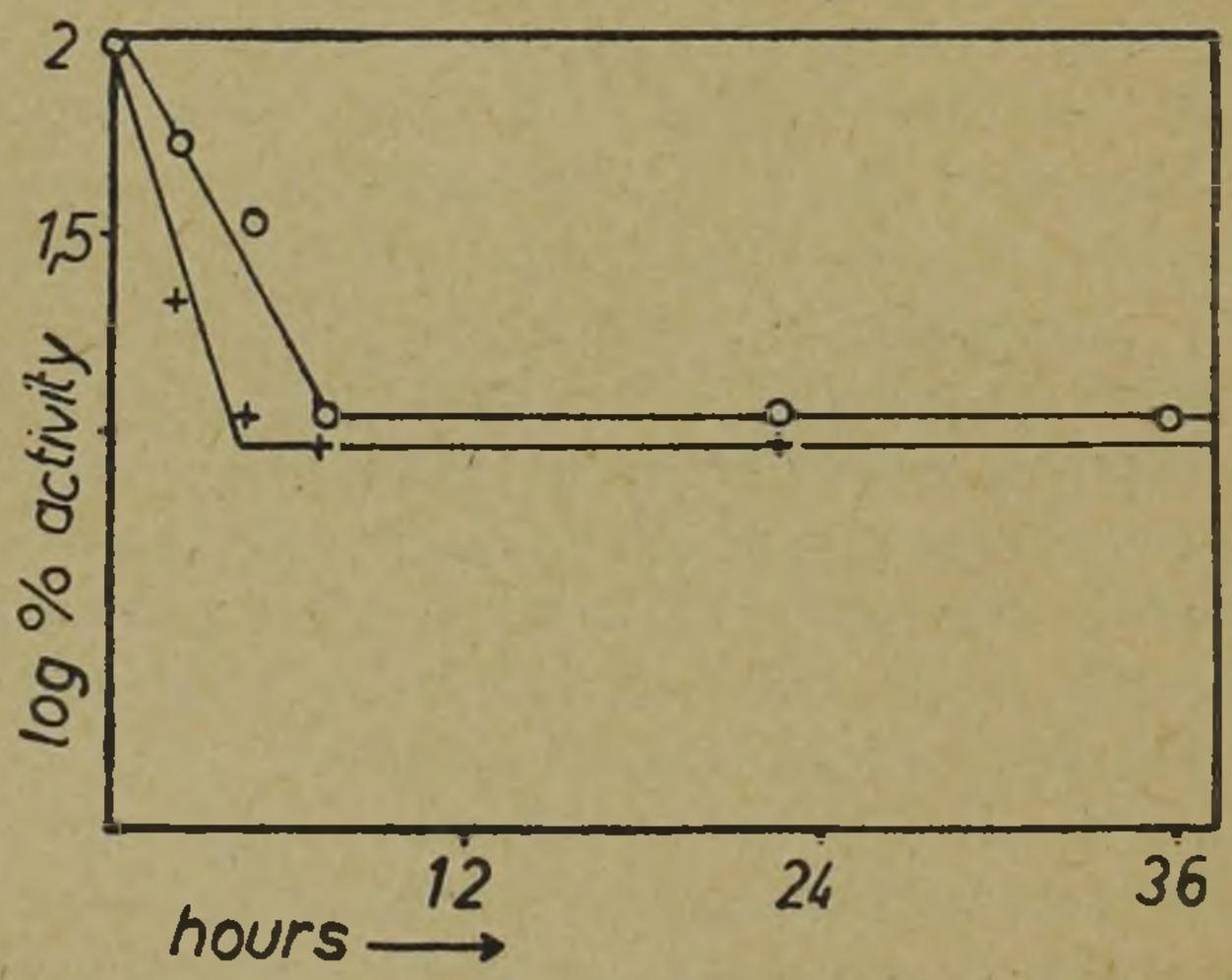

Fig. 1. Destruction of ATP and ADP in minced rabbit muscle, stored at room temperature. The activity of extracts prepared from the muscle at any given time is a measure of the combined ATP and ADP content. The ordinate represents the activity of the extracts on a logarithmic scale. The activity found in freshly excised muscle was taken as 100%.

original activity remained unaltered even after 3—5 days of storage, although the breakdown of ATP obviously came to an end after 6—8 hours at room temperature.

The possibility had to be considered, that there is another substance present in the muscle tissue, having identical effect on the frog heart as ATP, but which is not enzymatically split by the tissue enzymes. In support of this consideration we have found the residual activity mostly bound to the muscle tissue, several washings with water did not remove considerable amount of activity.

As shown below, this is however not the case. Instead, we find that a portion of the ATP present in the muscle tissue is strongly bound to proteins and is not decomposed by the adenosinetriphosphatase system.

We have isolated the active principle from horse muscle tissue in which the breakdown of ATP was apparently completed and which contained only the "residual activity", some 10% of that originally present. The tissue was taken from the slaughter house 10—48 hours after the animal was killed and no precautions were taken to keep it at low temperature. The activity of the extracts prepared from such material does not diminish any more if the tissue is kept at room temperature for some period.

2,5 kg of such muscle tissue were minced and suspended in 25 litres of tap water, stirred for 15 minutes and pressed through cheese cloth. The washing was repeated two more times. The washed mince was dropped into 2 volumes of boiling distilled water. The mixture was brought to a boil and then, when still hot, filtered through several layers of cheese cloth and left to drain by its own weight. The resulting fluid was pale yellow and water clear, when the drainage was slow and the boiled muscle debris acted as a filter.

To this filtrate were added 10 g $CaCl_2$ per liter and enough concentrated NH_3 to make it alkaline to phenolphtalein. The resulting precipitate was centrifuged off and dissolved by the addition of concentrated *HCl*. Enough *HCl* was added to reach a *pH* of 1. The addition of 15 ml 10% Na_2WO_4 brought down a heavy protein precipitate, which was centrifuged off and discarded. The clear yellow solution was again brought to *pH* 8,5 by the addition of NH_3 and the precipitate, containing the active principle centrifuged off. This was partially dissolved by the addition of *HCl* until the solution was acidified to *pH* 4. The volume of this fraction was 66 ml. The addition of one volume of acetone precipitated the active substance. This precipitate was removed and the fluid discarded. The precipitate was again dissolved by the addition of *HCl*, this time the *pH* was brought to 1,5. The acidified solution (26 ml)

was cooled to 0 C° and 1 volume of cold acetone was added. The resulting precipitate was discarded and 3 more volumes of cold acetone were added. The active principle was now found in the precipitate, which was removed in the centrifuge, washed with acetone several times and then dried in a dessicator. From the 2,5 kg muscle tissue 90 mg of a white airdried product was obtained.

23% of the activity present in the boiled extract was found in the final fraction. When compared in the frog heart test with a *Ca* salt of ATP, the isolated substance proved to be 40—50% as active as ATP, i. e. it was able to restore the normal amplitude of the quinine treated frog heart in a concentration of 1,0—1,5 μ g/ml.

The isolated substance showed the following composition: Adenin content (spectrophotometrically determined): 30,3%; ribose content: 34%; total *P* content: 10,5%, inorganic *P*: 1,15%, inorganic *P* after 30 minutes hydrolysis in 0,25 *N* H_2SO_4 at 100 C°: 4,2%. Ratio of hydrolysable *P* to total organic *P*: 1 : 3,06. The rate of hydrolysis in 0,25 *N* H_2SO_4 at 100 C° was exactly identical with the rate of hydrolysis of pyrophosphate under similar conditions.

The absorption spectrum of the substance is identical with that of adenine.

The high ribose and adenine content and the ratio of hydrolysable *P* to total *P* suggest the substance to be a mixture of adenylic acid, adenosinediphosphate and possibly adenosinetriphosphate. Further resolution of this mixture was not necessary from our point of view, as it is easily shown that the activity shown by this substance in the heart test is entirely due to its ADP and ATP content.

When the isolated substance (2 mg) was incubated with myosin and myokinase for 15 minutes at room temperature, inorganic phosphate was split off and the substance became inactive in the frog heart test.

It is therefore clear that the activity of the isolated substance is due to ADP. ADP is thus present in muscle tissue after prolonged storage. It can be estimated that stored horse muscle tissue contains 0,2—0,3 mg ADP/g wet tissue.

Significance of the presence of ADP not split by the adenosinetriphosphatase system. The question naturally arises as to the mechanism through which a seemingly constant part of ADP escapes destruction after the death of the cells.

One way of explaining the situation is to suppose that this part of ADP is bound to protein, notably to the protein of actin in case of muscle tissue.

Another possibility is to suppose that some modification of ADP different from that isolated and not susceptible to enzymic breakdown is present in the tissues. In this case it must be further assumed that the hypothetical modified ADP is transformed into the known modification of ADP during the process of isolation.

The existence of protein-bound ADP naturally is of very great importance. As ADP is regarded to be an energy reservoir of the tissues, its presence in a bound form in a protein concerned in muscle contraction may indicate the mechanism by which the chemical energy is directed into the contractile matter.

Similarly the presence of protein-bound APD in other organs may point to the proteins in those organs which take part in specific energy consuming processes (e. g. excretion and resorption in kidney tissue, etc.).

Further experiments to elucidate these problems are now in progress.

SUMMARY.

The active substance in muscle extracts and actin solutions, which restores the normal function of the quinine treated isolated frog heart, is identical with ATP.

ADP in the tissues is never completely broken down. A constant % of the total ATP of skeletal muscle, heart muscle, liver and kidney is never split by the tissue enzymes even on prolonged standing. This fraction is probably bound to proteins as ADP.

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THE EFFECT OF ACTIN AND PHYSICO-CHEMICAL CHANGES ON THE MYOSIN ATP-ASE SYSTEM, AND ON WASHED MUSCLE.

WITH 8 FIGURES IN TEXT.

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(RECEIVED FOR PUBLICATION 19. 11. 1948.)

According to the work of *Engelhardt* and *Lyubimova* (1) it seems most probable that myosin, the protein which takes an essential part in muscular contraction, is identical with the ATP hydrolysing enzyme of the muscle. Whether the myosin itself is the enzyme or a very good absorbent of it only (Kalckar, 2), the work of the Russian authors makes it possible to approach the relation between physico-chemical state and enzymic activity of this protein.

The ATP-ase property of myosin has been investigated by many authors. It was shown that *Ca* is a powerful activator of the system (Banga (3a), Bailey (7), Singher and Meister (8) etc.).

Szent-Györgyi and coworkers were able to separate "myosin" into two components: actin and myosin (3/b, c, 4). Actomyosin prepared from myosin and actin shows a very strong dehydratation under the effect of ATP in low *KCl* concentration: (*A. Szent-Györgyi* 3/d, 4, 5). This dehydratation may appear in different forms, depending on the physical state of actomyosin. If an actomyosin solution is squirred into distilled water through a narrow capillary, a thread is formed, which, in adequate salt solution, after addition of ATP, shortens violently in all directions: "contraction". In the form of a suspension "superprecipitation" takes place (*A. Szent-Györgyi:* 3/d, 4, 5). At higher *KCl* concentration the viscosity of the actomyosin, — which is now dissolved, falls on addition of ATP (*A. Szent-Györgyi:* 3/c, 4, 5: *Needham* and coworkers; 6). The effect of ATP on actomyosin is extremely specific. (Buchthal, Deutsch and Knappeis: 9).

Banga and *A. Szent-Györgyi* (3/f, 4) found that the addition of actin alters the behaviour of myosin-ATP-ase towards $MgCl_2$, i. e. $MgCl_2$ decreases the ATP-ase activity of myosin, and increases that of the acto-

myosin in low *KCl* concentration, where the actomyosin shows a strong superprecipitation.

It is very probable that the ATP is closely connected with muscular contraction, it may represent the immediate source of energy of it.

The aim of the present experiments was to investigate in what way the addition of actin and the physico-chemical changes of myosin alter the ATP-ase properties of myosin and if these changes are also to be found in the minced, washed muscle, which contains similarly acto-myosin and in which the physico-chemical state of myosin is unknown.

We measured the enzymatic activity of myosin, actomyosin, and washed muscle at different *KCl*, ATP-concentration, temperature and *pH*.

METHODS.

Muscle. Rabbit muscle was used as material. The animal was decapitated, rapidly eviscerated and skinned. After through cooling (cca. 25 min. in ice-water) the deep back muscles were cut, minced and washed twice by suspending it in 10 vols. of distilled water, all this being done at O°C.

Actin-free myosin (3/g, 4), *actin* (3/c, h, 4), *myosin B* (3/e, 4) and the ATP (5) were prepared according to the standard methods of our Institute.

The composition of reaction mixtures. In 3 ml final volume 0,6 ml, *M*/35, veronal acetate-buffer of *pH* 7 (if not otherwise stated), *KCl* to reach the concentration desired, enzyme and the ATP-solution (containing 10—12 mg ATP per ml)were added.

The ATP was neutralised with *KOH* to *pH* 7. The potassium salt introduced with the buffer and ATP was taken into consideration in the calculation of the final *K* ion concentration. Buffer of 0 C° was added to the cooled myosin solution and thus it was transferred into the cooled reaction mixture. In case of actomyosin the cooled *F*-actin was added to the myosin at 0 C°. The further procedure was quite the same as with myosin. Measurements of *pH*, where mentioned in the text, were carried out with the hydrogen electrode.

The reactions took place in test tubes. The mixtures containing all the components except ATP, were incubated in a water-bath of desired temperature to reach temperature equilibrum. In the same time the ATP, in a separate tube, was incubated also. (The temperature was maintained with a *Hoeppler*-ultrathermostat and controlled to ± 0,2°.) The reaction was started by adding the ATP, and was stopped by adding 3 ml 3,3% trichloreacetic acid.

The concentration of myosin was determined viscosimetrically, according to the experimental curve of *Straub* and *Guba* (3/h, 4), the content of actin solutions by dry weight measurement.

The same amount of myosin was always used in the experiments when the ATP-ase activity of myosin and actomyosin were compared; in case of actomyosin, actin was added separately.

In their earlier works, *Szent-Györgyi* and coworkers termed the drop of viscosity, caused by ATP, on myosin *B*, as "100% activity". This actomyosin is called a "100%active actomyosin". After the islolation of actin it turned out that the maximal drop of viscosity observable on actomyosin, prepared from pure myosin and actin, is greater than that of myosin *B*. With the above mentioned definition maximal activity is about 170%. In the 170% active actomyosin, — as well as approximatively in the muscle, — the ratio actin to myosin is 2 : 5 (3/k, 1, 4). In what follows by "170% active actomyosin" we mean an actomyosin with a ratio of actin to myosin 2 : 5. In those experiments in which the actin content of actomyosin was varied in addition to the amount (in mgs), of actin and myosin present, the viscosimetrical determinations are given also, expressed as % activity.

The ATP-concentration was determined by the easily hydrolysable phosphorus, P_7. Free phosphorus was estimated in 2 ml of the 6 ml trichloracetic acid filtrate according to the modified method of *Fiske—Subarrow* with *Pulfrich* photometer. The splitting of ATP was calculated from the increase in free phosphorus.

So much of ATP was added that the phosphorus split off should be less than 25% of the 7 min. *P* in case of myosin resp. actomyosin and less than half of the 7 min. *P* in case of washed muscle.

The superprecipitation of actomyosin was measured semiquantitatively. It was observed separately in small test tubes, or during the enzyme experiments (which were carried out in a thermostat having glass windows). The observations of superprecipitation were made according to the method of our Institute (5; p. 87, 93).

The "contraction" of washed muscle was determined as it is described by *L. Varga* (10) in a reaction mixture of the same composition as used for the determinations of enzyme activity. The degree of "contraction" was determined after 5 minutes under the microscope. The "contracted" and "relaxed" state of washed muscle fibers were well observable after some experience and the results were constant and reproducible.

RESULTS.

1. THE PHYSICO-CHEMICAL STATE OF ACTOMYOSIN.

A 170% active actomyosin, at 20 C°, in 0,1 *M KCl*, at *pH* 7, is insoluble. If to such a precipitated actomyosin ATP is added in a medium concentration (not over 0,007 *M*) the actomyosin dehydrates strongly, and settles to a small volume. This phenomenon will be called "superprecipitation" (3/g, 4, 5).

If we raise the concentration of *KCl*, the actomyosin precipitate will become more and more hydrated and will finally dissolve. On addition of ATP the precipitation will gradually be less marked. If to the dissolved actomyosin ATP is added, either a further clearing up will take place, or nothing will occur. In the latter case the well known decrease of viscosity (3/b, 4, 5) will point to the dissociation of actomyosin.

In what follows the superprecipitation and the dissolution (connected with the dissociation) will be considered as two different states of the actomyosin-ATP-system.

It is not only by altering the concentration of *KCl* that this actomyosin-ATP-system (actomyosin of 170% activity; 20 C°; 0,1 *M KCl*;

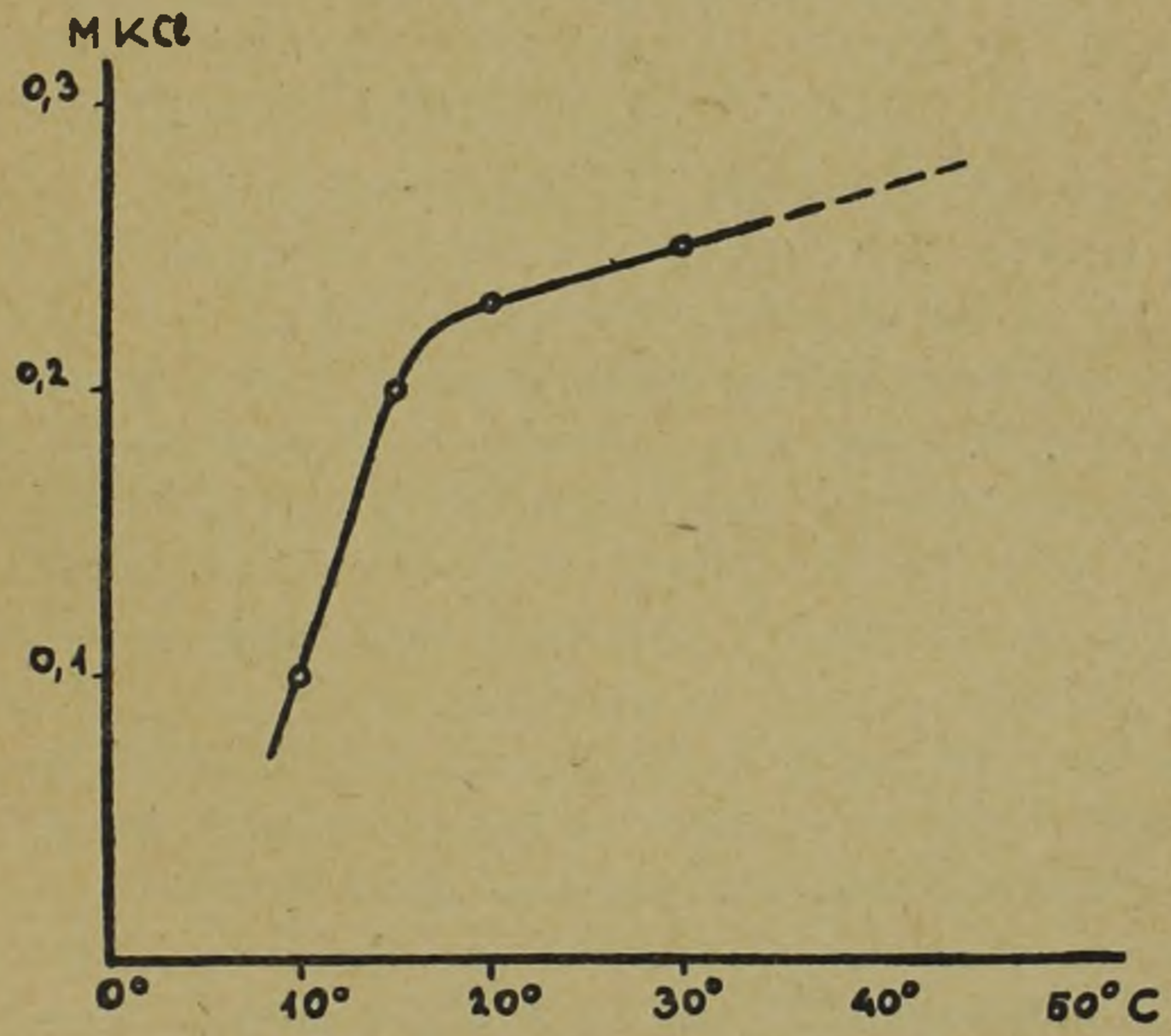

Fig. 1.

Superprecipitation of actomyosin depending on temperature and KCl-concentration.

2,66 mg myosin and 0,53 actin per ml. (A 100% active actomyosin).
0,06 *M* veronal-acetate buffer, pH 7; 0,6 m*M* ATP.

The points represent the *KCl*-concentrations at which the superprecipitation begins at any given temperature.

pH 7; ATP-concentration less than 0,007 *M*) can be transferred from the superprecipitated state to the dissolved one.

Dissolution will occur if anyone of these factors will be suitably varied. Thus dissolution, or at least a substantial diminution of superprecipitation can be attained:

1. By significant decrease of the actin-content.
2. By lowering of temperature (e. g. to 15 C°).
3. By increasing the *KCl*-concentration (e. g. over 0,20—0,23 M).
4. By increasing the *pH* (with approximately 1 unit).
5. By increasing the ATP-concentration (over 0,007 *M*).

These factors varied separately, the results obtained are shown in the lower diagrams of figg. 3, 5, and 6, representing the superprecipitation. (See below.) More factors varied at the same time will have an additive effect. Only a few of these more complicated cases have been studied. Such a case is represented in fig. 1, where the effect of simultaneous variation of temperature and *KCl*-concentration is shown. With increasing temperature the zone of superprecipitation becomes more extended towards the higher concentrations of *KCl*.

A similar double effect can be seen in the diagram of superprecipitation of fig. 5 (temperature and ATP-concentration).

2. ATP-ASE ACTIVITY OF MYOSIN AND ACTOMYOSIN.

I. Banga (3/a) found the ATP-ase activity of actomyosin to depend greatly on whether it is superprecipitated or dissolved. Further study of this phenomenon has revealed factors which definitely alter the ATP-ase activity of actomyosin having no influence on that of actin-free myosin.

The enzyme activity of actomyosin, if superprecipitated, is far greater than that of the myosin it contains. If actomyosin is shifted from superprecipitation to dissolution, its rate of ATP-splitting will shift towards that of myosin. (At the same time actomyosin dissociates to actin and myosin.)

This basic difference has been studied by changing the conditions which control the physico-chemical state of actomyosin and by determining the corresponding change of ATP-ase activity.

a) *Variation of actin content.* If increasing amounts of actin are added to myosin, the ATP-ase activity will increase rapidly. (20 C°; 0,1 *M KCl*; 0,0038 *M* ATP; *pH* 6,8.) (Fig. 2.)

It is well known that the actin-free myosin cannot be superprecipitated. The degree of superprecipitation of actomyosin is — to a certain extent —, proportional to its actin content.

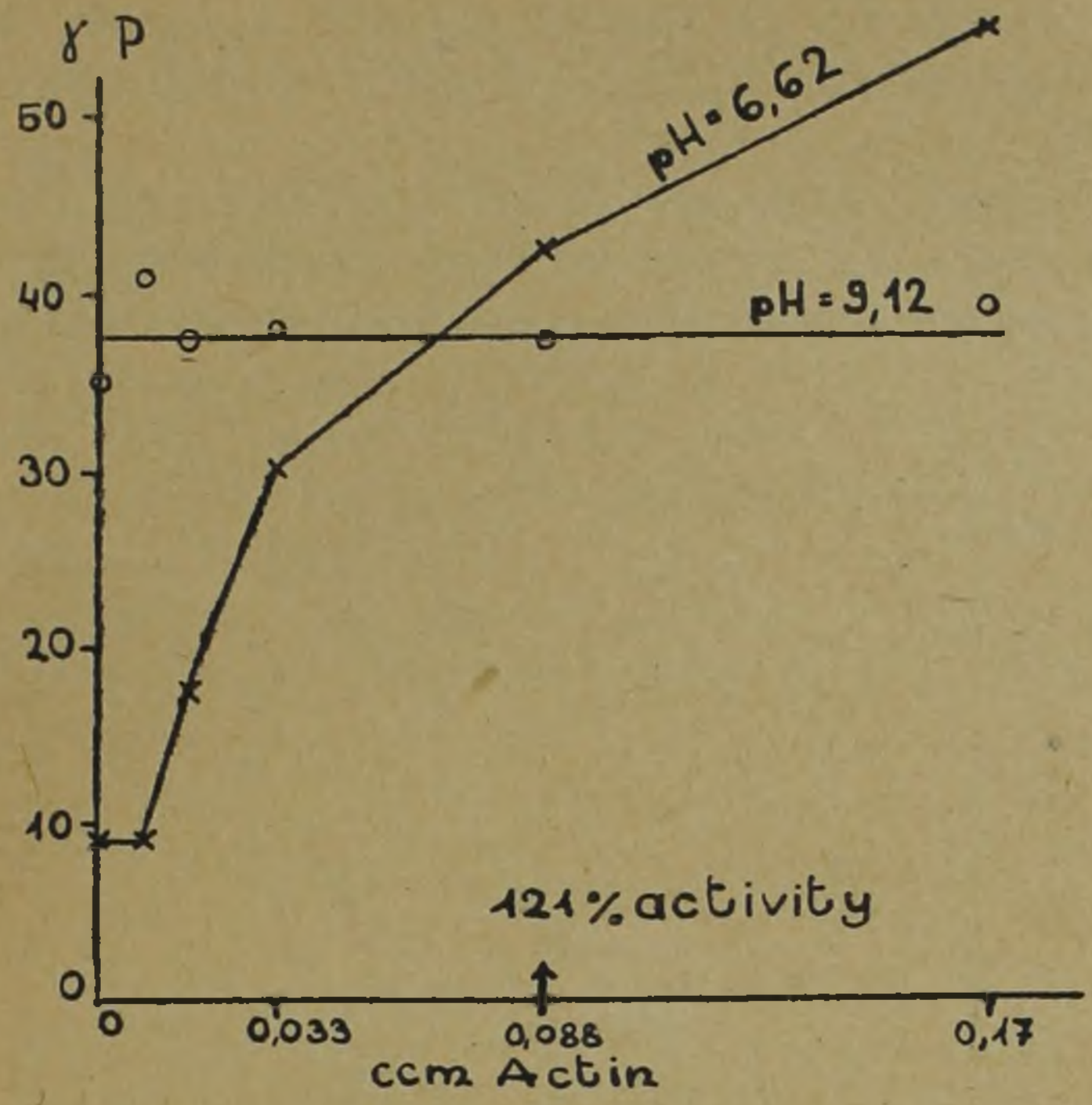

Fig. 2.

ATP-ase activity of actomyosine of different actin content at two different pH-s.

1,9 mg myosin, different amounts of actin.
0,1 *M* KCl; — 3,8 m*M* ATP; — a mixture of veronal acetate (0,012 *M*) and glycine (0,017 *M*) buffers
Abscissa : ml -s of actin-solution given to 1 ml of reaction mixture (to 1,9 mg myosin[1]).
Ordinata : γ phosphorus liberated in 5 min.

At alkaline *pH* values (*pH*=9·12) the addition of actin has no effect (fig. 2). According to viscosity measurements at this *pH* and *KCl* concentration, actin and myosin are entirely dissociated: on addition of ATP no decrease of viscosity is observed.

b) *Specificity of the effect of actin.* This effect of actin cannot be attributed to its *Ca* content. According to *Straub* and coworkers (11) there is in actin 0,2% *Ca* on dry weight basis, strongly bound to protein. In the trichloracetic acid filtrate of actin no *Ca* could be found. Supposing the present *Ca*, in spite of being bound to actin, would affect the myosin, the total concentration of *Ca* will not exced, with the actin given, 4.10^{-5} *M*. In such a dilution *Ca* has no effect on the ATP-ase activity of myosin.

Actin alone does not split ATP at all.

[1] At the composition of 0,083 ml actin per ml we observed viscosimetrically an activity of 120%.

Boiled actin did not increase the ATP-ase activity of myosin. Neither did glycogen, fibrinogen, egg-albumin, serum-globulin, myokinase and cystein.

c) *Temperature.* On addition of ATP (0,001—0,006 *M*), in the *KCl*-concentrations employed, at *pH* 7 and 0 C°, the actomyosin dissolves. With the increase of temperature it will shift gradually to superprecipi-

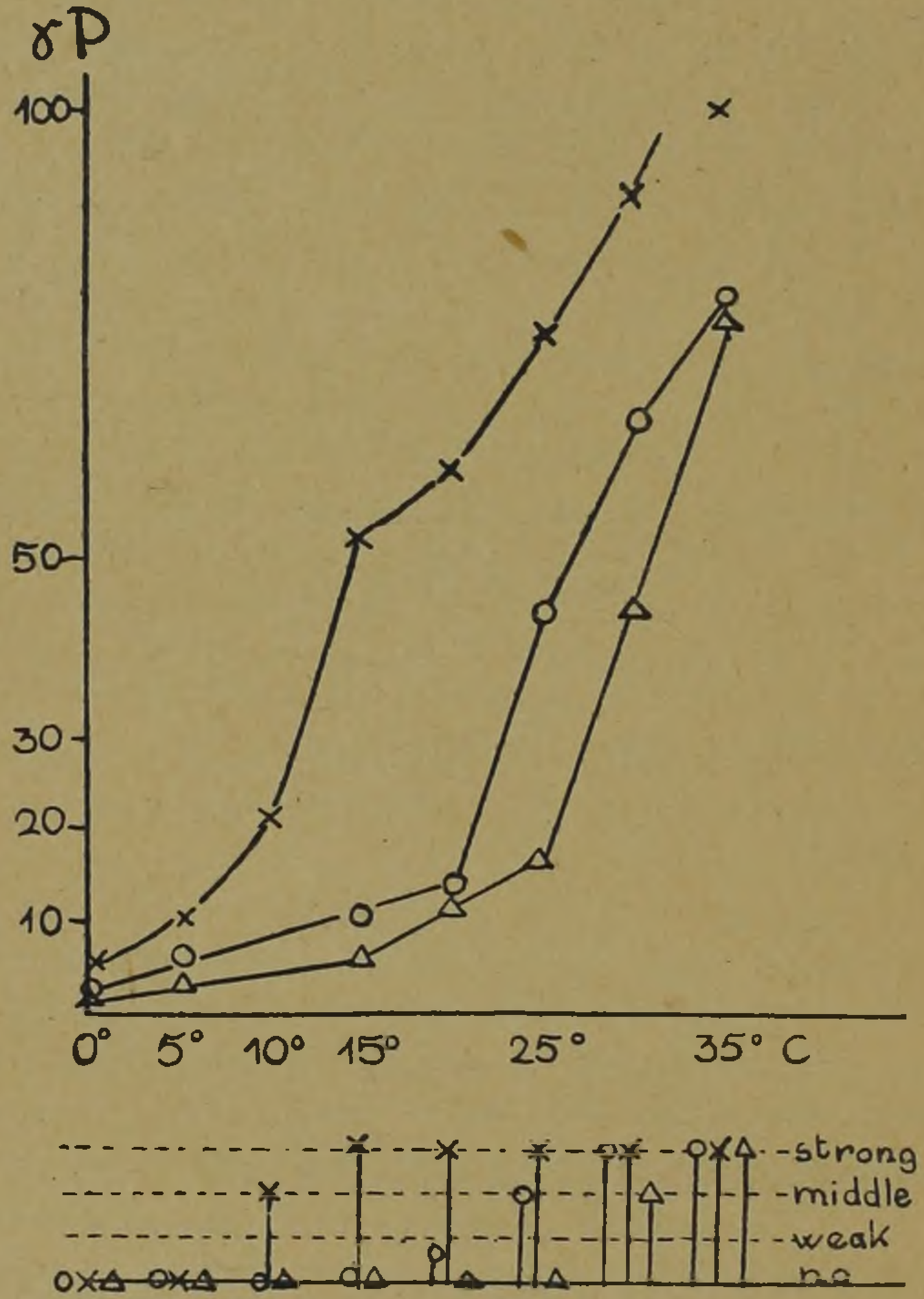

Fig. 3.

ATP-ase activity of actomyosin depending on temperature at different concentrations of KCl.

2,2 mg myosin and 0,53 mg actin (a 100% active actomyosin).
0,006 *M* veronal-acetate buffer of pH 7; — 7,6 m*M* ATP.

Abscissa: temperature in C°.
Ordinata: γ phosphorus split in 5 min.

×—×: 0,1 *M KCl* o—o: 0,2 *M KCl* ◁—▷: 0,23 *M KCl*

The diagram below shows the degree of superprecipitation: the four levels corresponding to: no, weak, middle and maximal superprecipitation.

tation. The greater the concentration of *KCl*, the higher will be the temperature at which the transition takes place. (See fig. 1.)

The temperature dependence of the ATP-splitting of actomyosin at different concentrations of *KCl* (*pH* 7; about 0,006 *M* ATP) showed the general exponential form of enzyme reactions. It is striking however that the substantial rise of splitting falls at each *KCl*-concentration exactly in the same interval of temperature in which the superprecipitation begins. (Fig. 3.) This is well noticeable with help of the lower part of the digrams, where our simultaneous observations concerning the superprecipitation are presented.

The temperature dependence of ATP-ase activity of myosin — on the other hand —, is entirely independent from the concentration of

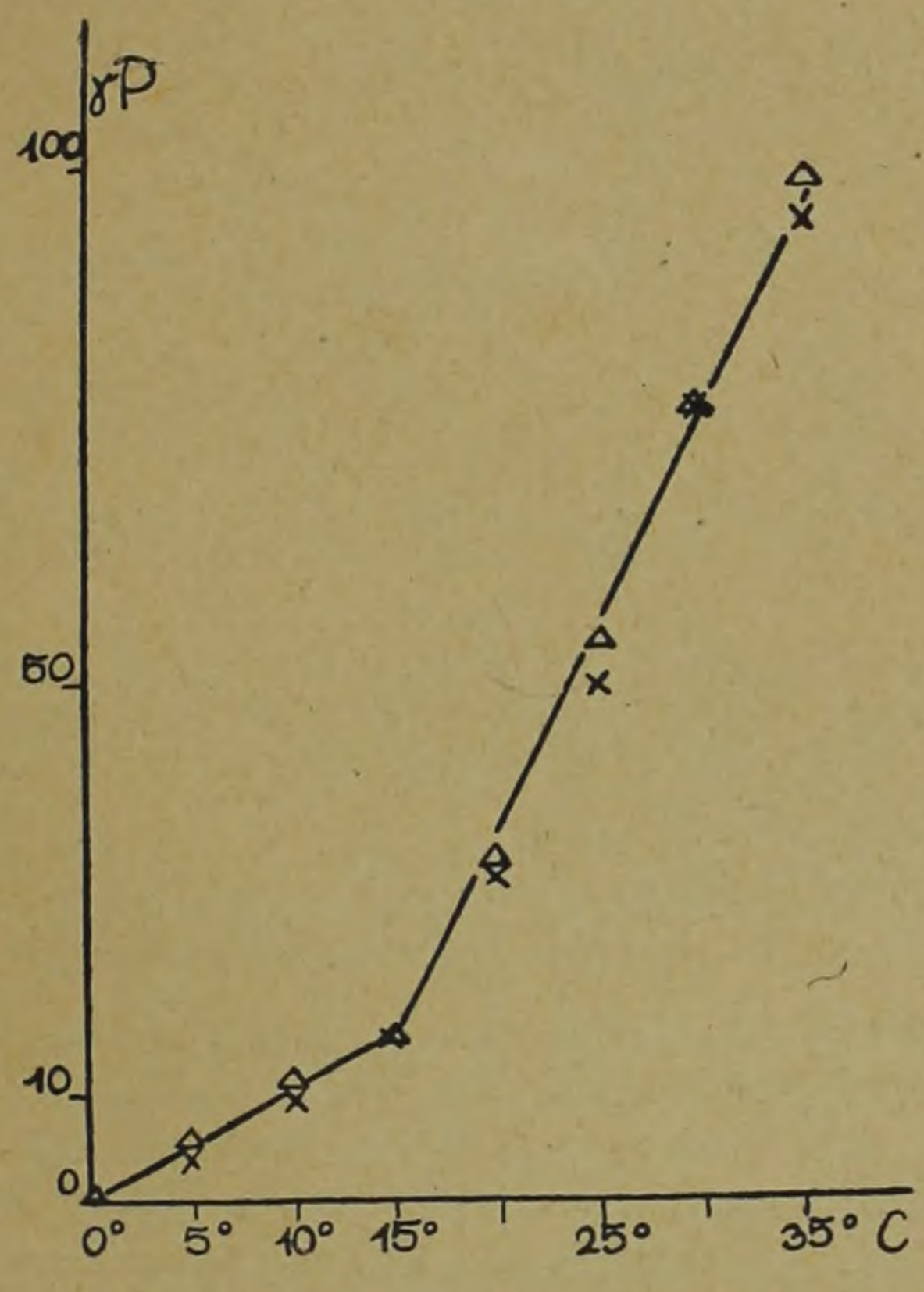

Fig. 4.

ATP-ase activity of myosin depending on temperature at different concentrations of KCl.

2,66 mg myosin.
0,066 *M* veronal-acetate buffer of pH 7; — 9 m*M* ATP.

Abscissa: temperature in C°.
Ordinata: γ phosphorus split in 5 min.

× × 0,1 *M KCl*; Δ Δ 0,3 *M KCl*

KCl (fig. 4). The curves obtained in *KCl* of 0,1 resp, 0.3 *M* are so similar that they can be darwn by the same line.

d) *Concentration of KCl.* In fig. 5 the ATP-splitting of actomyosin (a) and of myosin (b) was plotted against the concentration of *KCl.* (20°; *pH* 7; 0,006 *M* ATP). In case of actomyosin the splitting drops sharply by increasing concentration of *KCl,* and reaches at about 0,20 *M* the value of myosin. The superprecipitation shifted simultaneously from its maximal value into dissolution. (See diagram below.)

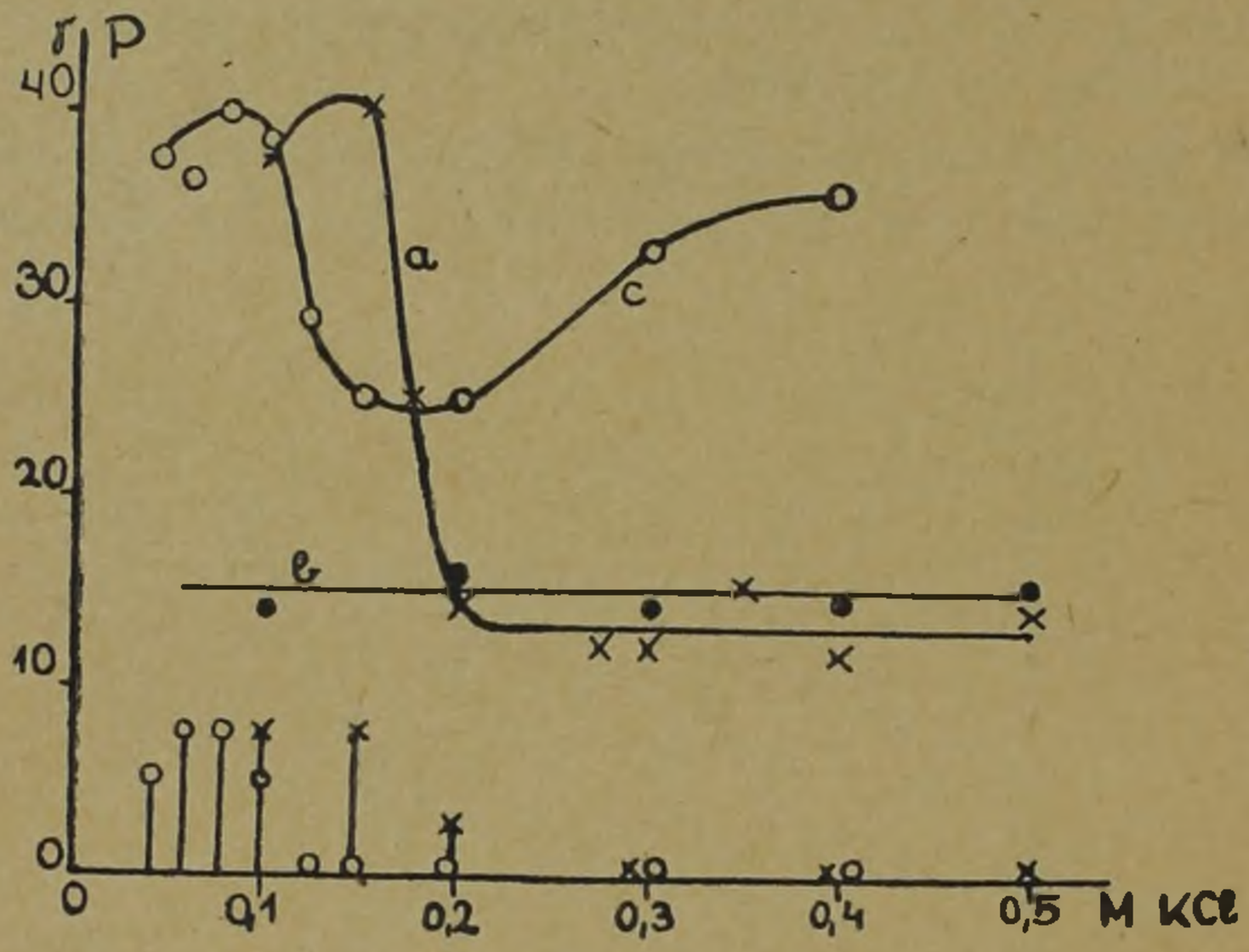

Fig. 5.

ATP-ase activity of actomyosin and of myosin depending on concentration of KCl.

2,3 mg myosin, and in case of curves of *a* and c, 0,46 mg actin. (A 100% active actomyosin.) 20 C°; 0,06 *M* veronal-acetate buffer of pH 7. (In case of c a buffer of pH 7 was used as well, but the ATP was brought before the experiment to pH 8,6.) The pH of these reaction-mixtures was determined electometrically in parallel samples — 6,6 m *M* ATP.

Abscissa : concentration of *KCl* in *M.*
Ordinata : γ phosphorus split in 5 min.
a: actomyosin at *pH* 7—*b :* myosin at *pH* 7—*c :* actomyosin at *pH* 8,2.
The meaning of-the lower diagram (superprecipitation) is the same as in fig. 3.

The enzymatic activity of myosin, contrary to that of actomyosin, is constant throughout the whole measured interval of *KCl* concentration.

e) *Effect of pH.* Curve *c* of fig. 5 shows the *KCl*-dependence of ATP-ase activity of actomyosin at a more alkaline *pH* (*pH*=8,2), which can be compared with curve *a* (*pH*=7). The maximum of splitting, as well as the maximum of the superprecipitation has shifted to a lower concentration of *KCl,* as compared with *pH* 7. The splitting measured at a higher *KCl*-concentration is far greater than the corresponding part of the actomyosin curve at *pH* 7. This is in agreement with the fact that the optimum *pH* of myosin-ATP-ase is at about 9.

f) *Concentration of ATP.* Fig 6 represents the dependence of ATP-ase activity on the concentration of ATP at different temperatures (*pH* 7; 0,08 *M KCl*). In case of actomyosin, by increasing the concentration of ATP we can reach at any measured temperature a level of ATP-concentration, above which the splitting begins to decline. This limit coresponds with the concentrations of ATP, at which according to our observations, the superprecipitation begins to decrease; in presence of still higher concentration of ATP the actomyosin will dissolve. (See diagram below the curves.) The splitting of myosin, on the other hand, is constant in the whole range of ATP-concentrations examined, as it is shown in curve "my" of fig. 6.

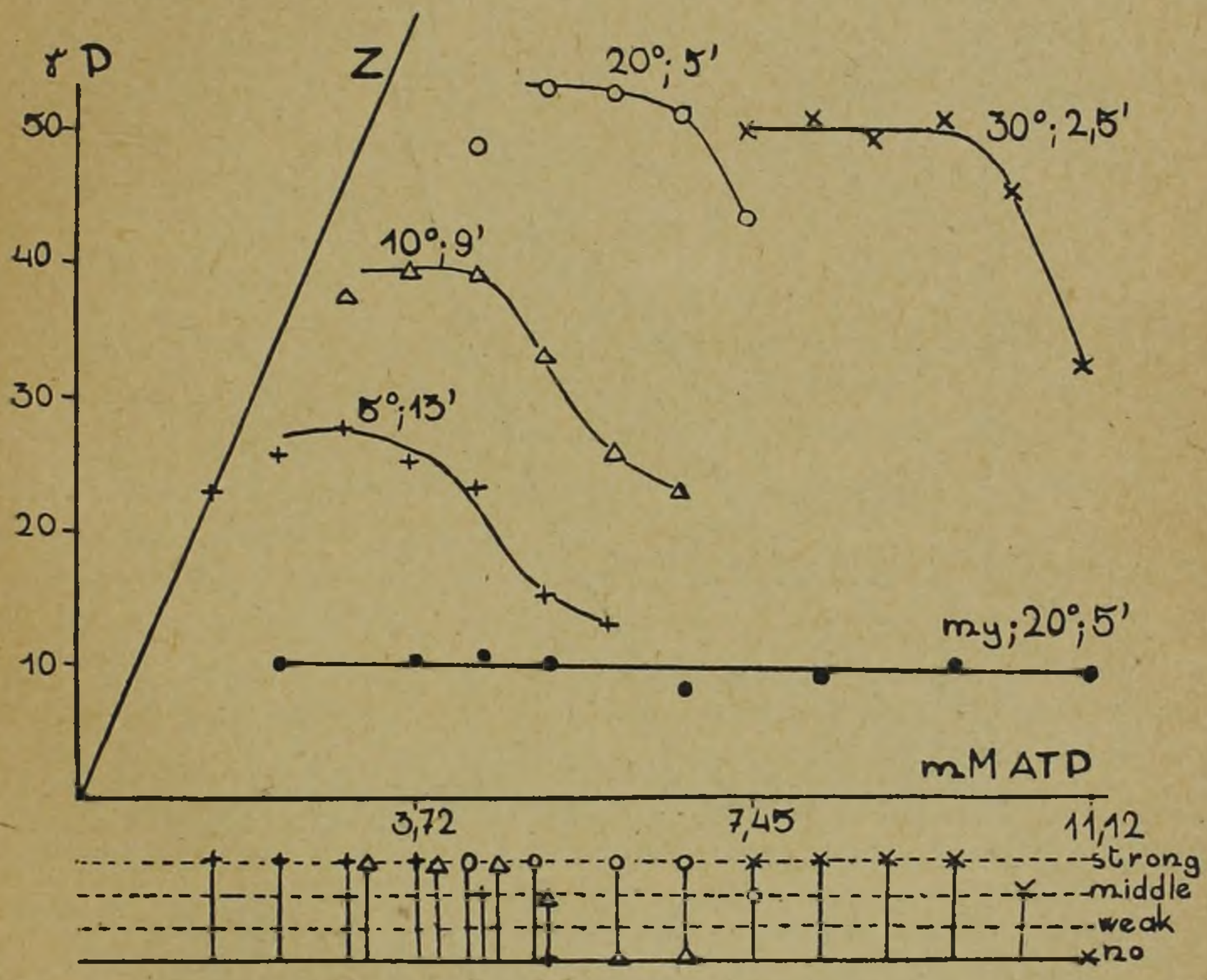

Fig. 6.

ATP-ase activity of actomyosin and myosin depending on ATP-concentrations at different temperatures.

1,97 mg myosin and 0,79 mg actin (a 170% active actomyosin).
0,08 *M* KCl; — 0,006 *M* veronal-acetate buffer of pH 7.

Abscissa: concentration of ATP in *mM.*
Ordinat: γ phosphorus split.
The straight line „Z" denotes the 25% of hydrolizable phosphorus, to the right of which the hydrolysis goes proportional with time.

The curve marked with „my" represents the splitting of the same amount of myosin, without actin at, 20 C°; in 5 min.

The meaning of the lower diagram is the same as in fig. 3.

3. ATP-SPLITTING OF WASHED MUSCLE.

As shown above, the actomyosin, in the state of maximal superprecipitation, is far more active an ATP-ase, than in the state of a diminished precipitation or of dissolution. There might be some correlation between the phenomena of superprecipitation and the physiological contraction of muscle. Therefore it seemed of interest to extend the study of these phenomena to other systems, the conditions in which approach more closely those prevailing in muscle. As one of those, myosin

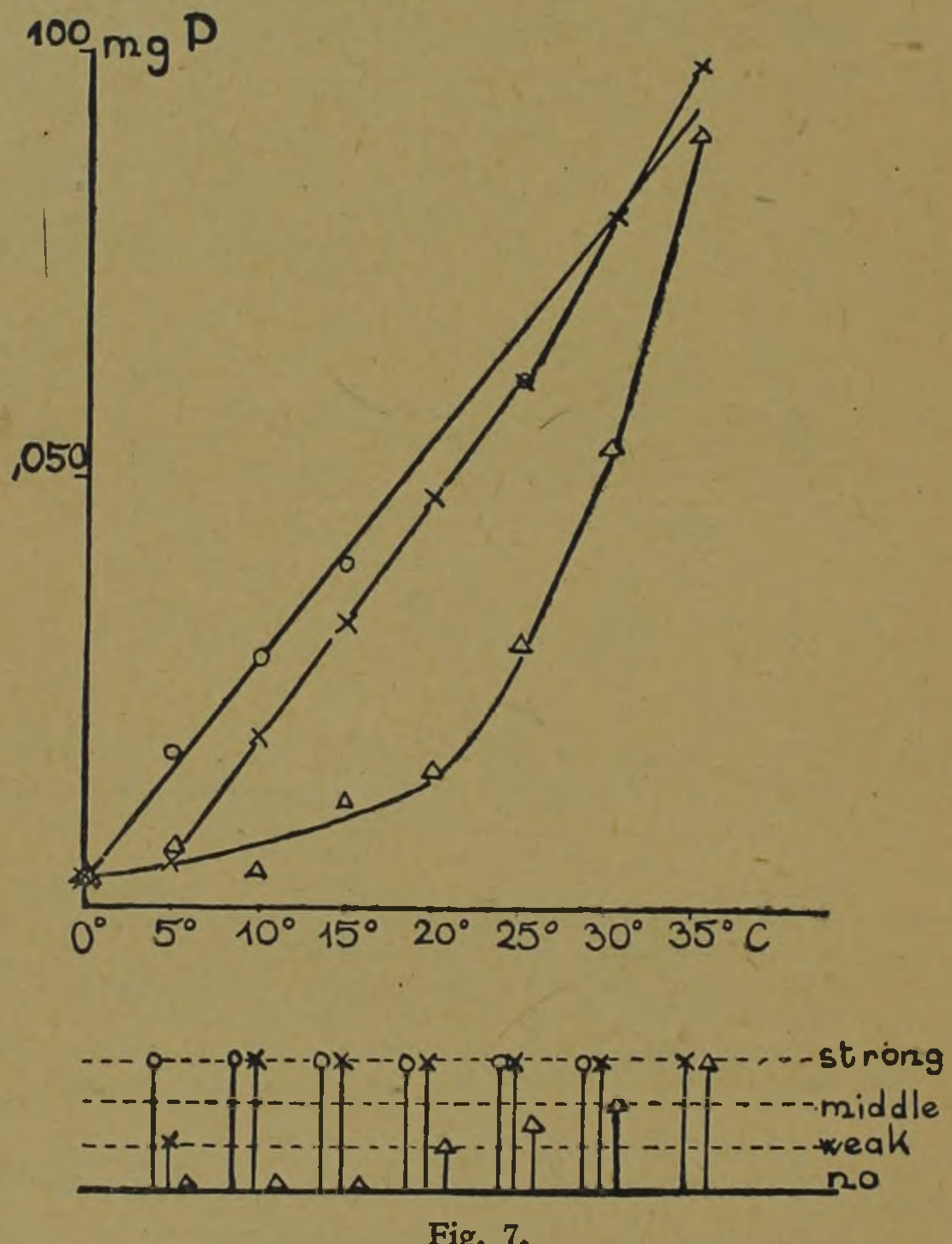

Fig. 7.

ATP-ase activity of washed muscle depending on temperature at different concentrations of KCl.

100 mg of washed muscle.
0,006 *M* veronal-acetate buffer of pH 7; — 6,6 m*M* ATP.

Abscissa: temperature in C°.
Ordinata: γ phosphorus split in 5 min.

The meaning of the lower diagram is similar to that of fig. 3. The four levels showing the degree of „contraction„ of the washed muscle.

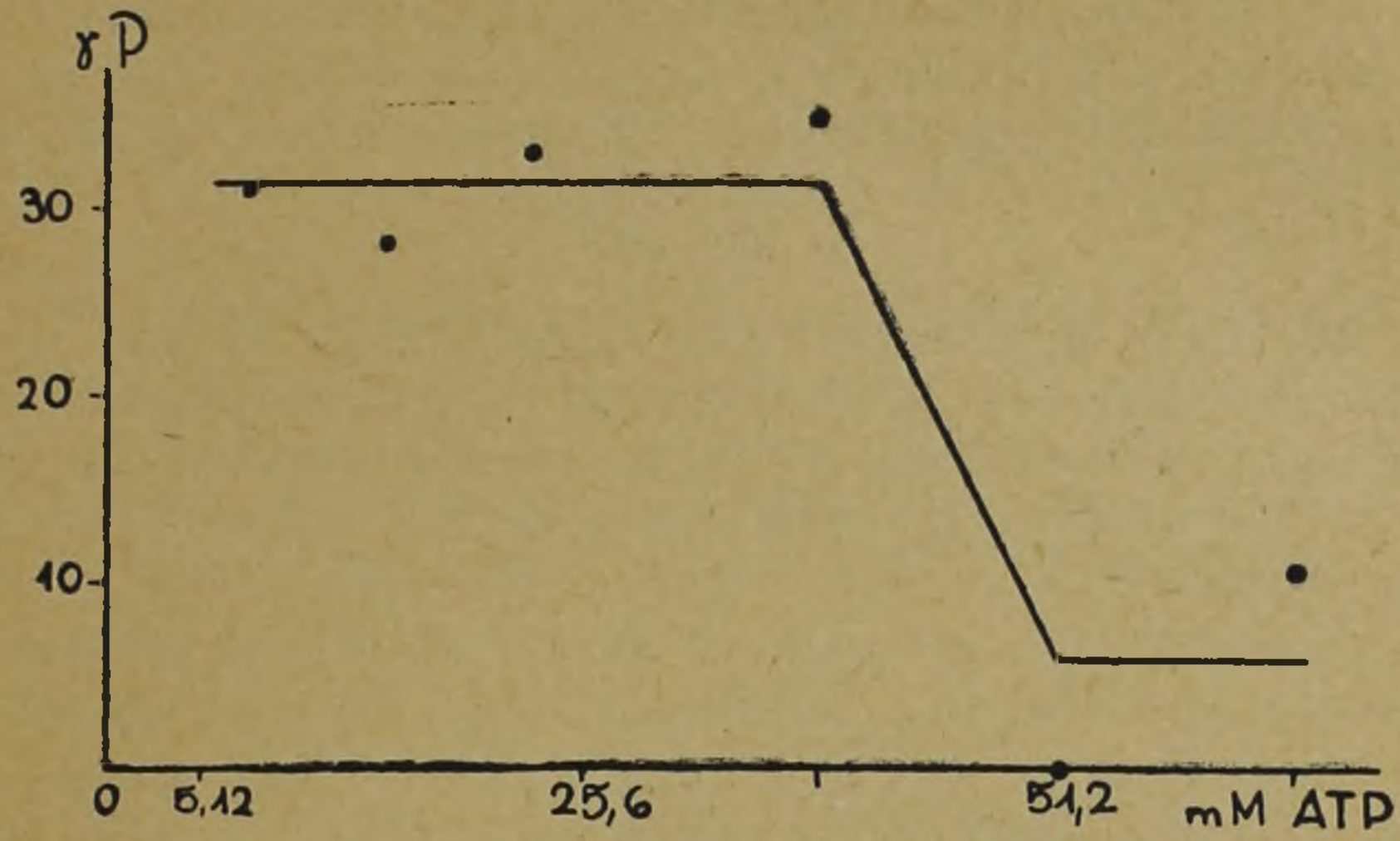

Fig. 8.

100 mg of washed muscle.
20 C°; 0,327 *M* KCl; 0,006 *M* veronal-acetate buffer of pH 7.
Absissa: concentration of ATP in *mM*.
Ordinata: γ phosphorus split in 5 min.

B could be considered, an actomyosin of 100% activity, which is extracted directly as such from the muscle. Investigations showed only slight differences between the dependence of temperature, resp. of *KCl*-concentration of the enzyme-activity of myosin *B* and actomyosin, prepared from actin and myosin.

A second, more essential approach was to investigate washed muscle on the same basis.

Our results with washed muscle are qualitatively in complete agreement with our experiences on actomyosin, if we replace superprecipitation by "contraction", produced by the effect of ATP on washed muscle. Varying the ATP-concentration or the temperature, the maximum of enzyme activity and the maximum of "contraction" of washed muscle occur at the same values and the decrease of "contraction" is always followed by the decrease of ATP-splitting. The limit of "contraction" (in temperature, *KCl* or ATP-concentration) shows however a significant numerical difference, compared with the values obtained by the superprecipitation of actomyosin.

Fig. 7 shows a series of experiments on washed muscle, corresponding with fig. 3: splitting of ATP depending on temperature at different concentrations of *KCl* (*pH* 7; 0,006 *M* ATP). Compared with the diagram below, we can see that the essential rise of ATP-splitting begins in each concentration of *KCl* at the same temperature at which the contraction has started.

Fig. 8 shows ATP-splitting of washed muscle plotted against the concentration of ATP. Above a certain concentration, ATP-splitting will suddenly fall: in these mixtures "contraction" does not take place.

DISCUSSION.

The results of these experiments differ from those of other investigators in two respects: 1. The major part of previous experiments reported in the literature were made with myosin containing indefinite amounts of actin. 2. The superprecipitation of actomyosin was formerly not taken in to consideration.

In general there are a number of data which were confirmed by our own experiments. E. g. *Singher* and *Meister* (8) found no effect of *KCl*-concentration on the ATP-ase activity of actomyosin with low atcin content, but the lowest *KCl*-concentration employed by them was as high as 0,19 *M*, where dissolution occurs.

Banga (3/a) found the *pH* optimum of a actomyosin-ATP-ase at *pH* 6. *Engelhardt* (12) described a double *pH* optimum.

Our findings are in disagreement with the papers of *Mommaerts* (13), who found that the ATP-ase activity of myosin is more dependent on *KCl* than that of actomyosin. *He* does not make any remark on superprecipitation. *He* concludes that the *pH* optimum is at *pH* 9 in presence of *Ca*, and at *pH* 7 in absence of *Ca*. *He* finds the same *pH* optima with myosin and actomyosin. We find that with superprecipitated actomyosin the optimum *pH* of ATP-ase-activity is at *pH* 6,5—6,7, whereas dissolved actomyosin and myosin have an optimum at *pH* 9, both in presence and in absence of *Ca*. According to *Mommaerts*, the physico-chemical changes of actomyosin have nothing to do with its enzymatic activity, but his conclusion is based on experiments done at high *KCl* concentrations. Under such conditions we could not find any effect either.

We are unable to offer a proper explanation of the mechanism by which superprecipitated actomyosin can be more active enzymatically than the dissolved one. The effect is the more striking, because during precipitation the surface diminishes and therefore it is possible that the effect is even greater than that measured. Moreover it seems that the enhancing effect goes parallel with the intensity of superprecipitation.

It had to be ascertained that the effects described above are not due to the removal of an inhibitory mechanism, i. e. with loss of water the dehydrated actomyosin does not loose an inhibitor, with circumstance would counteract the lessening of surface. Centrifuging the superprecipitated actomyosin the precipitated protein showed an enhanced enzyme

activity and the supernatant liquid had no inhibitory effect on the splitting of ATP.

Our results do not support the view of *Bailey* and *Perry* (14) according to whom there is a competition between ATP and actin for the *SH*-groups of myosin.

The experiments with washed muscle show that it behaves almost like actomyosin.

If the dehydration of actomyosin and the "contraction" of washed muscle can be accepted as the basis of the physiological muscle contraction, that should mean that the muscle splits ATP mostly in its contracted state.

SUMMARY.

1. Experiments on actomyosin show that there is a parallelism between superprecipitation and ATP-ase activity, superprecipitated actomyosin being enzymetically more active.

2. Washed muscle behaves in much the same way as actomyosin, its ATP-ase enzyme-system being more active in the "contracted" state than in the "relaxed" one.

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THE ROLE OF PHOSPHATE IN THE POLYMERIZATION OF ACTIN FROM FROG-MUSCLE.

WITH 5 FIGURES IN TEXT.

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Two proteins participate in the contraction of muscle: actin and myosin. The binding of these two proteins produces the formation of actomyosin. This in the form of a thread looses a great amount of water and contracts when *KCl* and adenosintriphosphate are present. (*Szent-Györgyi* 1.)

The actin was isolated by *Straub* (2). This solved in distilled water has a globular structure, but under the influence of certain salts it is transformed from a globular to a fibrous structure, i. e. it polymerizates. During the transformation from globular to fibrous actin the low viscosity of the solution rises. So the process of polimerization can be followed by measuring the viscosity. The physiological role of the structural transformation of actin was shown by *Straub, Feuer* and *Lajos* (3). In the contraction of actomyosin the actin is present in this polymerizated form.

It is proved by *Straub* and al. (4) that polymerization takes place in the presence of *Mg* ions and a prosthetic group only. Polymerization can be prevented by oxydizing agents. Our investigations resulted in the fact, that there is some difference between the properties of actin extracted from frog-muscle, and those of the actin of rabbit-muscle. In the course of our experiments performed on frog-muscle, we received results which proved that phosphate takes an important part in the polymerization.

METHODICS.

Preparation of actin. We employed the somewhat modified method of *Straub* and al (4). The spinal chord of the decapitated frogs were destroyed. The frogs were cut through beyond there sternum, then they were skinned, eviscerated and the hind limbes were packed in ice.

After five minutes the muscle of the hind limbes and the abdomen were cut off the bones and pressed through a mincer.

1. Every 100 gr. of minced muscle was suspended at 0° in 200 ml. of a solution containing 0,3 *M KCl* and 0,15 *M* phosphate-buffer of *pH* 6,5. After ten minutes 800 ml. glassdistilled water of room-temperature was added and the mixture pressed through a cloth.

2. The remaining myosin-free muscle-stroma was suspended in 300 ml. 0,4% $NaHCO_3$ at room-temperature. This solution contained 0,0001 *M* $CaCl_2$. After thirty minutes the mixture was pressed through the cloth.

3. The residue was extracted with 70 ml. of a mixture of 0,01 *M* $NaHCO_3$ and 0,01 *M* Na_2CO_3 at 0°. This mixture contained 0,0001 *M* $CaCl_2$. After ten minutes the mixture was diluted with 700 ml. of glass-distilled water of room-temperature and squeezed through the cloth.

4. 100 gr. of the non-solved residue was suspended in 300 ml. of water-free aceton for ten minutes. After straining 100 ml. of fresh aceton was added and the suspension was filtered after ten minutes. The residue was dried on filter-paper at room-temperature.

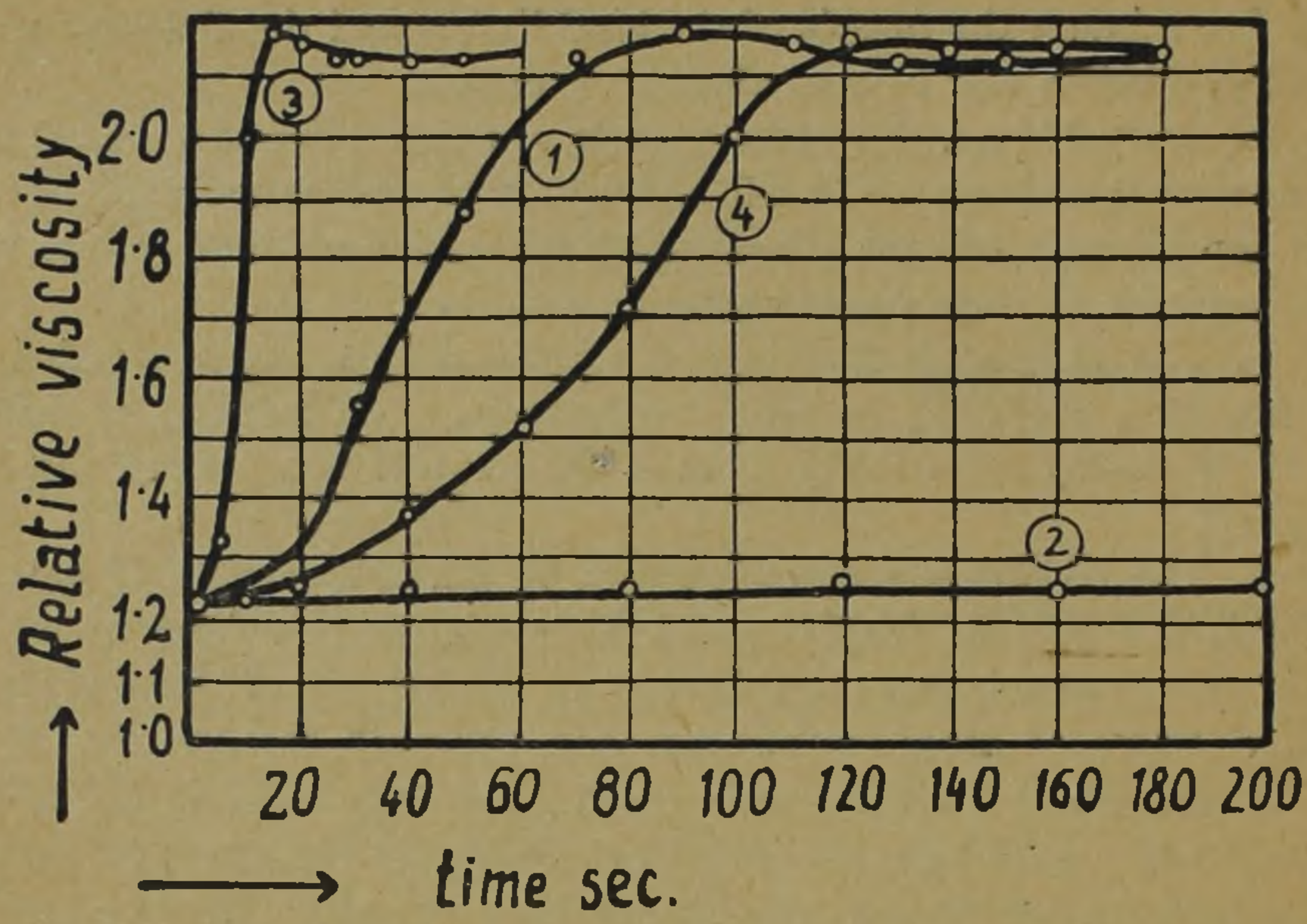
Suspending 1 gm. of this dry muscle-powder for thirty minutes at room-temperature in 20 ml. boiled distilled water and filtering it, we received the aqueous solution of the globular actin. The dry contents of the solution varied between 0,4—0,7%.

The measuring of the polimerization of actin. The change of viscosity of the actin solution was measured with the *Ostwald* viscosimeter. The results are given in relative viscosity values, calculated by the ratio of the measured flowtime of the actin solution /the time of outflow of the same volume of 0,1 *M KCl.*

In the cases, where we had to measure the influence of pressure on the solution, we employed the viscosimeter described by *Straub* and al (4). Our results here, too, are given in terms of relative viscosity.

RESULTS.

1. It is proved by *Straub* and al. (4) that the polymerization of rabbit-actin takes place in the presence of 0,1 *M KCl.* The polymerization is catalysed by *Mg* ions. Giving only 0,1 *M KCl* to the solution, here too, the effect of the *Mg* ions, resp. these, which are bound to the actin, and which were not washed out during the preparation, come into force. In the case of frog-actin at 0°, 0,1 *M KCl* did not induce polymerization, because the rise of viscosity started only at adding of *Mg* ions. This shows that the *Mg* ions left in the frog-actin after the procedure of extraction did not suffice to start the polymerization. The results obtained



1. figure. 1. curve: Viscosity of 4 ml. (22 mg. dry contents) actin solution after adding 1 ml. 0,005 *M* $MgCl_2$ and 0,5 *M KCl* at 0°.
2. curve: The same at the addition of 1 ml. of 0,5 *M KCl* at 0°.
3. curve: The same at the addition of 1 ml. of 0,005 *M* $MgCl_2$ and 0,5 *M KCl* at 14°.
4. curve: The same at the addition of 1 ml. 0,5 *KCl* at 14°.

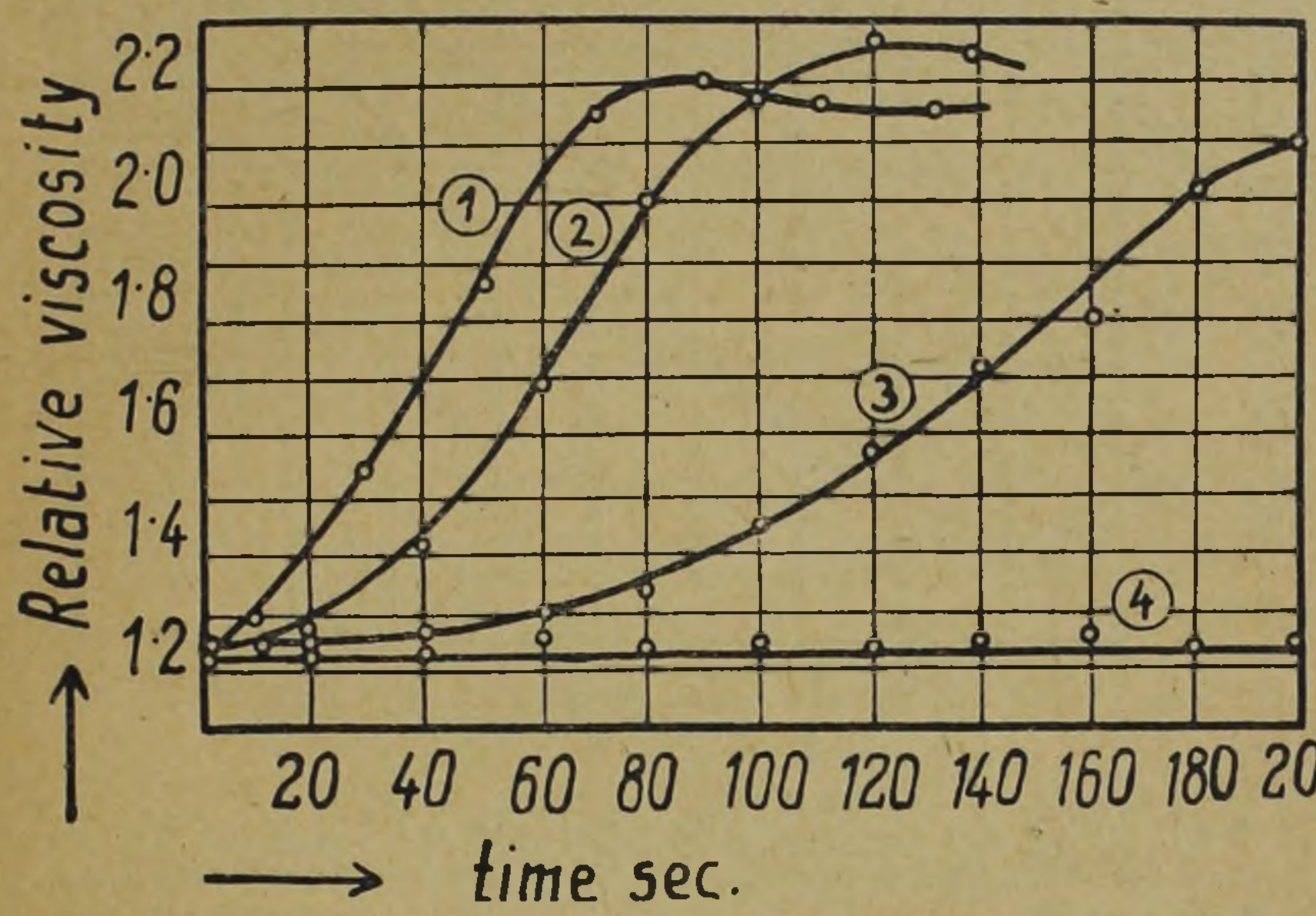
at higher temperature (14°) were quite similar to those of rabbit-actin (figure 1). The viscosity of the polymerizated frog-actin was only $^1/_3$ of that registered on the rabbit-actin.

2. It was found by *Straub* and al. (4) that polymerization of the rabbit-actin could be inhibited by weak oxydizing agents. Therefore they concluded that reducing groups play a part in the polymerization. The polymerization of frog-actin on the contrary, takes place with unchanged speed in the presence of 1 : 1000 methylenblue and even in the presence of the stronger oxydative $n/50$ H_2O_2. These results exclude the possibility of oxydative and reductive prosesses taking part in the polymerization of frog-actin. A difference of the biochemistry of frog and rabbit-muscle was shown also by *Varga* (5).

3. In consequence of the results mentioned above, we had to suppose another mechanism. It is a well known fact, that *Mg* ions have an important role in the organism: the activation of phosphatases. As the *Mg* ions catalyse also the polymerization, we had to assume that phosphate groups took part in the polymerization. The decision of this question met with some difficulties. The dialysis of the actin solution or the precipitation and washing of actin at the isoelectrical point, would

remove not only the phosphate but also the prosthetic group, without which polymerization cannot take place. Ammoniummolybdate, as we know, binds the phosphate even in very small concentrations in the form of a heteropoliacid of varying composition. We therefore investigated the effect of ammoniummolybdate on polymerization.

At 0° 0,05—0,06 ml./mg. actin of a 0,5% ammoniummolybdate solution totally prevents the polymerization that takes place in the pre-

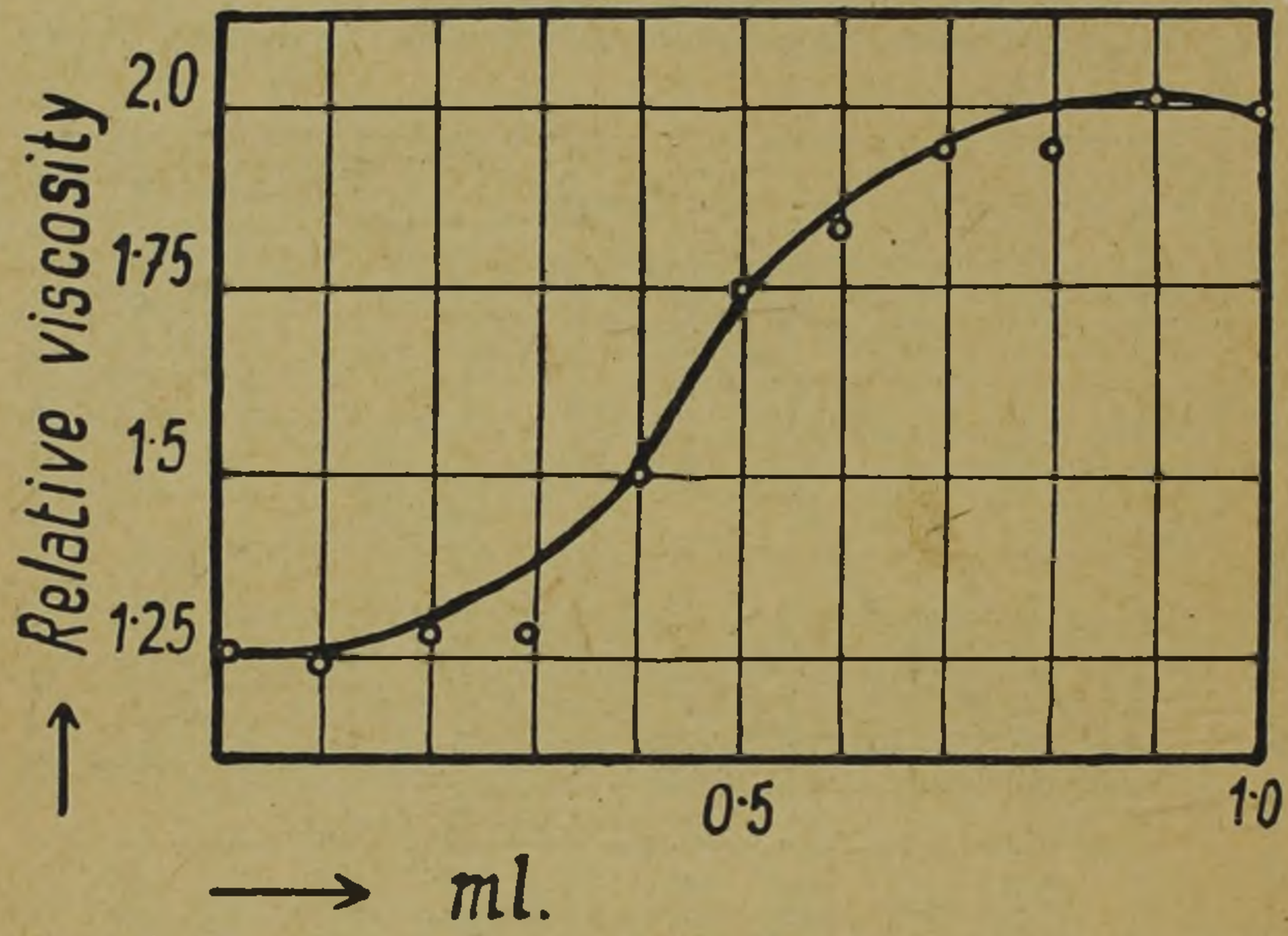


2. figure. 1. curve: 3 ml. (21 mg. dry contents) actin solution + 1 ml. of the mixture of 0,005 *M* $MgCl_2$ and 0,5 *M KCl* + 1 ml. H_2O at 0°.
2. curve: The same amount of actin + 1 ml. of the mixture of 0,005 *M* $MgCl_2$ and 0,5 *M KCl* + 0,3 ml. 1% ammoniummolybdate + 0,7 ml. H_2O at 0°.
3. curve: The same amount of actin + 1 ml. of the mixture of 0,005 *M* $MgCl_2$ and 0,5 *M KCl* + 0,5 ml of 1% ammoniummolybdate + 0,5 ml. H_2O at 0°.
4. curve: The same amount of actin + 1 ml. of the mixture of 0,005 *M* $MgCl_2$ and 0,5 *M KCl* + 0,6 ml. of 1% ammoniummolybdate + 0,4 ml. H_2O at 0°.

sence of saltmixture. (0,1 *M KCl* and 0,001 *M* $MgCl_2$.) Smaller amounts of ammoniummolybdate cause partial inhibition (2 figure). The effect of the amount of ammoniummolybdate, just enough to inhibit, was abolished by adding further amounts of actin to the solution, the whole quantity of actin present polimerized.

At 14° ammoniummolybdate has a contrary effect. At this temperature the amount of ammoniummolybdate totally inhibiting at 0°, induces polymerization, even in the absence of salts. The polymerization takes place faster than in the presence of saltmixture, it is so rapid, that the speed of polymerization cannot be followed on the *Ostwald* viscosimeter.

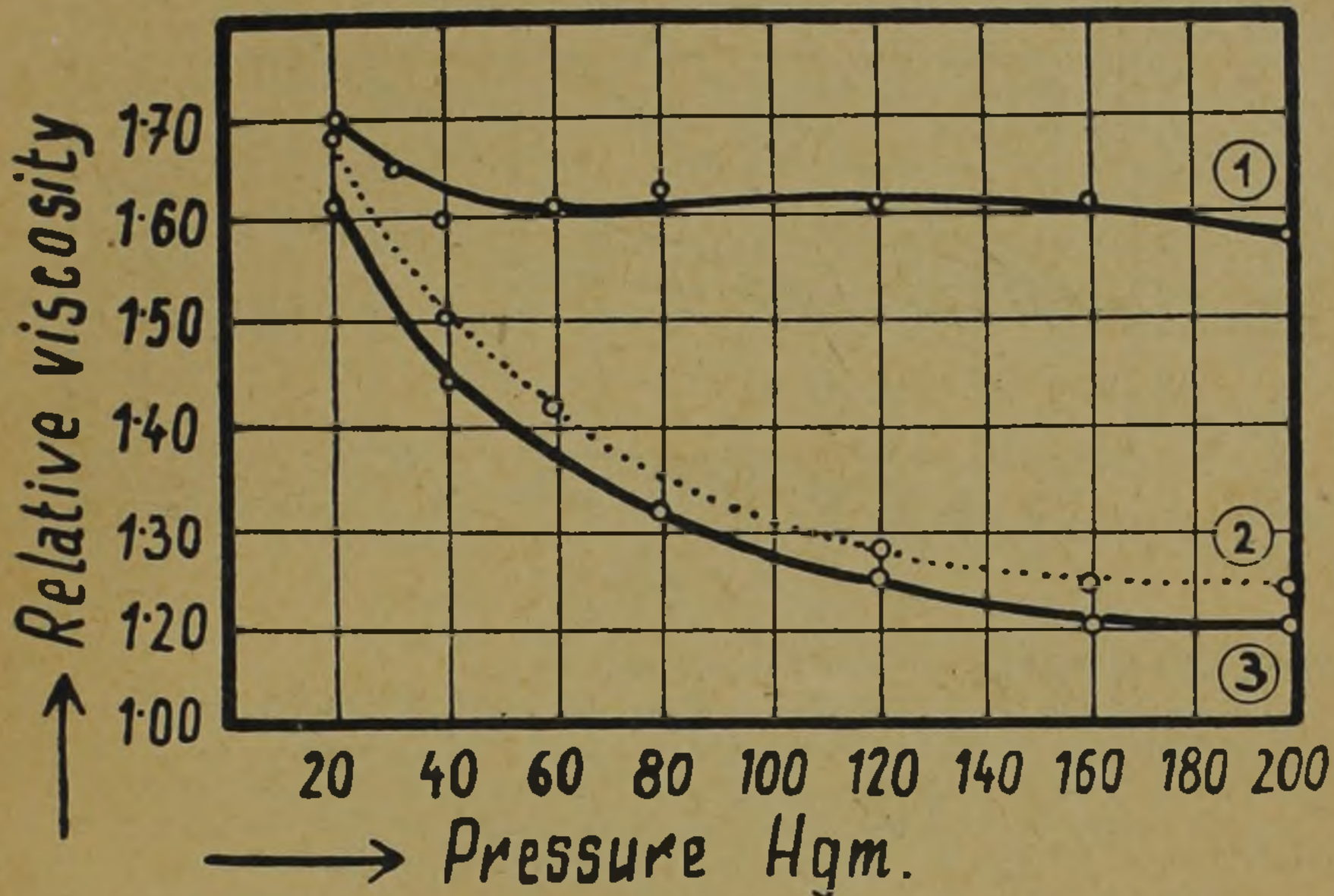
The rate of polymerization depends on the amount of ammoniummolybdate (3 figure). With smaller ammoniummolybdate quantities partial polymerization can be induced. In the case of saltmixture partial polymerization could not be obtained, the process either did not start at all, or it went on, till the whole amount of actin present was polymerizated.



3. figure. 3 ml. (21 mg dry contents) actin solution + 0,5% ammoniummolybdate in amounts as recorded on the abscissa, at 14°. The solutions are completed with distilled water at 5 ml.

Ammoniummolybdate has, in greater concentrations, a precipitating effect on proteins. Other precipitating and denaturating agents, too, increase the viscosity of the actin solution. Our aim was to find out whether the effect of ammoniummolybdate was precipitation, denaturation or real polymerization. We decided this question by the following considerations. Viscosity of a solution depends among others on the shape of the particles solved in it. We supposed that in the polymerization induced by salts, and in the rise of viscosity caused by denaturating agents, the actin had a different structure. This difference must manifest itself in the dependence of viscosity on pressure. We therefore measured at different pressures the viscosity of actin polymerizated by saltmixture, ammoniummolybdate and ethanol (4 figure). The viscosity of actin polymerizated by saltmixture and ammoniummolybdate depends in the same way on the pressure, unlike actin polymerizated by ethanol, which is more similar to Newton-fluids. These results show that ammoniummolyb-

date induces a process similar to that caused by saltmixture. Here we mention, that frog- and rabbit-actin polymerizated by ammoniumolybdate, could be bound to myosin. We do not give here the details, because these experiments will be described in another paper.



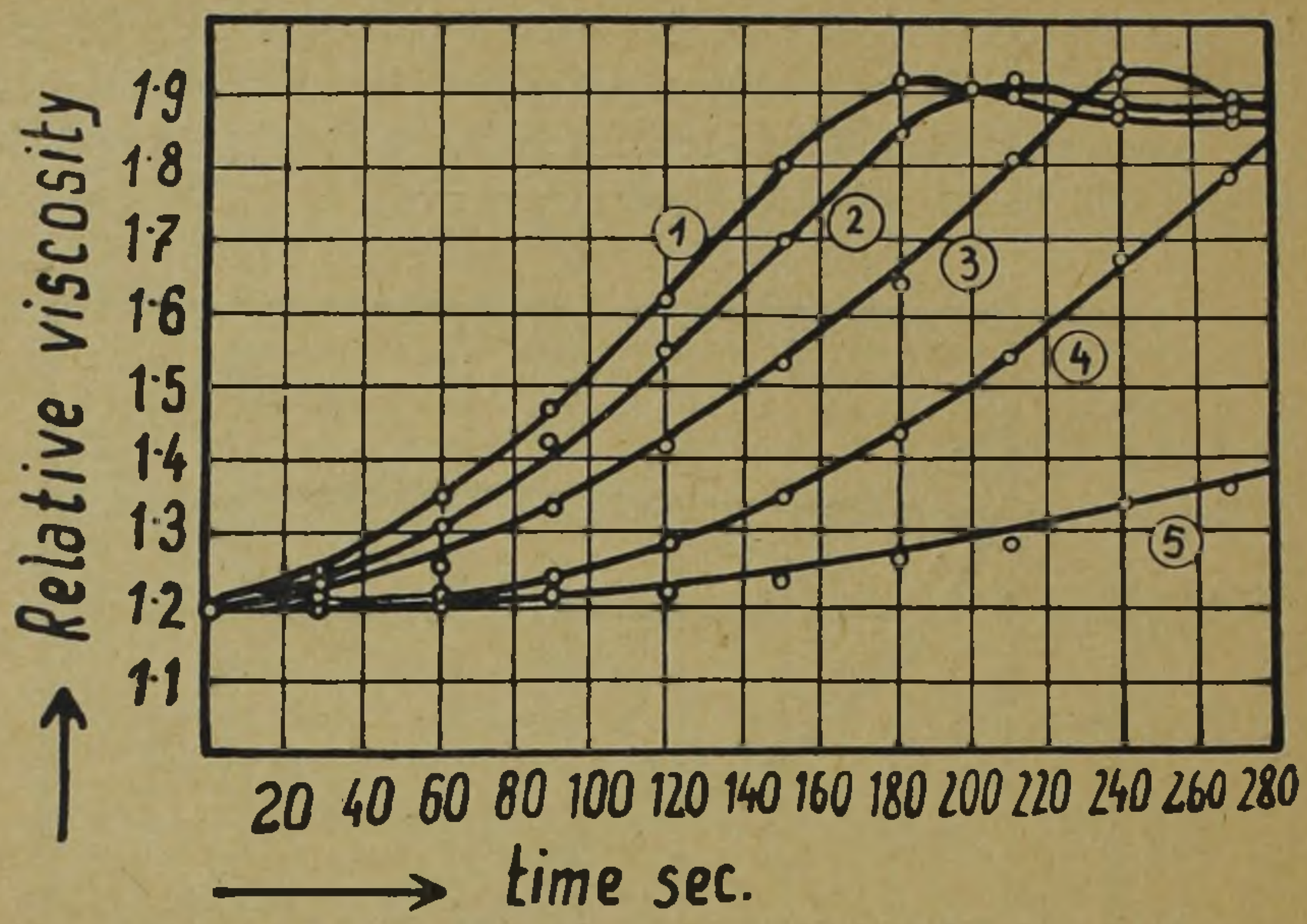
4. figure. 1. curve: 3 ml. (18 mg. dry contents) actin solution polymerizated by 0,5 ml. 96% ethanol at 14°.

2. curve: The same amount of actin polymerizated by 1 ml. of 0,5% ammoniummolybdate at 14°.

3. curve: The same amount of actin polymerizated by 1 ml. of the mixture of 0,005 *M* $MgCl_2$ and 0,5 *M KCl*, at 14°. Each solution was completed with distilled water at 5 ml.

4. We investigated if the ions which could substitute *Mg* ions in the case of the activation of phosphatases, were capable to replace the *Mg* in the polymerization. In the presence of 0,1 *M KCl* at 0°, the ions tested induce polymerization in a concentration of 0,0002 *M*. The order of activation is: *Mn, Co, Mg, Fe, Ni* (5 figure). If we compare these data with those obtained by *Bamann* and al (6), in the case of different phosphatases, we can see, that the activating effect on polymerisation of the ions investigated can be compared mostly with the activating effect on pyro- and metaphosphatases.

The results of our experiments described above may be summarized as follows: In the case of frog-actin reducing groups play no part in the polymerization. The polymerization is caused by a reaction in which the phosphate group has a central role.



5. *figure.* 3 ml. (18 mg. dry contents) actin solution +
1. curve: 1 ml. of 0.5 *M KCl* + 1 ml. of 0,001 *M* $MnSO_4$ at 0°
2. curve: „ „ „ „ „ „ + „ „ „ „ „ $CoSO_4$ „ „
3. curve: „ „ „ „ „ „ + „ „ „ „ „ $MgSO_4$ „ „
4. curve: „ „ „ „ „ „ + „ „ „ „ „ $FeSO_4$ „ „
5. curve: „ „ „ „ „ „ + „ „ „ „ „ $NiSO_4$ „ „

SUMMARY.

1. Actin extracted from frog-muscle polymerizates at 0° in the presence of 0,1 *M KCl* only after adding *Mg* ions to the solution. At 14° the reaction takes place without *Mg* being added.

2. Polymerization of frog-actin is not inhibited by oxydizing agents.

3. Ammoniummolybdate at 0° prevents polymerization, at 14° it induces polymerization. The viscosity of actin polimerizated by ammoniummolybdate depends in the same way on pressure as the viscosity of actin polymerizated by *KCl* and $MgCl_2$.

4. In the polymerization of frog-actin *Mg* can be replaced by *Mn, Co, Fe, Ni* ions.

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XIV. ANNUAL MEETING OF HUNGARIAN PHYSIOLOGICAL SOCIETY, PÉCS. JUNE 18. AND 19. 1948.

After the opening presidential adress of *K. Lissák, G. Szántó* ministerial councillor in the name of the minister of education, *E. Weil* the president of the Hungarian Public Health Council and of the Trade Union of Hungarian Physicians in behalf of hungarian Physicians and *L. Lajos* the dean of the Medical Faculty greeted the congress.

J. TIGYI (BIOPHYSICAL INST. UNIV. PÉCS): THE TENSION AND VOLUME DIMINUTION OF MUSCLE.

According to *Ernst*, the volume diminution by isometric and isotonic contraction is equal, while according to *Meyerhof* the volume diminution of isometric contraction is 3—4 times that of the isotonic one.

This difference seemed explainable by measuring the volume diminution of isometric contraction completed at different length. A small apparatus was devised which makes it possible to regulate muscle length in the volumeter. Using this, method for examining the frog's gastrocnemius, semimembranosus, and sartorius it was found, that the volume diminution of the isometric contraction is a function of length. The maximum of the function is at the resting length and diminishes gradually whether the muscle is longer or shorter during isometric action.

As Ernst measured the volume diminution of isometric contraction at resting length to be 25%, and Meyerhof found about the resting length, the difference can be explained.

After having cleared the correlation between length and volume diminution, the function of length and isometric tension was examined and was found to correspond to a similar curve, which shows maximum at resting length (Hill, Doi, Beck). Hence volume diminution changes paralelly with tension.

E. ERNST, (BIOPHYSICAL INST., UNIV. PÉCS): THE AUTOMATIC PART OF MUSCLE CONTRACTION.

The result of the foregoing communication raised the question: whether, and to what an extent the volume diminution is a function of tension? The gastrocnemius (Rana esc. Hung.) was stimulated by make and breake shocks indirectly in the volumenter, the Achilles tendom was doubly tied: in the beginning of activity the muscle could shorten isotonically but after having shortened 2—3 mm, it was prevented in further shortening, and so was forced (by the second binding) to exert tension ("ditonic" twitch or "Anschlagszuckung"). The result of the experiment is a superponed volume diminution of which the first, smaller part corresponds with isotony, the greater, and longer lasting part is superposed after 10—15 msec, corresponding to the tension.

Since the single stimulus and the excitement caused by it are already gone after 10—15 msec, i. e. when the tension and the second part of the volume diminution appears, one is obliged to ask what a process could possibly cause these two phenomena? The explanation proposed, is that the tension brought about by the impossibility of further shortening causes *crystallization* in the muscle, according as it is known in the case of stressed rubber. By stressing the rubber one finds volume diminution, increased double refraction, *X*-ray-diffraction, increase of light absorption, increased hardness, decreased solubility increasing irreversibility, phenomena all caused by crystallization. But these are already known since

long to occur in muscle activity. The phenomenon that in the active muscle crystallization sets in and causes certain effects, i. e. these effects — being not directly caused by excitement — is termed: the autonomic part of muscle contraction.

J. BALÓ, (INST. OF PATHOLOGY AND EXP. CANCER RESEARCH. UNIV. BUDAPEST): LYSIS OF ELASTIC FIBRES.

Elastin belongs to the scleroproteins. All investigators agree that elastic fibres are very resistant to chemicals. Neither acids, nor alkalis attack these fibres. Lysis of elastic fibres can be attempted in animal experiments, but also in vitro. Ewald and Kühne employed digestion as a histological procedure for the study of elastic fibres. The action of acids and alkalis as well as that of urea, Weber solution and *KJ*, has been studied with this method. All these substances showed very little effect. Among organ-extracts and body fluids pancreatic extract proved to be the most effective. One could suppose, that this is due to trypsin, but having prepared pure trypsin according to the procedure of Northrop, this specimen proved to be inactive. If we extract from pancreatic powder with dist. water the trypsin and then a second extraction with slightly alkaline phosphate-buffer is performed, an extract is obtained which does not posses a tryptic effect, but dissolves promptly the elastic fibres.

These investigations prove that a specific ferment, the elastase, which is independent from the trypsin, but which is produced also in the pancreas, serves for the lysis of elastic fibres.

I. BANGA, (INST. OF PATH. ANAT. AND EXP. CANCER RESEARCH. UNIV. BUDAPEST): THE QUANTITATIVE DETERMINATION OF ELASTIN.

In histology, staining methods serve to distinguish the elastic fibres from the collagen ones. These are based on the fact that while elastin is a protein of an acid character and has reducing power, collagen does not reduce. In histology the most frequently used staining methods are those of Unna and Weigert. Yet these are not quantitative and so the disappearence or increase of elastic fibres of a minor degree cannot be determined by them. This is accomplished by the method of the author, which renders possible the quantitative determination of the elastic fibre. The principle of the method is, that only elastic fibres bind chemically resorcin-fuchsin, by the other tissue proteins, such as collagen, myosin etc. it is not bound chemically, only absorbed. The absorbed dye is dissolved from the tissues by differentiation with acid alcohol. The chemically bound stain of elastin can be dissolved by heat coagulation of the protein. In this way the chemically bound stain is liberated from the denatured elastin which is dissolved quantitatively in 75% alcohol at 82 C°. The concentration of the stain solution is determined colorimetrically comparing it with known standards and so the quantity of dye bound by measured dried weight of tissues may be given. The results, gained by this method, show that among human tissues the aorta contains most elastic fibres.

M. FABINYI AND J. SZEBEHELYI (PHARMACOLOGICAL INST. UNIV. BUDAPEST): THE EFFECT OF HISTAMINE ON THE SWELLING OF COLLAGEN.

Inflammatory and anaphylactic phenomena are characterized by the swelling of the connective tissue. According to the investigations histamine — in pathologically occuring concentration — induces marked swelling of isolated collagen fibres in vitro. The mechanism of the swelling caused through histamine, and that brought about by a change in the *pH* values (acetic acid) is not the same, because the individual ions have different effects upon the two kinds of swelling. Sodium salicylate in therapeutically employed concentration, not only prevents the swelling effect of histamine on the collagen fibres, but dehydrates also the swollen fibres, while it leaves the swelling induced by acetic acid unaffected. Histamine reduces the ostomical resistance of the red blood cells in vitro, but this influence is not affected by sodium salicilate. Sodium salicylate, histamine and antihistamine drugs (Antistin and Pyribenzamin) influence not at all the activity of hyaluronidase in vitro, and hyaluronidase is not able to produce swelling of the collagen fibres. These results suggest, that the effect of sodium salicylate in rheumatic diseases may be the consequence of its antihistamine-effect too.

P. ADLER AND G. CSOBÁN (STOMATOLOGICAL CLINIC AND PHARMACOLOGICAL INST. UNIV. DEBRECEN): PROTECTIVE EFFECTS ON ENAMEL SOLUBILITY.

Solution of *Ca* and *P* from pulverized enamel was examined after previous treatment by different electrolytes, in veronal buffer solution of *pH* 3,95. Dissolving of *P* is most markedly lessened by action of *Al*, while simultaneously solution of *Ca* is promoted. *Mn* ions exert a bi-phasic action. Fluorides exert a protective action upon solubility of *Ca* and *P* of approximately 30%. — The protective effect of lead-nitrate was less marked.

J. J. STRAUB (MEDICOCHEMICAL INST. UNIV. DEBRECEN): DATA TO BIOLOGY OF FLUORINE.

Several Hungarian drinking waters were examined and proved to be poor in fluorine. In 90% the fluorine content is below 0,3 mg %. Scarcity of fluorine in water and soil may be a causative factor of high caries frequency in Hungary.

Enamel treated by fluorides adsorbes readily *F*-ions in considerable quantity and in 10 minutes the *F*-content increases from 7,6 mg % to 12 mg % in 60 minutes up to 34,8 mg %. *F*-ions enter into apatite molecules instead of hydroxilions.

I. PORCSALMY, (MEDICOCHEMICAL INST. UNIV. DEBRECEN): MICROVOLUME DETERMINATION OF NICOTINE IN TOBACCO.

Basis of the micromethod is the following: solution of the nicotine from the alkalized tobaccopowder with a mixture of ether-petrolether. The simultaneously delivered ammoniak can be removed without loss, determining the content per cm^3 of the residue, we receive directly the percentage of nicotine. The results of the micromethod correspond with the results of the *Pfyl-Schmitt's* standard method, modified by *Pyriki*.

F. GUBA, AND B. HORVÁTH, (BIOCHEMICAL INST. UNIV. BUDAPEST): NEW METHOD FOR THE DETERMINATION OF THIOL GROUPS OF PROTEINS.

Principle of the determination: — Bromacetophenone (BAP) combines with the *SH* groups of proteins. This reaction is specific and irreversible. The protein-bound BAP is removed from the solution by precipitating the protein with sulphosalycilic acid, and the excess BAP is determined as a ketone. In alkaline solution BAP forms a pink compound with *m*-dinitrobenzol (DNB). The intensity of the colour is estimated in the Pulfrich Stufen Photometer using the *S* 53 filter. Effects of *pH*, salt concentration, time and temperature of incubation on the intensity of the pink colour were studied. Of these only the effect of the salt concentration appears to be significant. *SH* groups of twice crystallized egg albumin were determined by this method. The results are in good accordance with the findings of *Anson*.

J. SÓS, (PATHOPHYSIOLOGICAL INST. UNIV. BUDAPEST): THE EFFECT OF ORALLY ADMINISTERED METHYL-RED AND NIGROSINE.

Methyl-red (4-dimethylanilin — azobenzene — carbonic acid) in daily doses of 0,25 mg caused in 120 days hepatitis, cirrhotic degeneration and praecarcinotic changes in the liver of mice. Spherocytic infiltration, wildly grown connective tissue, moreover atypical multipolar mytoses, syncitic polynuclear cells, anaplasia disorder of the liver structure was observed. The induction of liver changes was observed in all the dyestuff fed mice (50). The livers of the control animals remained normal. Nigrosine does not cause any significant pathological changes.

S. JÓNÁS, (PHARMACOLOGICAL INST. UNIV. BUDAPEST): MODIFIED METHOD FOR THE TITRATION OF PENICILLIN WITH SERIAL DILUTION IN URINE.

A new method is described for the estimation of penicillin with serial dilution in both glucose buffered at *pH* 7,6 containing phenol red as indicator against subcultures of a Staphylococcus *H* strain. The method was applied to measure the penicillin content of steril urine. By a much more simplified technic then that of Fleming's the method ennables the estimation of 0,02 units of penicillin.

J. KOVÁTS, (2.ND MED. CLIN. UNIV. BUDAPEST): ON THE MYOSIN OF THE HEART.

The myosin/actin ratio of normal healthy pig-, rabbit- and sheephearts was tested. Myosin was extracted by the method of Á. Csapó. Determination of viscosity were carried out in the *Ostwald* viscosimeter as well as in a modified *Couette* viscosimeter. Differences were found in the myosin-actin ratio between the right and left ventricles and the auricles. Most actin was found — counted for equal amounts of myosin — in the left ventricle, less in the right one, and only a very small quantity in the auricle.

F. GUBA, (BIOCHEMICAL INST. UNIV. BUDAPEST): THE ROLE OF *SH* GROUPS OF THE MYOSIN.

Crystalline-myosin is a protein containing free *SH*-groups. According to the quantitative determination of its *SH*-groups, 17,600 grs, unity weight of myosin contain about one *M SH*. The *SH* groups of myosin are considered as bridges connecting myosin with actin. Closer examination of *SH* groups has proved this statement only in part. About 80 per cent of the *SH* groups are taking part in the combination of myosin and actin the remaining 20% serve to bind ATP. Probably the *SH* groups involved in the actin-myosin combination are responsible for the ATP-ase activity of myosin. These statements are supported by *SH*-determinations, viscosimetric and enzymatic experiments.

G. MANSFELD (PHYSIOLOGICAL INST. UNIV. OF BUDAPEST): NEW INVESTIGATIONS ABOUT INFECTION AND IMMUNITY.

Former experiments showed that in infectious diseases the viruses and toxins accumulate in the midbrain and reach the peripheral organs by wandering along the nervous pathways. This made it likely that the cause of immunity is the blockade of the nerve cells against these toxins rather than the result of the appearance of immune-bodies in blood and tissue fluids. This hypothesis is supported by the following experiments:

1. If a rabbit is inoculated with variola vaccine and, after immunity has developped, is sewn together with another rabbit so that their circulations communicate (parabiosis), then the second rabbit will not develop immunity.

2. If however, the first rabbit is inoculated when he is already in common circulation with the other, both animals will become immune.

3. If rabbits are inoculated *once* with the brain of rabbits infected with tuberculosis, and 2 weeks later are infected with the same tuberculosis strain as the "brain-donor", the animals will die within 1 to 12 days. If, however, they are inoculated with the brains of infected animals throughout a period of 3 weeks, $^2/_3$ of them will develop immunity against tuberculotic infection.

D. BAGDY, (BIOCHEMICAL INST. UNIV. BUDAPEST): TRANSITION OF FIBRINOGEN TO FIBRIN.

The clotting of fibrinogen with thrombin is only possible at *pH* values above the isoelectric point of fibrinogen. At about *pH* 5,0—5,3 fibrinogen does not clot, but it can be shown, that thrombin has an effect on it even at this *pH*. After neutralisation clotting will be brought about the sooner the longer fibrinogen and thrombin are kept together and after a certain incubation period, depending on the quantity of thrombin, the reaction mixture clots immediately when neutralized. This proves that the transition of fibrinogen to fibrin takes place in two steps. Experiments elucidating the nature of the primary reaction between fibrinogen and thrombin show that *SH*-groups cannot play any role in the coagulation process. The first period of the reaction can be inhibited by oxalate and fluoride. This fact is analogous to the observation of *Laki* and *Lóránd* who isolated a copper-containing, bluish coloured thrombin with an oxydase activity, which could be inhibited by the mentioned substances.

E. KOKAS, AND I. BANGA, (PHYSIOLOGICAL AND BIOCHEMICAL INSTITUTES, UNIV. BUDAPEST): THE BIOLOGICAL DEFINITION OF "CORHORMON" AND ITS CHEMICAL PROPERTIES.

Authors established that "Corhormon" shows a positive chronotropic and inotropic effect on the quininized frogheart. This effect developes gradually and

remains on its maximal level for a very long time. The "Corhormon" effect results from many components. A substance with positive chronotropic and inotropic effect can be isolated from "Corhormon" by dialysis. This substance ist thermostabile. The well known nuclein-acids, like the nucleinacids of the liver, thymus and yeasts show generally a negative effect on the quininized frogheart. The positive effect, if it is observable at all, is not stronger than 10—15 per cent of the "Corhormon"-effect. The nuclein-acid obtained from pulverized heart shows a much weaker effect, than "Corhormon". The strongly chronotropic and inotropic substance, which could be isolated from "Corhormon" contained 67 per cent *N* and 7 per cent *Mg*, but neither organic phosphorus nor strongly reducing substances could be found. Authors come to the result, that the effective substance of "Corhormon" does not belong to the group of the thus far known nucleosides or nucleotides, it has to be a base. The pure chemical structure of this base could thus far not to be detected, but further investigations are going on. This substance can not at all identified with histamine. Authors investigate the effect of the isolated substance on heartcells in kernelcultures to decide whether it can be identified with "Corhormon" or not.

I. GÁL, (MED. CLIN. UNIV. DEBRECEN): NEPHELOMETRIC METHOD FOR SERUMCHOLINESTERASE DETERMINATION. PHARMACOLOGICAL AND CLINICAL EXPERIENCES.

A nephelometric method for determination of serumcholinesterase activity is described. The hydrolysis of acetylcholine follows the monomolecular reaction type. Esterase activities of normal human sera have a Gauss-variation-like distribution. The effect on cholinesterase activity of any watersoluble agent can be readily and graphically demonstrated. Though the serumcholinesterase activity and the autonomous nervous system are in close relation, the former is also dependent on the function of he liver and the serum-protein-spectrum.

P. NÁNÁSI, AND B. TANKÓ, (INST. OF ORG. CHEM. UNIV. DEBRECEN): INVESTIGATIONS ON THE CHEMISTRY OF ADENINE NUCLEOTIDES.

For the preparation of pure cozymase *Ohlmeyer's* method was combined with *Williamson* and *Green's* method and so better yields were attained by simpler procedure. For the preparation of AP (Adenylic acid) and ATP and to obtain better yields several modifications on *Kerr's* procedure were made in converting ATP into AP in part. — Splitting of ATP by bone phosphatase was studied to explain the discrepancies between *Barrenscheen* and *Joachimowitz's* result (Biochem. Zeitschr. *292*, 350, 1937) and the *Lohmann* formula corroborated by the recent synthesis of an ATP by *Baddiley, Michelson,* and *Todd* (Nature *161*, 761, 1948). It was found that contrary to *Barrenscheen* and *Joachimowitz's* observation all three *P* atoms were set free (as it seemed, simultaneously) without any sign of the more acid resistant *P* being split separately and the labile *P* remaining in organic linkage. Since the bone phosphatase acted on inorganic pyrophosphate as well, the findings of the Austrian authors still remain unexplained. So further investigation is wanted, to prove whether the ATP synthetized by *Todd* et. al. represents the only form, which is active biologically.

J. BODNÁR, Ö. SZÉP AND GRUSZ, E. MEDICOCHEMICAL INST. UNIV. DEBRECEN): NEW VIEW-POINTS IN THE DETERMINATION OF ARSENICPOISONING.

The examination of the usual organs has not given a positive result in two lethal cases of chronic poisoning. The hair and fingernails contained much arsenic (maximum 6120%). Therefore it is very important to determine arsenic in the hair and fingernails which are regulary disregarded.

G. STERN, K. BIRÓ, AND PETTKO, E. (BIOCHEMICAL INST. UNIV. SZEGED): THE INTERACTION OF ACTOMYOSIN AND ADENOSINETRIPHOSPHATE.

Adenosintriphosphate (ATP), inorganic triphosphate, pyrophosphate and calgon form dissociating complexes with actomyosin, but only in presence of *Mg* or *Ca* ions. The function of these ions is to decrease the negative charge of the

molecule, thus facilitating its association with the negatively charged protein. At least 140.000 g of myosin are needed to bind 1 g mol of ATP. There is no valid evidence to show that myosin is able to bind more than this amount of ATP. It is concluded that most of the ATP in the muscle tissue is in the free state.

G. FEUER, AND F. B. STRAUB, (BIOCHEMICAL INST. UNIV. SZEGED): THE PROSTHETIC GROUP OF ACTIN.

The prosthetic group of actin, which is lost on dialysis or isoelectric washing of actin, was isolated in pure form. The substance is similar to adenosine triphosphate, but not identical. When a purified preparation of adenosintriphosphatase from potato is added to an actin solution, the latter protein loses its ability to polymerize on addition of inorganic ions. This property is however restored on addition of the purified prosthetic group preparation. The prosthetic group is split by adenosinetriphosphatase and by myosin.

F. B. STRAUB, AND G. FEUER, (BIOCHEMICAL INST. UNIV. SZEGED): THE PHYSIOLOGICAL SIGNIFICANCE OF THE POLYMERISATION OF ACTIN.

Actin polymerizes on addition of inorganic ions and the rate of the polymerisation depends on the nature of the ion added. There is a distinct antagonism of *K* and *Ca* ions at certain concentrations. Of the drugs which act directly on the muscle, adrenaline greatly enhances, veratrine, quinine and strychnine significantly reduce the rate of polymerisation. These effects however, are variable according to the ionic milieu, they are especially dependent on the ratio of *K* and *Ca*. These observations lend further support for the assumption that the polymerisation of actin plays an important role in muscle contraction.

N. A. BIRÓ, AND A. E. SZENT-GYÖRGYI (BIOCHEMICAL INST. UNIV. BUDAPEST).

The ATP-splitting activity of actomyosin depends to a great extent on factors which control the "contraction" of actomyosin as *K*, *Cl*, ATP, *H*-ion concentration, — temperature and actin content. The enzymic activity is optimal under circumstances which are causing maximal contraction. According to our experiments we may suppose the completely uncontracted actomyosin to be entirely inactive enzymatically, although in the presence of ATP it is not quite possible to a realize this state of actomyosin experimentally. The addition of actin to myosin can thus have a changing or an inhibiting effect on the enzym activity depending on the extent to which the formed actomyosin contracts under the prevailing experimental conditions. If these conditions are favourable to the disocciation of actomyosin i. e. if there is no formation of actomyosin at all, the actin has no effect on the splitting of ATP. We presume the manyfold ways of control of the enzym activity of actomyosin to play perhaps a role in the physiological control of the energy-transport of the muscle.

J. KUCHÁRIK, (2ND MED. CLIN. UNIV. BUDAPEST): EXPERIMENTS RELATING CARBONIC-ACID-ANHYDRASE.

Quantitative estimation of carbonicacid — anhydrase in human blood was carried out by the method of *Roughton* and *Boot*, which is precise enough for clinical purposes. Carbonic acid was determined according the method of *Van Slyke*. In 37 subjects the quantity of ferment in 100 cc blood was found to range from 2—3 thousand to 18 thousand units, the carbonic acid content varying from 30—53 per cent without any correspondence between them i. e.: low ferment content was not associated with high quantities of carbonic acid. It is presumed, that the role of the carbonicacid-anhydrase is more important in other metabolic changes such as decarboxyilation, synthesis of carbamid, calciumcarbonat deposition etc. than in the regulation of the carbonic acid content of blood.

B. LUTTER, AND B. TANKÓ, (INST. OF ORG. CHEM. UNIV. DEBRECEN): ON PARTICIPATION OF PHOSPHATASES IN THE AMYLOLYTIC BREAKDOWN OF STARCH.

Flower and malt from wheat were extracted with 5% *NaCl*, preparations made by fractionating with various concentrations of alcohol and their splitting action on starch and fructosediphosphate was followed. In the fraction obtained

with 57—62% alcohol from malt extract the phosphatatic power was accumulated, amylolysis being fairly low. This preparation split starch into products giving no colour with iodine, and having low reducing power. Purified beta-amylase (from wheat) combined with this preparation split 88% of starch into maltose. The results suggest, that to the breakdown of starch besides alfa and beta amylase a phosphatase is required, whose action may consist of splitting phosphate attached to side chain linkages.

I: ZÁDOR, (PSYCHIATR. AND NEUROL CLINIC. UNIV. BUDAPEST) NEUROPHYSIOLOGICAL INVESTIGATIONS WITH EVIPAN.

The use of Evipan for neurophysiological experiments is based on the property of the nervous system that Evipan affinity of the different layers of the nervous system follow phylogenetic evolution in inversed order. In every case it is possible to provoke a Trömner—Hoffmann reflex with Evipan, and other deficiency symptoms of the different layers also appear. After use of Evipan the minimal neuropathological symptoms appear markedly. It can be applied both for diagnostical and therapeutical purposes. The symptomes appear immediately and are completely reversible.

J. SZENTÁGOTHAI, (ANATOMICAL INST. UNIV. PÉCS): THE ANATOMICAL BASIS OF CENTRAL INHIBITION.

One of the most important advances of modern neurophysiology is the work of D. P. C. *Lloyd* (1946), which has given evidence that reflex inhibition may occur by interaction of two neuron reflexes, when an afferent volley arising from large, low-threshold afferent fibers coming from an extensor was found to inhibit the two-neuron-arc reflex discharge in the motor nerve of a flexor acting on the same joint. Time relations were excluding the primary activity of any internuntial neurons. These results have thrown new light on observations made by the author for several years, when working with the bouton-degeneration-method. The author noticed, that in several brainstem and spinal reflex arcs, when the forelast neuron was destroyed, generally more degenerating end-boutons were to be found on each of the ganglion sells supplying the agonist, while on the ganglion cells supplying the antagonist generally few (rarely more, than one) degenerating boutons could be detected. Inferences concerning the significance on these few boutons on the ganglion cells of the antagonist were impossible while inhibition was thought to be due primarily, to internuntial neurons, but since several examples of direct inhibition became known, which must be ascribed to the synapses on the motor ganglion cell, the author draws attention upon the possibility of opposite (excitatory) boutons — belonging to a certain path- on the soma of the ganglion cells.

I. HUSZÁK, AND E. DOMOKOS, (NEUROLOGICAL CLIN. UNIV. SZEGED): HISTAMINOLYTIC PROCESSES IN THE CENTRAL NERVOUS SYSTEM.

In order to clear up the pathomechanism of the allergic and hyperergic reaction of nervous tissue the authors examined histamine production and decomposition in the different parts of the nervous system. In the present work they deal with histaminolytic processes. With the help of chemical and biological methods they stated that under aerobic conditions a considerable decomposition of histamin can be observed only in the white matter, the histamin decomposing capacity of the gray matter is about one tenth of the white.

I. HUSZÁK, AND M. WOLLEMANN, (NEUROLOGICAL AND PSYCHIATRIC CLINIC, UNIV. SZEGED): CONCERNING THE ORIGIN OF ACETYLCHOLINE ESTERASE IN PATHOLOGICAL CEREBROSPINAL FLUIDS.

Authors have determined the acetylcholine esterase (*AChE*) content of 50 pathological and 10 normal cerebrospinal fluids, employing titration and Warburg's manometrical method. They established that in normal cerebrospinal fluids acetylcholine decomposition does not essentially exeed in 1 hour the values of autohydrolysis (average 1—2%). The amount of *AChE* shows a parallelism with the protein content of pathological cerebrospinal fluids. From a clinical diagnostical point

of view it is of importance to investigate where under pathological circumstances the *AChE* of the cerebrospinal fluid originates. The fact has been established that there are specific and non specific *AChE*-s *(Mendell, Mundel* and *Rudney)*. Specific *AChE* hydrolyzes only acetylcholine and can specifically be inhibited by caffeine *(Nachmansohn, Schneemann, Bodansky)* the brain *AChE* represent the specific *AChE*. Serum *AChE* is nonspecific as it also splits other esters besides acetylcholine e. g. tributyrine. This physiological observation seemed an appropriate method for deciding the origin of enhanced *AChE* in pathological cerebrospinal fluids, as to whether it originates in the blood or in the brain parenchyme. The obtained results were as follows: amongst cerebrospinal fluids with high *AChE* content pronounced inhibition with caffeine was observed in general paralysis, malignant intracerebral tumors and emollition of the brain. In the initial stage of Heine—Medin, there is no caffeine inhibition, after the first week, however slight caffeine inhibition could be observed. In the case of other diseases with an increased protein content and an enhanced *AChE* activity (meningitis, encephalitis, apoplexia, polyneuroradiculitis, complete block) no caffeine inhibition was found or if it occured the degree of inhibition was within the limits of experimental error. Authors find their method to be an appropriate expedient for estimating in vivo the destruction of brain tissue.

S. LÁNG, (PSYCH. AND NEUR. CLINIC. UNIV. BUDAPEST): WORK-EXPERIMENTS IN HYPNOSIS.

The red blood-corpuscle count and blood-sugar level of hypnotized patients, to whom slight physical work has been suggerated, shows no change, while after suggeration of heavy work, there is an increase in both cell count and blood sugar level. These results coincide with the effects of actual work as found in animal experiments.

A. RAZGHA, (GENERAL PATHOL. AND PHARM. INST. UNIV. BUDAPEST): INCREASE OF THE EFFECT OF ADRENALIN ADMINISTERED INTO THE SUBCUTIS.

A quantity of adrenaline inefficacious alone, may, in man, bring about an increase of 30 to 60 mm in the blood pressure if histamin is added to it. The quantity of histamin adhering to the syringe after it has been washed with a 1‰ solution is sufficient for this action. The effect is missing if the two drugs are injected to different different places in separate syringes. It may be seen from these facts that there is no true synergism but the absorption of adrenalin from the subcutis is promoted by histamin which abolishes the local vascular spasm due to adrenaline. The assumption that histamin has, in this case no sensitising effect, may also be seen from the fact that other vasodilating drugs have the same action (acetylcholin padutin, adenosinetriphosphoric acid) if they are added in adequate quantitaties to adrenaline.

A. TIGYI, AND J. MARTIN (PHYSIOLOGICAL INST. UNIV. PÉCS): THE EFFECT OF DENERVATION ON THE BRONCHIAL REACTION OF ADRENALINE.

The adrenaline and acetylcholine sensibility of sympathectomized and parasympathectomized bronchi of cats, was estimated. The corresponding vagus fibres were divided in cats. After ten days of degeneration the adrenaline, acetylcholine and histamine sensibility of the denervated and normal animals was compared. Bronchial sensibility was asseyed by the method of Went—Drinker. It is concluded that there is no difference in the adrenaline and acetylcholine sensibility of denervated and normal cats.

A. ÁNGYÁN, AND E. VARGA, (PHYSIOLOGICAL INST. UNIV. PÉCS, PHYSIOLOGICAL INST. UNIV. DEBRECEN): ACTION OF ADRENALINE ON DIFFERENT STAGES OF NEUROMUSCULAR TRANSMISSION.

On the Sherrington mammalian gastrocnemius nerve-muscle preparation of dogs and cats, it was found with frequent tetanic stimulation (60—80/sec) of the sciatic nerve, that adrenaline, sympatol and sympathetic stimulation causes typical changes in the tension of fatigued muscle, related to the five stages of neuro-

muscular transmission, first described by *Cannon, Lissák, Rosenblueth* and *Luco*. Small amounts (2—10 ug/kg) of adrenaline and stimulation of the lumbal sympathetic chain evoked a transitional rise of the fatigue curves in the 3d stage only, while a depressing effect was seen by intravenous administration of larger (20—25 μg/kg) doses of adrenaline in both the 3d and the 5th stages whereas during the 4th stage of complete fatigue, all above mentioned stimuli were without effect. In the fifth stage (definite rise of the contraction) adrenaline, pressor substances and sympathetic stimulation cause depression. These changes coincide with general blood pressure changes, however they are more characteristically altered by changes in the so called five stages of neuromuscular transmission and they can suggest a direct action on the neuromuscular synapsis as assumed by *Orbeli* and recently by *Bülbring* and *Burn*.

D. NAGY, (PHYSIOLOGICAL INST. UNIV. PÉCS): THE MECHANISM OF CHEMICAL TRANSMISSION IN THE UTERI OF DOGS AND CATS.

Stimulation of the nervus hypogastricus in pelvectomized pregnant and nonpregnant dogs and cats caused the liberation of an adrenaline-like substance the effect of which was tested on blood pressure and on isolated uteri. In parallel experiments, carried out by stimulation of the hypogastricus or the pelvicus on normal and stimulation of the pelvicus on sympathectomized animals, the liberated substances, being estimated in the same way, showed no definite action. Thus the chief motor nerve of the uterus in dogs and cats is the nervus hypogastricus, through which impulses to postganglionic adrenergic fibres are conducted, arising from the small ganglia of the plexus hypogastricus. At least, the impulse acts by liberation of adrenaline, whether the animal is pregnant or not. Acetylcholine, however is also liberated in the sympathetic ganglia and may affect the results.

K. LISSÁK, AND C. MARTIN, (PHYSIOLOGICAL INST. UNIV. PÉCS): THE ANEURINE CONTENT OF CHOLINERGIC AND ADRENERGIC NERVES.

Aneurine determinations were made with the method of *Westenbrink* and *Karrer* on adrenergic, cholinergic and mixed nerves of dogs. The pure adrenergic fibres of the superior mesenteric plexus contain aneurine. The Wallerian degeneration of the n. ischiadicus results in a strong decrease or disappearance of aneurin. Compared to the retina the sensory nerves contain a high amount of aneurine. The fact, that sensory nerves contain a fair amount of aneurine but very little, acetylcholine and that even adrenergic fibres lacking acetylcholine contain aneurine permits the inference that either the role of aneurine is not confined to the acetylcholine-cholinesterase system and participates as an active substance in the adrenergic mechanism as well or that aneurine is not a substance of action at all, and serves in both kinds of nerves only as cocarboxylase in carbohydrate metabolism.

C. MARTIN, AND K. LISSÁK, (PHYSIOLOGICAL INST. UNIV. PÉCS): THE ANEURINE CONTENT OF THE NERVES OF B_1-AVITAMINOTIC AND NORMAL RATS.

Aneurine determinations were made with the methode of *Westenbrink and Karrer* on the brain and n. ischiadicus of normal and B_1-avitaminotic rats. The aneurin content of the n. ischiadici were found to be 80%, the brain 56% lower in B_1-avitaminotic rats. These experiments compared with previous observations of the authors show, that a considerable loss of acetylcholine causes no perceptible change in nerve function, these results show that a loss of aneurine may leave the function of peripheral nerves apparently unaltered. Decrease of aneurine content of the central nervous system in agreement with results of earlier investigations support the view that the well known serious nervous symptoms of B_1-avitaminosis are caused by functional disturbances, arising from the loss of aneurine in the central nervous system.

J. MARTIN, K. LISSÁK, AND A. ÁNGYÁN, (PHYSIOLOGICAL INST. UNIV. PÉCS): ACTION OF ANEURINE ON MUSCLE FATIGUE.

Contrary to the widespread opinion, that vitamine B_1-exerts a beneficial effect on muscular exercise and fatigue, the authors showed, on Sherringtons nerve-

muscle preparation, of cats that in chloralose narcosis previous or simultaneous i. v. administration of different amounts of aneurine causes a characteristic, transient depression of the fatigue curves. The contraction of the gastrocnemius after the Waller degeneration of the sciatic nerve is, contrary to *Chauchard*, uninfluenced and the fatigue curves are depressed, when aneurine is given. A marked depression was seen after aneurine injections during the fifth stage of neuromuscular transmission. Possible interpretation of these depressing effects is the inhibition of acetylcholine (or that of cholinesterase activity) at the neuromuscular synapsis, as shown contrary to *Minz* by various authors. These results confirm earlier observations (*Lissák, Varga:* Berichte über die gesamte Physiologie 134 : 181, 1943) according to which in B_1-vitamine depleted rats neuromuscular transmission is unaltered and indirect excitability rather augmented.

T. STÜRZER, (PHARMACOLOGICAL INST. UNIV. SZEGED): A METHOD, USED TO HINDER OR RELEASE STRYCHNINE CONVULSIONS.

If we fixe the hind legs of a mouse, rat or rabbit, to whom lethal doses of strychnine were given, convulsions do not appear and the animals remain alive, but by giving up this position, a tetanus develops and the animals die. The development of this tetanus could be inhibited or stopped, by fixing the fore and hind limbs, and by a slight compression of the thorax, even if $2—2^1/_2$ lethal doses were injected, to the animals. Applying the method described here, only a part of the animals was killed by a respiration paralysis, without any convulsions after the administration of large amounts of the drug; another part of them remained alive.

I. KARÁDY, AND A. KOVÁCS, (MED. CLIN. UNIV. SZEGED): ADAPTATION MECHANISM OF THE ORGANISM TO DAMAGE: THE ROLE OF "RESISTINE".

It could be shown on rats and mice, using the Indian ink method devised by Jancsó, that the exposure of the organism to various damaging stimuli (surgical trauma, *X*-ray irradiation, starving, exposure to cold, injection of formaldehyde, adrenaline, agmatine, etc). leads always to local and often general release of histamine. It could be shown further that the histamine liberated by certain damages brings about as a basic factor all the changes characteristic of "alarm reaction" causes shock and plays also an essential part in setting in operation the defence mechanism of the organism, resulting finally in acquired increased resistence. In this stage of resistance both the prolongation of circulation time of the Indian ink injected intravenously (it circulates in the blood about twice as long as in normal control animals) and the diminished phagocytic activity of the surviving liver of a resistant rat particularly when the animal has been again exposed to damage just prior to the perfusion experiment (the amount of Indian ink absorbed during 40 minutes of perfusion was 25 per cent less and in case of repeated exposure of the animal prior to the perfusion experiment 50 per cent less than that absorbed by the surviving liver of normal control animals) indicate that the increased resistence of the organism is caused by an excess formation of a substance possessing antihistamine activity, called by us "Resistine". The antihistamine activity of resistine produced in the organism by its own cells in great amount, and stored, will be apparent especially when needed, that is, when the resistant organism has to face further damage. Experiments concerning the chemical nature of resistine, the mechanism of its formation and its patho-physiological importance, other than the role of rendering the organism resistant, are already under way.

IVANOVICS, GY. AND HORVÁTH, S. (PATHOLOGICAL INST. UNIV. SZEGED): THE OCCURENCE OF ANTIBACTERIAL SUBSTANCES IN THE MEMBERS OF CRUCIFERAE.

As it was found earlier by the authors, the seeds of radish are containing an inactive precursor which is transformed by a concomitant enzyme to a potent antibiotic which has been termed raphanin. In the present investigation an alcoholic extract of radishseeds was concentrated and purified, and preparations obtained contained about 30 per cent of the precursor. The concomitant enzyme was isolated from the watery extract of seeds by ethanol fractionation. The enzyme was found to be highly resistent to heat, however its action was destroyed by heating

for 30 minutes at 75—80 C°. The same enzyme is occuring in the seeds of other cruciferae, viz. Sinapsis alba, Brassica oleracea, Rorippa islandica, Cheiratus cheiri, Hesperis matronalis etc. and also in the root of radish which latter does not contain the antibiotic. By crushing and extracting seeds of some cruciferae an antibacterial principle was found in the watery extracts wich exerted a definite antibiotic effect on staphylococci and B. coli. The distribution of this antibiotic among the members of cruciferae was not paralelled with the presence of enzyme capable of transforming the precursor of raphanin. Thus the watery extract of seeds of Sinapsis alba, Rorippa islandica and Diplotaxis muralis was devoid of any antibacterial activity, although the presence of the enzyme could be shown in their extract. The authors believe that the enzyme is identical with myrosine and some of the antibacterial principles occuring in the seeds of cruciferae probably are mustard oils, however the raphanin can not be identified as mustard oil up to present time.

I. WENT, AND L. KESZTYÜS, (PHYSIOLOGICAL AND PATHOLOGICAL INST. UNIV. DEBRECEN): HISTAMINE ANTIGEN.

The biological effects of the antibodies produced by a histamine antigen (histaminazobenzol azoprotein) were studied. These effects have been tested on the body temperature and on isolated organs of white rats and on sensitized guinea pigs. On rats immunised with histamine azoproteins, the action of toxic histamine doses on body temperature did not develop. Sera of immunised rats protect normal animals against the temperature decreasing and other toxic effects of larger histamine doses. The inhibiting action of antihistamine could also be demonstrated on isolated organs of immunised rats. On sensitized guinea pigs immunised with histamine azoproteinc, the lethal bronchiolar spasm, characteristic of anaphylactic shock, never developed. This indicates that by immunisation with histamine azoproteins sensitized guinea pigs can be protected against the lethal consequences of asphyxiation, following the reinjection of the homologous protein.

GY. LUDÁNY, F. KOKAS, AND GY. VAJDA, (2D CHIRURGICAL CLINIC. AND INST. OF GEN. PATH. UNIV. BUDAPEST): SUPRARENAL GLAND AND THE PHAGOCYTOSIS STIMULATING CAPACITY OF THE BLOODSERUM.

It was ascertained in previous researches that the normal immune substances of serum (protective substances, complement, bactericid substances resp. capacity of stimulating phagocytosis) are subject to sympathetic stimulation and parasympathetic inhibition. In whatewer way sympathetic excitation is elicited, the phagocytosis stimulating capacity of serum increases considerably (Ludány). Phagocytosis was determined by Wright's method. In dogs under pernocton narcosis the phagocytosis stimulating power of adrenal venous blood increases even by 100—200% following direct excitation of the nervus splanchnicus. If the adrenal veins on both sides are ligated the excitation of the splanchnic nerve is ineffective. The increase of phagocytosis stimulating capacity is absent also following asphyxia (central sympathetic excitation) if the adrenal glands are exstirpated or the veins flowing thereof are ligated. Therefore the adrenal glands play a decisive role in the increase of phagocytosis stimulating capacity following sympathetic excitation. Adrenalin itself, resp. cortex extracts (Cortigen—Richter) in vitro have no effect on phagocytosis. In animals whose adrenal veins have been ligated adrenalin equally augments the phagocytosis increasing capacity of serum. Thus it has become evident that the level of protective substances of serum is also under the control of the vegetative nervous system. The phenomenon may be ranked among the Cannon sympathico-adrenal alarm reactions and its more precise mechanism is to be defined by means of further investigation.

I. TÖRŐ, (INST. OF HISTOLOGY, ANATOMY AND EMBRYOLOGY UNIV. DEBRECEN): THE "STORAGE-CAPACITY" OF THE RETICULOENDOTHELIAL SYSTEM.

Thus far an adequate and exacte procedure for the measurement of the storage capacity of the Res. was lacking. The author tried in his investigations to determine the storage capacity of the Res. and to measure the effect of a substance named "Resactor". "Resactor" is a substance, won from foetal liver pro-

ducing excitation of the Res. The essence of the authors procedure is, that after intravenous injection of a fixed quantity of "Collargol" one determines with dithisonic titration the quantity of the silver which is storaged in the weight-unit of the liver. From the weight unit and from the weight of the whole liver one can establish the whole silver quantity which is stored in the liver. This method makes it possible to determine, whether some substance has an "exciting" effect on the Res. or not. The author fixed that the so called "blockade" occurs only in 50 per cent and that colloidal copper can not increase the blockade. Resactor causes an increase of the storage capacity with 20—30 per cent. It was established that the liver needs proteins for the production of "Resactor" therefore during the absorption of proteins the storage-capacity of the liver increases. That fact indicates that the defensive capacity of the liver can be increased by proteins.

I. NIKODÉMUSZ, T. SZILÁGYI, AND KESZTYÜS, L. (PHYSIOLOGICAL INST. UNIV. DEBRECEN): THE ANTIGENIC PROPERTIES OF MYOSIN.

The authors produced a serum precipitating myosin of rabbits by treating dogs with rabbit-myosin. The myosin has antigenic properties and species specifity. The myosin antiserum does not precipitate actin. That myosin, produced according to Szent-Györgyi's method — is free from actin can be demonstrated by immunological tests. The rabbit's actin has also antigenic properties, but this is an isoantigen and organ specific. Actin antiserum does not precipitate myosin, so actin prepared according to Straub's method — is clear too. Seeing that both the actin — and the myosin-antiserum precipitate acto-myosin the authors suppose that either these parts of myosin and actin molecules which determine the antigenic character are unchanged in actomyosin or some quantities of actin and myosin do not take part in the junction. The *G*-actin antiserum precipitates *F*- and *G*-actin as well, therefore it is evident that the activation of actin passes over without any chemical change of antigenic character.

K. LAKI, AND L. LÓRÁND, (BIOCHEMICAL INST. UNIV. BUDAPEST): ON THE HAEMOPHILIAN FACTOR.

It is known that beef-plasma filtered through a Berkefeld-bacteria filter can be considered regarding its clotting proporties as haemophilic. The authors succeded in purifying from beef-plasma the factor which can correct this defect. The principle of the isolation is adsorption on caolin at a slightly acid *pH* and two subsequent elutions from the caolin with an alkaline borate solution. The active substance is thermolabile, the activating energy for heat-denaturation is about 50.000 cal. Therefore it is presumed that this substance is a protein compound. The isolated substance is able to correct the clotting defect of the real haemophilian blood too, as it has been checked by the authors in two clinical cases.

I. CSEFKÓ, (PATHOLOGICAL INST. UNIV. BUDAPEST) INTERPRETATION OF SHOCK PHENOMENA ON THE BASIS OF THROMBIN INACTIVATION.

Investigations carried out independently by various authors show that there is a close connection between RES function, blood clotting and the shock problem. Having demonstrated the effect exercised by histamine on blood clotting in vitro, or on thrombin inactivation the author investigated how thrombin inactivation changes under the effect of intravenous histamine and in the case of anaphylaxis and peptone shock. From comparison of the curves obtained in these experiments it can be stated that in shock histamine reduces inactivation at the beginning then, rising above the normal value, after several compensatory wawes, returns to the normal. In anaphylaxis and peptone shock the phenomenon occurs contrarywise. Here inactivation increases in the beginning then sinks below the normal value, and becomes normal again, after several periodic wawes. Decrease and increase in inactivation are therefore connected with one another, phenomena always succeeding one another, succeeding each other periodically after disturbances in the blood's histamine-heparin equilibrium.

F. SZONTÁGH, (OBSTETR. AND GYNAEC. CLINIC. UNIV. PÉCS): CAPILLARY PERMEABILITY, SERUM PROTEINS AND HAEMATOCRIT VALUES IN NORMAL PREGNANCY.

Using *Landis'* method, a significant increase of capillary permeability could be demonstrated in normal pregnancy. The high protein concentration of capillary filtrate is especially striking nearing term. Serum protein content and haematocrit values decrease in the course of pregnancy. Decrease of proteins is due to the albumin content of the capillary filtrate. In a few cases of eclamptic toxemia, investigated so far, the protein content of the increased capillary filtrate approaches the figures, found in the corresponding serum.

I. GÖNCZÖL, L. KESZTYÜS, (PHYSIOLOGICAL INST. UNIV. DEBRECEN) AND T. VÁLYI-NAGY, (PHARMACOLOGICAL INST. UNIV. DEBRECEN): THE METHAEMOGLOBIN-FORMING PROPERTIES OF ISOMER AMINOPHENOLS.

The ortho- and paraisomer phenols form much more methaemoglobin both in vitro and in vivo than accounted for stoechiometrically the metaisomer forms methaemoglobin too, but not through the same mechanism. Alcohol does not influence significantly the quantities of methaemoglobin formed by aminophenols. The isomer nitrophenols do not for methaemoglobin. Since the nitrophenols take up a chinoid-structure in alkaline medium and in spite of this do not react with haemoglobin it is evident that the β-hydroxylamine-nitrobenzole oxydo-reduction system is essential in the catalytic chain-reaction of methaemoglobin-forming aromatic *N*-derivatives and not in the transformation of chinoid structure of the molecules.

L. SZEKERES, S., KOVÁCS, L. POZSONYI, AND MÉHES, GY. (PHARMACOLOGICAL INST. UNIV. PÉCS): THE EFFECT OF THYMIN AND FOLIC ACID ON EXPERIMENTAL, ANAEMIAS.

The effect of Thymin and folic acid has been examined on experimental anaemias of rabbits. Neither Thymin nor the folic acid had any influence on the red blood cell level of normal animals. Both substances — given orally or subcutaneously — had a very good haematological effect on the saponin-collargol anaemia of rabbits and produced a considerable rise in red blood cells and haemoglobin, even in doses aequivalent to some mg daily. The same has been found in chronic anaemia of rabbits, produced by repeated bleeding. Accordingly very small doses of Thymin and folic acid had an excellent haematological effect also on animals with non macrocytic and megaloblastic anaemia. Thymin proved to be still more effective than folic acid. A longer preliminary treatment with Thymin and folic acid could not prevent the anaemic effect of saponin-collargol, on the contrary, the anaemia developed even faster and very often serious neurological symptoms could be observed. China ink is eliminated within a 40—60% shorter time from the blood of animals, which had a longer preliminary treatment with small doses of Thymin or folic acid, than from the blood of the control animals. Therefore it is obvious, that preliminary treatment stimulates the reticuloendothelial system.

R. BACKHAUSZ, AND GY. VAJDA, (INST. OF GEN. PATH. UNIV. BUDAPEST): THE OCCURENCE OF THE TYPE RH_0 OF HUMAN BLOOD AMONG THE POPULATION IN BUDAPEST.

Data are presented as to the relative distribution of the four blood groups and the property Rh_0 (*D*) of human blood among 1041 individuals tested in Budapest. Our series consisted of 30,0% (311) group "0" 45,2% (471) group "*A*", 17,0% (177) group, "*B*" and 7,9% (82) group "*AB*". The RH_0 (*D*) type occured in 83,1% of the individuals tested. The results showed no correlation between the incidence of RH_0 (*D*) factor and the other blood groups and sex.

L. UNGHVÁRY, (INST. OF GEN. PATHOL. UNIV. BUDAPEST): THE ELECTROCARDIOGRAPHIC DIAGNOSIS OF THE DISTURBANCES OF THE VENOUS CIRCULATION OF THE HEART.

In animal experiments the author has brought about venous congestion in the heart's own venous circulation by ligating, the sinus coronarius. As a result

of the experiments he proves that the venous congestion injures the ventricular muscle, and that as a result of it pathological electrocardiograms arise. Low-voltage and pathological changes of the *T* wave are typical, — for which reason the author has called them, in short "pathological low-voltages". At cessation of venous congestion, the pathological electrocardiograms, and thus the injury of the ventricular muscle return to normal within a short time. The author considers the described pathological low-voltages just as characteristic of venous congestion, as the infarct electrocardiogram is characteristic of the breaking of the arterial circulation. On the basis of his animal experiments the author presumes, that the cause of myocardial low-voltage occuring in human pathology is in many cases also venous congestion, alone, or in combination with other heart lesions.

I. PETRÁNYI, AND P. MEGYES, (INST. MED. CLIN. UNIV. BUDAPEST): INFLUENCE OF SYSTOLE ENERGY ON THE PERIPHERAL BLOOD FLOW OF PATIENTS WITH AORTIC REGURGITATION.

The investigations were made on patients with marked aortic regurgitation during the treatment with digitalis-preparations. Measurements of diastolic blood-flow in the art. digitalis with the method of Petrányi proved, that the aortic regurgitation became diminished in the course of digitalis treatment although the pulse rates lowered and so the duration of diastole lengthened. Digitalis-bradycardia favours diastolic regurgitation, however the gain in the increase of systole energy is so outstanding, that regurgitation is compensated by the greater filling of the large arteries. Perhaps a better functioning of the arterial muscular coats contributes also to this effect.

DONHOFFER, SZ. ANDIK, I. AND VÖNÖTZKY J. (MED. CLIN. UNIV. PÉCS): FOOD INTAKE AND SELECTION.

White mice were given free choice of three foods, each containing one third part of a in every respect satisfactory standard mixture and of two thirds starch, casein and lard respectively. Low environmental temperature was followed exclusively by an increase of the consumption of the starchy food while high ambient temperature, leading to a reduction of ingested calories, was followed by an equally exclusive reduction of the intake of the starchy food. Administration of thyroxin was followed by a very marked increase of food intake, in which during the first phase of the experiment in some cases all three varieties of food participated,while in the later stages the plus calories were furnished exclusively by the starchy food, the consumption of the others returning to, or in the case of the fatty food in some instances even below the original level. After termination of thyroxin treatment the original ratio between the consumption of the three kinds of food, was gradually reestablished. Reduction of the rate of metabolism following the administration of methylthiouracyl was associated with a corresponding reduction of the ingested calories. Reduced intake was confined to the starchy food, the consumption of the other two remaining unchanged. Termination of thiouracyl administration was followed by a gradual rise of the consumption of the starchy food until the original level was reached.

I. HORVÁTH, AND G. WIX, (PHARMACOLOGICAL INST. UNIV. BUDAPEST): A NEW METHOD TO MEASURE ABSORRPTION FROM THE INTESTINES.

The aim was to study the absorption from the intestines with the help of a method, where errors caused by diffusion are eliminated. The authors put a glass-canula into the proximal part of the jejunum and another into the coecum, and let flow a physiological salt solution containing 100 mg %, of glucose through the animal. The solution was coloured with haemoglobin and the resorption of water was measured with the colorimetric method. Daily variations of the absorption of glucose, on the same rat, where determined: at 8 *h* a. m. the animal absorbed 45—55% of the infused sugar, later this amount rose slowly and reached the maximum at 3 *h* p. m. with a value of 75—85%. Meanwhile the absorption of chloride was measured and the variations of the dry content and of the sugar content of blood.

I. SZÉKELY, T. SZILÁGYI, AND L. KESZTYÜS, (PHYSIOLOGICAL INST. UNIV. DEBRECEN): THE EFFECT OF THYREOID-FEEDING ON GUT MOVEMENTS.

It was shown that the sensitivity of rabbit's small intestine fed with thyroid substance (Thyreoidea sicca sec. Richter) diminished to adrenaline but increased to pilocarpine, histamine and acetylcholine compared with that of untreated animals. We attribute the decrease of adrenaline — sensitivity and the increase of pilocarpine — acetylcholine and histamine-sensitivity of gut, to the movement-increasing effect of thyroid hormone acting on the smoothmuscle of gut indenpendently of the autonomic nervous system. Thyroxine-adrenaline synergism does not exist with reference to movements of gut.

B. ISSEKUTZ, AND L. GYERMEK, (PHARMACOLOGICAL INST. UNIV. BUDAPEST): THE EFFECT OF DIHYDROERGOTAMINE, AND DIHYDRIERGOCORNINE ON THE METABOLISM.

0,4 mg/100 gr dihydroergotamine methylsulf. decreases a little the basal metabolism of rats anaesthetized with urethan. The effect of dihydroergocornine in a dosis of 0,2 mg/100 g is greater but both effects are rapidly passing away. They are hindering the increase of metabolism caused by phenylisopropylamine, but the effect of adrenaline is not altered. 0,4—0,6 mg/100 g dihydroergotamine has no effect on the increased metabolism of animals, treated with thyroxine. The fact that dihydroergotamine and dihydroergocornine are inhibiting the effect of phenylisopropylamine but leaves unchanged the effect of adrenaline, suggest that they have a central sympaticolytical activity.

I. FEUER, G. HETÉNYI, JR. B. ISSEKUTZ, JR. (PHYSIOLOGICAL INST. UNIV. SZEGED): DISCRIMINATION BETWEEN METABOLIC ACTION OF CENTRAL AND PERIPHERICAL ORIGIN.

The red blood-cells of ducks, having no neural connection with the central nervous system, show an increase of their metabolic rate only after administration of drugs, which exhibit their effect immediately on the oxydative mechanism of the cell. Such drugs are adrenaline, and 2,4-dinitrophenole. Drugs, which exhibit their effect through the central nervous system, do not alter the metabolic rate of the blood cells. To this group belongs benzedrine and the same effect is seen by cooling of the animals. Tested with this method, thyroxine seemed to exhibit its effect peripherically.

G. HETÉNYI, (MED. CLIN. UNIV. SZEGED): ON THE COMPENSATORY ROLE OF HYPERGLYCAEMIA.

Clinical observations and experimental data alike are in favour of the assumption, that diabetic hyperglycaemia is not a harmful, but a useful and compensatory phenomenon. Its consequences are: 1. improved sugar utilization, 2. a better insulin effect and 3. raising of insulin-sensitivity. To maintain an adequate sugar-utilisation in the tissues, the organism is able to regulate its level in case of diminished sugar utilization. The normal organism's way is: diminished sugar-utilization — (by humoral transmitted stimuli *(Heténvi)* liver (by raised sugar — output) hyperglycaemia — direct improvement of sugar-oxydation enhanced insulin-effect raised insulin sensibility — normalized sugar-utilization. In case of "hypergic" *(Heténvi)* diabetes (-want of insulin) the sequence of events is in mild cases the same, in severe cases, however the compensatory effect of hyperglycaemia is insufficient and the use of insulin is necessary. In case of "hypocrestic" *(Heténvi)* diabetes (-diminished insulin-sensitivity) diminished insulin-sensitivity leads to diminished insulineffect and so to diminished sugar-utilization. Compensatory — hyperglycaemia is followed by enchanced insulineffect and insulin — sensitivity and leads by this way to the restitution of sugar utilization. The possibilities of adequate compensation are different in the three groups: best they are in the healthy organism, bad in the severe hypergic diabetes-cases, whereas the chances of the hypocrestic diabetics lie between the two.

Z. HORN, AND S. ADLER-R., (KOLTÓI ANNA HOSP. LAB., BUDAPEST): EFFECT OF MEAT AND AMINOACIDS ON CARBOHYDRATE METABOLISM.

The bloodsugar-concentration examined through 5 hours after feeding meat or a mixture of aminoacids (of blood-proteins) does not show any change in healthy people and animals, or shows only slight increase in the 4th or 5th hour. The bloodsugar concentration of diabetic people shows an increase beginning in about the second hour after having eaten meat. Healthy animals that have been fed for 10—14 days ion mixed food plenty of meat, or a mixture of aminoacids, though characteristic changes are noticeable in their endocrin glands and so on their pancreas β-cells as well, have a normal blood-sugar concentration after fasting, and a normal response to sugar-ingestion. It is supposed that the cause of diabetes is to be searched for in the periphery, in the collapse of some ferment-system and in that case, the pathologic aberrations of the endocrin glands playing a part in sugar-metabolism may cause a further damage of the already pathologic carbohydrate metabolism. The experiments have shown that in some cases of diabetes large quantities of animal proteins do not only affect carbohydrate metabolism harmfully, in so far, as sugar and ketonbodies are produced from some aminoacids, but also by their specific effect on the endocrine-system.

K. FARKAS, AND Z. HORN, (KOLTÓI ANNA HOSP. LAB. BUDAPEST): HISTOLOGICAL CHANGES IN THE ENDOCRINE SYSTEM OF ANIMALS OVERFED WITH MEAT AND AMINOACIDS.

Rabbits, rats, and dogs were overfed with meatprotein and aminoacids. The typical histological changes were: hyperfunction of the pituitary, eosinophily of the thyreoidea cells and changes in the insular apparatus of the pancreas which were caused presumably by a hyperfunction of the insuline-producting cells'

A. SASS KORTSÁK, (PHYSIOLOGICAL INST. UNIV. BASLE AND INST. PEDIATRIC. CLINIC. UNIV. BUDAPEST): EFFECT OF DESOXY-CORTICOSTERONACETATE ON GLYCOGEN METABOLISM OF ADRENALECTOMIZED RATS.

Experiments were performed on male albino rats of the same strain, kept on a standard diet. Liver and muscle glycogen content of 18 normal and of 11 adrenalectomized rats was determined. The adrenalectomized animals received on the day of operation 2 mg, on the successive days 1 mg of desoxycorticosteron acetate (Percorten Ciba). The normally fed adrenalectomized rats treated with desocycorticosteronacetate show already on the 2 nd and even on the 20th day after operation the same glycogen level of liver and muscles as normal ones. By examination of the glycogen forming capacity of the same groups of animals it could be shown that the metabolism of glycogen remains normal after adrenalectomy if the animals are treated with desoxycorticosteron acetate.

L. KOCSÁR, AND L. KESZTYÜS, (PHYSI OLOGICAL INST. UNIV. DEBRECEN): THE EFFECT OF HOGIV AL ON THE BLOOD SUGAR LEVEL.

Small doses of Hogival (oestrone) increase the concentration of blood sugar with 30—60%, but large doses decrease bloodsugar by 25—30%. Middle doses, are followed by a transitional small rise which is followed by a slight decrease. Parallel with increased dosage of Hogival the hyperglycaemic effect decreases and the maximum of blood sugar rise follows sooner. Atropine, respectively ergotoxin, injected at the same time do not influence the hyperglycaemic effect of the small or large doses of Hogival. This excludes a direct effect on the vegetative nervous system. A stilbene preparation was followed always by hypoglycaemia ndependently of dosage showing no connection between the quantity of stilbene iand the degree of hypoglycaemia. If atropine was given at the same time the effects of the stilbene is not exhibited in every cases. Thus, the natural and the artificial oestrogenic substances influence carbohydrates metabolism at different phases.

E. STARK, AND C. NIKODEMUSZ, (PHYSIOLOGICAL INST. UNIV. DEBRECEN): THE EFFECT OF HOGIVAL (OESTRONE) ON LIVER GLYCOGEN.

Small and large quantities of oestrone (500—5000 international units/kg) injected i. m. during two days decrease the liver glycogen by 15—90% resp. 50—80%. This confirms *Eulers* theory that oestrone is an important factor in carbohydrates metabolism especially in the liver. The hypoglycaemia caused by large quantities of Hogival can be attributed partly to the elective glycogen-storing effect of oestrone, partly to an extremely rapid decomposition of carbohydrates.

M. JULESZ, (INST. MED. CLINIC. UNIV. BUDAPEST): NEW DATA ON THE QUESTION OF CONNECTION BETWEEN CARBOHYDRATE METABOLISM AND VITAMIN B_1.

In 24 experiments on 21 patients the insulin and vitamin B_1 sensitivity was determined and the influence of vitamin B_1 on insulin sensitivity was investigated. Among the 10 diabetic patients all being insulin sensitive, three cases of juvenile diabetes showed increased sensitivity against vitamin B_1, while 6 aged and 1 juvenile patient was found to be resistant to this vitamin. An insuline sensitive and vitamin B_1 resistant boy became insulin resistant and vitamine B_1 sensitive after transplantation of pituitary. This effect was absent in three other cases of pituitary transplantation but in this cases clinical improvement was not demonstrable either. A man who was kept on a ketogenic diet, was also found to be vitamin B_1 sensitive.

I. KERESE, AND S. KOVÁCS, (PHARMACOLOGICAL INST. UNIV. PÉCS): EFFECTS OF INSULIN AND VITAMIN B_1 ON THE SUGAR-METABOLISM OF ISOLATED FROG LIVER.

Isolated frog liver perfused with Ringer solution in a closed system delivers a certain amount of sugar to the perfusing fluid, — a fact stated already by *Issekutz* and *Szende*. Adrenalin increases, preliminary treatment with insulin inhibits the deliverance of sugar. This deliverance of sugar is not proportional to the glycogen content of the liver, nevertheless it can be stated, that in experiments made in autumn and winter the sugar deliverance is generally greater than in the spring and summer experiments. 15 mg % vitamin B_1 added to the perfusing Ringer-solution — increased considerably sugar deliverance. 1—3 mg % had no or a very small effect. If 1—3 mg of vitamin B_1 has been given to the frog subcutaneously 18—24 hours before the experiment, the sugar deliverance decreased considerably. The decrease is still greater if 0,25—0,5 I. U. Insulin is given simultaneously with vitamin B_1 administration. Accordingly a preliminary treatment with vitamin B_1 increased the antiglycogenolytic effect of insulin.

ST. PATAKY, AND D. SZENT-GYÖRGYI, (PHARMACOLOGICAL INST. UNIV. BUDAPEST): THE EFFECT OF THYROXINE ON CREATINE METABOLISM.

The creatine content of the muscle pulps of animals, treated with thyroxine, shows — within six hours after thyroxine administration — a higher total creatine value, than control pulps. This increase is greater in the pulps treated with arginine. It is supposed that in the muscle of thyroxine treated animals creatine synthesis is possible.

M. FÖLDI, AND G. SZABÓ, (1.ST MED. CLINIC. UNIV. BUDAPEST): STUDIES ON THE FUNCTION OF THE DISEASED RENAL TUBULI.

It was proved in tubular damage produced by different means, that normal tubular epithelium has a double function in the absorption of filtered substance, the first is the maintenance of active reabsorption (i. e. glucose) or passive rediffusion (i. e. urea) the second is the limitation of the maximal value of this active reabsorption or passive rediffusion. The cause of the "excessive reabsorption" in nephrosis described by *Frey*, is that there is a disfunction of the regulating mechanism which determines the normal rate of reabsorption and rediffusion. In the diseased kidney there may be a passive rediffusion of glucose.

G. SZABÓ, AND M. FÖLDI, (1. ST MED. CLIN. UNIV. BUDAPEST): CRITICISM OF CLEARENCE METHODS.

It was proved experimentally that clearence substances rediffuse passively through damaged tubular walls. Passive rediffusion depends on the physico-chemical properties of the substances that is, the reason why the clearence of insulin is the greatest and that of the other substances according to their molecular size-more or less lower. Inulin clearence does not show the proper value of glomerular filtration either, but presumably not significantly less. In the case of most severe kidney destruction and absolute asthenuria, clearence values gained with the different substances will be equal, but here the fluid filtered in the glomeruli is diffusing back in unchanged composition so we cannot draw any conclusion on the amount of the glomerular filtrate from the clearence value.

G. JR. HETÉNYI, AND B. ISSEKUTZ, JR (PHYSIOLOGICAL INST. UNIV SZEGED): EFFECT OF ATROPINE ON DIURESIS.

Atropine inhibits water, urea, sodium-sulphate and formoguanamine diuresis as well as diabetes insipidus. It increases probably the rate of reabsorption of water and chlorides. So it does not seem suitable for the discrimination of the modes of action of several diuretics, as proposed by *Kuschinsky*.

P. BÁLINT, L. HÁRSING, AND M. LENNER, (1ST MED. CLIN. UNIV. BUDAPEST): HYPERSALEMIC AZOTEMIA.

By injecting large quantities of a hypertonic *NaCl* solution subcutaneously to rabbits (3,6% resp. 7,2%) greatly elevated serum *Na* and *Cl* values for a langer period were obtained. (Serum *Na* 180 maeq. serum *Cl* 150 maeq.) In all cases where these elevated *Na* and *Cl* values could be maintained for at least 24 hours an azotemia developed. The glomerular clearence remained normal, resp. elevated throughout the whole experiment. Calculating urea filtration and absorption it was found that during the azotemic phase 80% of filtered urea was reabsorbed instead of the normal 30%. The reabsorption of sodium and chloride were also augmented. Histologic examination of the kidneys showed degeneration of the cells of the ascending limb of the loop of Henle and fat deposition in the whole tubular apparatus. Thus a lesion of the tubular apparatus with entirely intact glomeruli developed. The degenerated tubular cells lost their normal potency to resist over reabsorption of urea and sodium. The authors point out that they did not found such purely tubular asotaemias known in the literature, for all elevations of the NPN were connected with diminution of the glomerular filtration rate.

P. GÖMÖRI, P. BÁLINT, AND L. HÁRSING, (1ST MED. CLIN. UNIV. BUDAPEST): TUBULAR FACTORS IN EXTRARENAL AZOTEMIA.

By intraperitoneal injection and subsequent withdrawal of an isotonic glucose solution in rabbits, extrarenal azotemia and dehydration develops. *(Darrow* and *Yannet.)* During the dehydration, reduced glomerular filtration rate (measured by endogeneous creatinine-clearance) and reduced urea clearance was found of the latter being much more pronounced. In the control period the urea-clearence was 68 to 87% of the creatinine-clearence but during dehydration it diminished to 7 to 20%. The cause of this behaviour is to be sought in the augmented tubular reabsorption of urea. It seems to be certain, that besides the hemodynamic factor of dehydration azotemia, there are tubular factors operating as well. The great diffusibility of urea makes it apt for passive rediffusion. The latter view is supported by the fact, that reabsorption of urea is much more expressed, than the reabsorption of non-urea nitrogen. The histological changes, only recently found by *Gömöri* and *Romhányi* in the kidney of cases of experimental dehydration (medullar, hyperemia degeneration of the tubular cells) are in favour of such a rediffusion.

G. GOTTSEGEN, M. HÁRY, AND Z. HORN, (KOLTÓI ANNA HOSP. LAB. BUDAPEST): THE EFFECT OF LARGE DOSES OF VITAMIN *K* ON HEPATIC FUNCTION.

In cases of liver damage, the effect of large doses of vitamin *K* on the protrombin time of diluted plasma is inconstant, the usefulness of the test proposed by *Unger* and *Shapiro* is also limited by the length of time (at least a week) necesary

for its performance. However, the load imposed on the deteriorated liver by an overdosage of vitamin *K* reduces also the excretion of bilirubin, a sign that can be utilized far more advantageously for the estimation of hepatic function. The bilirubin level remains unchanged 1,2 and 8 hours following the parenteral administration of 70 mg of Vitamin *K* to healthy subjects and rises in presence of hepatic damage. Highest elevations (2—6, in average 3,26 mg%) have been observed in case of infectious hepatitis, during the stage of increasing jaundice. As soon as the icterus begins to decrease — a sign of reestablished hepatic function there is a substantially diminished response: 0—1,0 in average 0,45 mg %. In one case of calculous obstruction with liver abscesses, a rise of 1,0 mg % has been observed, in cases of anicteric cirrhosis 0,15—0,30, in chronic cholangitis 0—0,1 mg %. Every increase after the injection of vitamin *K* that exceeds 1,5 mg % is a sign of active hepatocellular damage.

M. HALÁSZ, (MED. UNIV. PÉCS): THE RELATIONSHIP BETWEEN THE SERUM BILIRUBIN CONTENT AND URINARY BILIRUBIN EXCRETION.

Former observations have shown a close relationship of urinary bilirubin excretion with the concentration in the serum and the rate of glomerular filtration. The correlation of the total bilirubin content of serum and bilirubin excretion is not linear. The bilirubin concentration of the glomerular filtrate is only a small fraction (e. g. 1/770 at 10, 1/400 at 30 mg bilirubin per ml serum) of that in the serum. In this respect no difference was observed between obstructive and hepatocellular jaundice. Total bilirubin and the direct type of bilirubin of serum showed the same high correlation with the rate of urinary bilirubin excretion though in cases of low bilirubin concentration in the serum it is evident that the excretion of bilirubin depends on the direct type of bilirubin. In dogs ten times more bilirubin is excreted at the same level of serum bilirubin and the same rate of glomerular filtration than in men.

A. HÁMORI, AND A. TOMPA, (MED. CLIN. UNIV. SZEGED): SEROLOGICAL ANALYSIS OF NOCTURNAL HEMOGLOBINURIA.

It could be shown in in vitro experiments that the specific pathogenic factor is absorbed by the erythrocytes. This factor might be perhaps regarded as an amboceptor in spite of its tight binding-ability due to which it can't be removed even by washing, contrary to the findings of *Hegglin* and *Maier*. A non specific factor present in every sera plays also an essential role and it seems not to be impossible that this latter is identical with the complement. The mechanism of action of the pathophysiological processes belonging to the field of the *Marchiafava* anaemia seems not to be uniform. According to experiments carried out in vitro the hemolysis could be entirely inhibited by vitamine *C* even when added in minute doses and the therapeutic administration of it proves also to be effective in in vivo experiments still under way.

I. MAGYAR, (1ST MED. CLIN. UNIV. BUDAPEST): EXAMINATION OF THE DECREASED EXCRETION OF VITAMIN *B* IN THE COURSE OF PROLONGED ADMINSTRATION.

Out of the thiamin and riboflavin given parenterally in daily doses for a long time, the organism excretes less and less in the urine. The examination of the phosphorylation of these vitamins leads to an explanation. Monoiodoacetic acid, paralysing phosphorylation decreases, adenosintriphosphoric acid increases excretion. The decrease of phosphorylation signifies therefore a decrease of excretion in the case of vitamins given in large doses and for a long time. The cause of decreased phosphorylation may be the exhaustion of its mechanism. To prove this supposition, the author examined the absorption rate of thiamin from the intestine of rats, when other vitamins of the *B* complex and dextrose were given in the same time or before the experiment. Adminstration of thiamin, riboflavin, pyridoxin, nicotininc acid, and dextrose decidedly decrease the absorption of thiamin from the intestine of rats. The exhaustion of phorphoralytion gives an explanation of the decreased excretion of vitamins given in large doses and for a longe time explains the symptomes of vitamin-deficiency manifested after administration of large doses

of pure synthetized vitamins and explains in the same time the deficiency symptoms which follow the continuous administration of dextrose, a substance which undergoes also phosphorylation in the tissues.

B. BERDE, AND A. FEKETE, (PHYSIOLOGICAL INST. UNIV. BUDAPEST): HORMONAL BODYTEMPERATURE REGULATION IN ACTIVE AND PASSIVE HYPERTHERMIA.

In the serum of animals (rabbits) in passive hyperthermia (when the rise of the body temperature is conditioned by an abnormally high environmental temperature) — as well known — a thyreogen substance, thermothyrin *A* is present, which injected into normal animals (rats) involves depression of O_2-consumption and CO_2-production. In these experiments the question was investigated whether thermothyrin secretion can also be demonstrated in active hyperthermia (fever) due to a change in the activity of the heat regulating centre. Active hyperthermia was produced in rabbits by heat puncture. Blood was taken from the jugular vein during the period of high fever (acmes) and during defervescence (lysis) respectively. The serum proteins were precipitated by alcohol and a great part of lipids eliminated by a short treatment with ether. This serum preparation injected into normal, male albino rats was not followed by any significant depression of the basal metabolic rate, i. e. secretion of thermothyrin *A* could not be demonstrated in active hyperthermia. This is a further difference between the mechanism of active and passive hyperthermia.

L. TAKÁCS, AND B. BERDE, (PHYSIOLOGICAL INST. UNIV. BUDAPEST): HEAT TOLERANCE AND CASTRATION.

Heat tolerance of rabbits was measured by the method of determining time-temperature-areas (Berde B.: Hungarica Acta Physiologica 1. 52. 1947 and Schweiz. Med. Wschr. 77. 1367. 1947) in overheating experiments at 34—35 C° environmental temperature. If a great number of experiments are carried out on several groups of litter mates, it is always possible to find pairs of animals which — in several experiments on different days — show congruent time temperature-areas: and have therefore equal heat tolerance. One member of such a pair was castrated, and the overheating experiments were repeated a week after operation. There was no significant difference between time-temperature-areas of castrated and uncastrated animals neither in male, nor in female rabbits. Heat tolerance is therefore not affected by castration.

I. PINTÉR, AND J. MÉHES, (PHARMACOLOGICAL INST. UNIV. PÉCS): CONTRIBUTIONS TO THE QUESTION OF JUVENILE HORMONAL HEATREGULATION.

Mansfeld and his collaborators have proved that in the hormonal heatregulation of the warm-blooded, the thyroid gland plays a part not only by producing thyroxin, but also by the secretion of two antagonist hormones of thyroxin: thermothyrin *A* and *B*. The production of these hormons depends on the physiological activity of the thyroid gland. Their effect of reducing basal metabolism can be demonstrated by giving the blood serum of a heated animal, the so called "Warm serum" to a normal animal and then the basal metabolism of this latter decreases. Following strictly *Mansfelds* prescriptions the metabolism reducing-factor was sought for in the blood of a severely hypothyreotic 6 years old girl, — before the introduction of substitution theraphy-, and after great clinic improvement following the treatment. According to the results — neither before treatment, nor after the great improvement, respectively after the disappearence of all symptoms did the blood serum, taken after heating, influnce the metabolism of rats. The blood serum of a normal healthy 4 year old girl proved also to be ineffective. Experiments with blood sera of 14—43 days old dogs, which did not yet reach puberty produced no decrease, but on the contrary a more or less marked increase of heat production. The blood serum of rabbits 75—95 days old just being at the age of puberty elicits already a small decrease of metabolism. "Warm serum" of mature dogs and rabbits reduced in all cases the metabolism of rats.

J. SZEBEHELYI, AND M. FABINYI, (PHARMACOLOGICAL INST. UNIV. BUDAPEST): THE ROLE OF HISTAMINE IN THE SYMPTOMS CAUSED BY OXYGEN LACK.

The inhalation of a gas-mixture containing 10% oxygen increases the rate of respiration of white mice. According to the authors investigations Antistin and desensibilization with histamine hinder this effect, but not by means of decreasing the irritability of the respiration-centre, because the increase in the rate of respiration caused by the inhalation of CO_2 remains unaltered. The histamine content of blood increases after the inhalation of a gas mixture containing 10% oxygen. This histaminaemia is in all probability connected with adrenaline metabolisation, because dihydroergotamine is also preventing the increase of the rate of respiration in lack of oxygen. Of adrenalinaemia and histaminaemia the former may be primary, because Antistin — which drug is counterbalancing the symptoms of oxygen lack — hinders not all the effect of histamin on the mobilisation of adrenaline. The fact that Antistin and desensibilization with histamine prevent the increase in the rate of respiration caused by lobeline too, suggests that in oxygen lack histamine increases the rate of respiration by means exciting the chemoreceptors of the sinus caroticus, or possibly histamine is the stimulus transmitting substance in the sinus caroticus.

D. SZENTGYÖRGYI, AND J. SIMONYI, (PHARMACOLOGICAL INST. UNIV. BUDAPEST): THE EFFECT OF THYROXINE AND BENZEDRINE ON THE TEMPERATURE.

Benzedrine releases the lowering of temperature caused by amidazophen antipyrine and novocain. Giving sevenal and urethan together with benzedrine a collaps-like fall of temperature developes. If one drug is given former and the other after the development of the effects, the effect of sevenal, respectively urethan prevails, that of the urethan is greater, while sevenal causes a smaller decrease in temperature and there is no collapse. Benzedrine causes a letal hyperpyrexia after thyroxinising which is repulsed by fever-diminishing substances which defend the animals from perishing with the exception of novocaine. Thyroxinising inhibitis the temperature lowering effect of novocain and antipyrin and decreases the effects of sevenal, urethan and amidazophen.

B. ISSEKUTZ, JR. G., HETÉNYI, H. NAGY, AND LUNG, M. (PHYSIOLOGICAL INST. UNIV. SZEGED): TEMPERATURE REGULATION OF THE DENERVATED LIMB.

A new method for the determination of the blood flow is described. It is possible to measure during 10—12 hours the oxygen consumption of the muscles of the hind leg of dogs under wholly physiological circumstances. After section of the cervical spinal cord, the hind legs posses a certain temperature regulation. They react to refrigeration with an increase of their metabolic rate of about 40%. The denervated limb of dogs, whose thoracic spinal cord was transected, showed an increase of the metabolic rate of about 30—50% when the upper part of the body was cooled. This remaining temperature regulation can be easily abolished by narcotics. From this it has been concluded that it is established by the stimulation of the temperature regulating center.

I. LICHTNECKERT, H. NAGY, B. ISSEKUTZ, JR. (PHYSIOLOGICAL INST. UNIV. SZEGED): CALORIMETRIC EXPERIMENTS WITH HISTAMINE AND CAPSAICINE.

By means of their new calorimeter the authors performed experiments on mice treated with histamine and capsaicine. The effect of small doses of these drugs are alike. Both excite the end organs sensitive to heat. This stimulates the heat center and according to this the rectal temperature decreases. Independently from this, the heat loss increases by way of axonreflexes, which cannot be narcotised, and so in narcosis the metabolic rate follows passively the temperature of the rectum. In the case of histamine the well known shock effect causes the fall of blood pressure and of the metabolic rate, and so covers the specific effect.

G. GELEI, I. LICHTNECKERT, B. ISSEKUTZ, JR. (PHYSIOLOGICAL INST. UNIV. SZEGED): CONTRIBUTIONS TO THE MODE OF ACTION OF SOME DRUGS CAUSING LOSS OF HEAT.

The action of pyribenzamine (PBA) on several drugs causing loss of heat was investigated. Mice received 16 γ/g PBA s. c. This caused a fall in their rectal temperature of 2 C°, lasting appr. 45 minutes. After an hour when the mice regained their original temperature the drug in question was given. The loss of heat caused by histamine, anilin or carbolic acid was suppressed signaficantly by PBA. No suppression was seen in the cases of novocaine or cardiazol. Capsaicine proved to be a transition between the two groups. From these it can be concluded that PBA exhibits its effect in the case of such drugs, which cause loss of heat by producing shock, like histamine.

S. SIMON, ("MAGYAR PHARMA" LAB. BUDAPEST): NEW OBSERVATIONS ON THE PHARMACOLOGIC PROPERTIES OF ALCOHOLIC EXTRACTS OF HUMAN URINE.

In continuation of his published experiments (Arch. f. exp. Path. u. Pharm. 198, 528. 1941 and 203, 171. 1944) the author states that the low NH_3 (0,13—45 mg/sec) content of the extracts gives no explanation for the bloodpressure effect caused by these extracts. Neither pepsin nor angiotonase destroyed the substance responsible for the circulatory phenomena. The extracts paralysed in a reversible manner the automatic contractions of the uterus of rats. Intravenous injections caused convulsions on mice and the animals died in respiratory paralysis. Rapid i. v. administration is much more toxic, than slow injection.

J. MÉHES, (PHARMACOL. INST. UNIV. PÉCS): THE PHARMACOLOGY OF THE 1(α NAPHTYL)— AND 1 (β NAPHTYL) — 2 AMINOPROPAN.

If the alkyl-amin side-chain being present in the phenyliso-propylamin (Aktedron, Benzedrin) is joined to a naphtyl radical — the very tipical central motoric irritation effect of the phenyl-isopropylamin is greatly reduced and generally it appears only in form of premortal convulsions, but very expressed salivation and exophtalmus can be observed. A solution more concentrated than 1 : 400.000 has a negative chronotrop and dromotrop effect on the Straub heart. This effect can be removed by great doses of atripon. The formation of impulses is not affected. The drug causes vascular constriction on Laewen—Trendelenburg preparation, which can be removed partly by ergotamin. The effect of the α derivative is stronger and more adrenalinlike. Both derivatives raise the blood sugar level. They have a sympaticomimetic effect on isolated intestine. They raise the blood pressure of the warmblooded, 0,1—0,3 mg/kg has a one phasis — greater doses have a double phasis-effect first a little sinking, then a long lasting rise. Doses of 1 mg/kg and more have especially a depressive, effect. Section of the vagi, or decapitation did not change the effect considerably. Atropin does not remove the depressive effect. Ergotamin decreases the pressoric effect. The phenomenon of tachyphylaxia could not be observed. Cocain did not increase the effect. Owing to the experiments, the change of the phenyl radical with a naphtyl radical did not alter substantially the character of the effect, typical to the phenyl-alcylamin group, but modified it in many respects.

L. LAJOS, (OBST. AND GYN. CLIN. UNIV. PÉCS): BIOLOGICAL EFFECTS OF VERNIX CASEOSA.

With the collaboration of *F. Szontágh* and *J. Görcs* it could be established that Vernix has a strong oestrogenic action. Assay with the Allen—Doisy test showed an activity corresponding tn 3000 I. U. folliculin per gramm. So vernix contains folliculin in the highest concentration observed sofar in biological material. Comparison of normal and spayed rats treated through months showed the expected effect on the growth of the uterus and the usual oestrogenic action, while the inhibiting effect on the ovary and the deterioration of general health of the animals observed after comparable doses of folliculin were absent. Therefore, vernix caseosa contains beside folliculin another factor. This factor is, as preliminary experiments suggest, identical with progesterone.

J. URI, AND P. ADLER, (PHARMACOLOGICAL INST. AND STOMAT. CLIN. UNIV. DEBRECEN): SENSIBILISATION OF THE ACTION OF SUPRARENINE BY PROCAINE.

The effect of procaine on the action of suprarenine was examined in cats under chloralose anesthesia receiving artifical respiration, applying non-toxic dosage of suprarenine and procaine. — Procaine enhances the effect of suprarenine on blood pressure. This phenomenon is observed after atropin and after decapitation as well after the extirpation of the suprarenal glands it is more pronounced. In agreement with some data of the literature, these results prove — contrary to general opinion — that procaine has sympathicomimetic effects and is capable of sensitizing the protoplasma to suprarenine. Procaine acts in all probability in a similar way as do other epinephrine sensitizing agents.

D. GYÜRE, AND B. KOVÁCS, (PHARMACOLOGICAL INST. UNIV. SZEGED): THE ANTIHISTAMINIC EFFECT OF TANNINE.

Very good therapeutic results were gained by parenteral administration of tannine in many cases of allergic disorders. The single toxic dose of tannine in guinea pigs and rats i. p. proved to be 250—300 mg/kg. The bronchiolospasm produced by histamine spray could be suspended for $1^1/_2$—$1^3/_4$ hours by previous i. p. injection of 20—30 mg/kg tannine. According to *Jancsó* the chemosis caused by a a 2 per cent solution of histamine can be specifically abolished by antihistamine drugs. By i. p. injections or local treatment of tannine the appearence of this chemosis could be inhibited. *Jancso's phenomenon* — the ability of histamine to turn the resting endothel cells into active phagocytes — prevented by antihistamine drugs. This phenomenon was also suspended by 80—100 mg/kg tannine administered intraperitoneally. The prevention of letal shock occurs only in about 50 per cent of the animals by i. p. adminstration of 20—30 mg/kg, tannine. The action of histamine on the blood pressure of cats could not be inhibited. The authors suppose that between tannine and histamine chemical interaction takes place in the organism resulting in a neutralization of histamine. In vitro tannine and histamine give rise to the formation of a fine precipitate.

F. HERR, (PHARMACOLOGICAL INST. UNIV. BUDAPEST): THE EXAMINATION OF SOME ANALGETIC DRUGS.

"Methadon" (6-dimethylamino-4, 4-diphenyl-heptan-3-on) and the new *Chinoin* compound, called "St". (3,3-diphenyl-1-pyperidino-hexan-4-on) was examined with the help of a new method. The analgetic effect upon white mice was determined by measuring the decrease of pain caused by radiated heat. The irritability of the respiratory centre was also estimated by a new method. The effects of these two compounds are similar to that of morphine, but they are able to inhibit the muscle-contraction caused by *BaCl* and pilocarpine too.

Z. DIRNER, (PHARMACOLOGICAL INST. UNIV. SZEGED): EXPERIMENTAL DATA ON THE MECHANISM OF ACTION OF CONVULSIVE DRUGS.

Myanesin (α,—β-dihidroxypropan) alters characteristically the development of strychnine poisoning on mice: a very frequent tremor of the limbs and the trunk is alternating with restng periods. The tremor lasts at the beginning only some msecs and is followed by a longer resting interval but later the tremor period is extended on account of the resting phase and finally stabilized. This is followed by a shortening and frequency decrease of the tremor attacks and the mice recover. The whole event lasts 2—3 hours. The doses administered, where: 1,5 γ/g strychnin nitr. s. c. and 0,2 g/kg Myanesin i. p. (after 4 min.). Stimulation, applied at the resting interval exhibits the tremor, while stimulation during tremor causes an immediate ceasing of it. Tetanic convulsions caused by (1—1,5 γ/g s. c.) strychnine on mice, compelled to swimming develope in the mean only after 16,5 minutes, while on the controls, convulsions appear after 8 min. If one lifts them from the water or stimulates them during swimming after the 10th minute of poisoning, the tetanic convulsions become immediately manifested.

M. ARATÓ, AND L. SZEKERES, (PHARMACOLOGICAL INST. UNIV. PÉCS): RELATION OF THE FREQUENCY OF HEART-CONTRACTIONS TO THE DEVELOPMENT OF THE EFFECT OF DIGITALIS.

The influence of changes of frequency of the rhytmic electric shocks on systolic heart stoppage caused by Digitalin and Digitalis Lanatanglycosides was studied on isolated ventricles of a frog heart with a Stannius II. ligature. Solutions 1 : 40.000 of Digitalis lanata and 1 : 15.000 of Digitalin were administered, which stopped the contractions of the spontaeously beating, and at the rate of 25 stimuli per min. stimulated hearts after 6 min. Stimulation rate of 50/min. shortens the poisoning time (to 5—6 min.) but not significantly, whereas at a frequency of 15/min. the poisoning time is considerably prolonged. (Over 30 min.) A cumulative effect of the Digitalis, put into the cannula appears only if during the poisoning period, the ventricle makes some spontaneous contractions or is stimulated by very rare stimuli (4—8) min. and then by a 25/min. frequency (heart stoppage follows after 2—3 min.). The non contracting ventricle does not bind glycosides or the glycoside affects not the inactive heart muscle cells. The condition of the development of the Digitalis effect seems to be a certain frequency of the heart contraction. It is supposed, that the Digitalis influences chemical processes in connection with changes of the state of heart myosin.

A. SZENDEY, L. MOSONYI, AND J. PORSZÁSZ, (PHARMACOLOGICAL INST. UNIV. BUDAPEST): THE EFFECT OF PENICILLIN ON THE HEART.

A great dose of penicillin (5.000—10.000 U.) stops Straub's heart preparation. This effect is reversible and it is not the result of changes of ions. Such doses are not administered in human therapy, but in patients with heart diseases, intravenously given great doses, can approach them.

E. VARGA, AND L. KESZTYÜS, (PHYSIOLOGICAL INST. UNIV. DEBRECEN); AND D. BAGDY, (BIOCHEMICAL INST. UNIV. BUDAPEST): CORTINLIKE EFFECT OF QUABAIN I.

Quabain decreases the potassium concentration of blood but increases the sodium level of rabbits and dogs. The sodium diminishes the potassium increases in the urine. The chloride and blood sugar level of rabbits is increased by Quabain. Hyperchloraemia in dogs is much smaller and the blood sugar value rather diminishes. It was not succesful to state the characteristic change caused by Quabain, because the chloride value in excreted urine shows extreme differences. The effect of Quabain on salt metabolism seems to confirm the hypothesis of *Zwemer* et al. according to which Quabain — similar to cortin — extends the life of the adrenalectomized animals. Opposite observations of *Dorfmann* cannot be confirmed theoretically.

E. FÜZES, E. VARGA, AND L. KESZTYÜS, (PHYSIOLOGICAL INST. UNIV. DEBRECEN): CORTINLIKE EFFECT OF QUABAIN II.

The glycogen phosphorylation in muscles of rabbits and cats results in a decrease of 55—80% of the original inorganic phosphorus in vitro in one hour. Quabain has no influence on these values. The phosphorylation decreases with 24—40% in adrenalectomized animals. Quabain does not increase in vitro this decreased phosphorylation. Quabain is unable to influence similary the phosphorylation in dog's cardiac muscle. According to Verzár's theory the criterium of cortin-action is restitution of the intensity of decreased phosphorylation, so these data do not confirm the hypothesis of Zwemer et al, which attribute to Quabain a cortinlike effect.

K. LUDMÁNY, T. SZILÁGYI, AND E. VARGA, (PHYSIOLOGICAL INST. UNIV. DEBRECEN): FE++-ADRENALINE-ANTAGONISM. I.

On the isolated hypodynamic frog's heart, ferrous iron in a proportion of 1 : 10 markedly suppresses the effect of adrenaline and in a proportion of 1 : 20 likewise that of ephedrine. In the Trendelenburg frog, the vasodilatatory effect of adrenaline is also annulled by ferrous iron. The ferrous irons effect on adrenaline is not influenced by atropinisation thus the parasympathetic nervous system has no part in the adrenaline *Fe*++-antagonism. The blood pressure action of adre-

inaline in dogs and the contractions of the spleen are markedly depressed by ferrous ron. Ferrous iron abolishes also the blood pressure reducing effect of adrenaline occuring after ergotamine and yohimbine, in contrary to the data of *Ivancevic* and *Stern*. The hyperglycaemic effect of adrenaline remains unaltered. The blood-pressure lowering effects of histamine are also essentially weakened in the presence of ferrous iron. Following the author's opinion the ferrous iron affects the smooth muscle by lowering the irritability of the musculature and that of the blood vessels regarding both dilatative and constrictory impulses.

T. SZILÁGYI, ST. SZÉKELY, AND E. VARGA, (PHYSIOLOGICAL INST. UNIV. DEBRECEN): FOLLOW UP REPORT ON THE INFLUENCE OF FERROUS IRON ON THE ACTION OF ADRENALINE.

By intravenous infusion of ferrous iron (*Fe*++) in quantities much smaller than the lethal minimum dose, the capacity of the dog's vascular musculature to respond to various stimuli is markedly reduced, the Lovén reflex is absent, stimulation of the spinal chord by adrenaline and electrical stimuli causes no increase of blood pressure, the depressive action of histamine, acetylcholine and that of adrenaline after previous treatment with ergotamine or yohimbine, on the blood is no longer observed. This action is specific to the bivalent ferrous ion. Other metal ions — ferric, stannous — manganous and compounds of arsenic — do not influence in any way the action of adrenaline on the blood vessels, only the cobaltous ion (*Co*++) blocks adrenaline's effects on blood pressure. This action of *Co*++ lasts considerably longer, than that of *Fe*++, in conformity with this action, the *Co*++-ion is demonstrable in the dog's blood for a much longer period than the *Fe*++-ion. Isolated organs provide the most suitable test objects for the investigation of the ferrous ion-adrenaline antagonism. In order to inhibit the action of adrenaline on both the isolated frog's heart and the isolated intestine preparation, for each adrenaline molecule, 25—30 ferrous ions must be applied. From these stoechiometrically identical quantities, one can infer with great probability that the mechanism of the functional antagonism between ferrous iron and adrenaline is identical in the heart and intestine. The observations of *Ivancevic* and *Stern* that *Fe*++-ions do not block the inhibitory actions of adrenaline on active organs, could not be confirmed.

G. HAHN, AND T. SZILÁGYI, (OPHTALMOLOGY, UNIV. AND PHYSIOLOGICAL INST. UNIV. DEBRECEN): EFFECT OF FERROUS-IRON UPON PUPILLARY REACTIONS.

Authors proved besides the antagonism between ferrous-iron and suprarenine a similar effect to several pharmacological and biological agents in experiments on 116 rabbits. The effect of suprarenine, privin eserin and cocain is lessened or suspended by ferrous-iron in six times larger dosage. For the same effect of atropin, scopolamin and acetylcholin an essentially higher dosage — 300—600 times more — is required. Sympathetic denervation of the iris has no effect whatever upon these phenomena. This is indicative of a direct action of ferrous iron upon smooth muscle. Reaction of the mebrana nictitans in cats caused by epinepherine is diminished too. — It is concluded that ferrous iron in high dosage considerably lessens the reactivity of smooth muscle, in moderate dosage it suspends the effect of the agents aforementioned, without acting upon smooth muscles and small dosage augment the mydriasis caused by the atropin group (paradoxical reaction).

E. ENDRŐCZI, AND J. SZŐKE, (PHYSIOLOGICAL INST. UNIV. PÉCS): INHIBITION OF ADRENALINE BY EXTRACT OF RED PEPPER.

The biologically effective substances of alcoholic and etheric extracts of sweet red pepper were examined. Etheric extract inhibits the effect of adrenaline on the isolated frog's heart and on mammalian blood-pressure. This inhibitory effect was observed also on the action of acetylcholine, aleudrine, adrianol and sodium-oleinate. The inhibition lasts on the isolated frog's heart over hours. The effective substance was destroyed in mammalians in fifteen minutes.

B. KELENTEI, AND T. VÁLYI NAGY, (PHARMACOL. INST. UNIV. DEBRECEN): THE CHANGE OF THE LACTIC ACID CONTENT IN BLOOD PRODUCED BY THE THREE ISOMERS OF AMINO- AND NITROPHENOL.

In cats the lactic acid content of the blood increases parallel with the haemiglobin produced by the three isomers of aminophenol. In case of nitrophenols- which do not produce haemiglobin, — the increase of blood lactic acid is not observed or is only very low compared with that following the administration of aminophenols. These experiments confirm in vivo the results of *Kiese* and *Wendel* obtained in vitro.

E. VIRÁGH, GY. SZABÓ, AND GY. MOLNÁR, (PHARMACOLOGICAL INST. UNIV. DEBRECEN): POISONING OF KALIUMCHLORITE ($KClO_2$) IN IN VIVO EXPERIMENTS.

The chlorite radical is thousand times more poisoning than the chlorate. The experiments prove the suggestion of *Heubner* and his school according to which the poisoning effect of chlorate is due to his reduction products, mainly chlorite.

K. GARAY (BIOCHEMICAL INST. UNIV. BUDAPEST): THE IN VITRO EFFECT OF ULTRASONIC VIBRATIONS ON FIBRILS OF CROSS-STRIATED-, SMOOTH- AND HEARTMUSCLE.

Based on the depolymerizing effect, observed on myosin and other highly polymer zed chainmolecules in the autumn 1946, the author supposed, that similar effects could be produced at the musclefibrils also. Striated musclefibres from grinded rabbit muscle were treated 2 hours with Weber's solution. These fibres and other ones not treated with Weber's solution were irradiated with ultrasonic vibrations produced by an apparatus of high energy, built by the principle of the pyeso-effect. The ultrasonic vibrations had a frequency of 300 kHz and an energy of 10 Watt/ccm², which could be increased by focusing tp 1000 Watt. For heart muscle fibres of cattle-heart were used suspended for irradiation in physiologic saline or in a phosphate buffer of *pH* 7,2. In muscle fibres, treated with Weber's solution, parallel fissures in a distance of 7 μ, crossing the whole fibre rectangularly to the length axes of the filaments, were observed already after 10—20 minutes of irradiation. After 60 minutes of irradiation, a complete desintegration of the muscle structure could be seen. On the other hand, in muscle fibrils not treated with Weber's solution, the author found fissures parallel with the fibrils length axis showing, that the connecting side laminae were split. In native smooth muscle fibres fissures parallel, rectangular to the lengthaxis were produced the constant distance being 4 μ. In the native heartmuscle both types of splitting could be observed. It is concluded that a new aspect of the structural properties of muscle filaments, and for calculating the junctions between the contractile unites was found. Inferences regarding atoms, respectively atomgroups takeing part in the junctions may be drawn (dipol. energies, magnetic and other energies) and data concerning the mechanism of energy conduction in biological systems may be obtained. Following the author's opinion, the described effects prove the energy spreading of *Zimmer—Rompe* whereas the splitting effects found can be explained by the splitting theory of *Schmidt*.

I. HORVÁTH, J. KOCH, AND J. SZERB, (PHARMACOLOGICAL INST. UNIV. BUDAPEST): INVESTIGATIONS ON POLYMERISATION OF ACTIN EXTRACTED FROM FROG-MUSCLE.

There are essential differences between the qualities of actin extracted from frog muscle and the qualities of rabbit-muscle-actin prepared by *Straub* and coll. Frog-actin is able to polymerise also in the presence of oxydising agents. Ammonium molybdate at a temperature of 0 C° inhibits polymerisation. This effect can be hindered by the presence of phosphate ions. After giving exactly the inhibiting amount of ammonium molybdate we added more actin to the solution, the polymerisation of the whole actin took place at a lower speed. Ammonium molybdate has a contrary effect, at 14 C° actin solution polymerises also in the absence of salts at a very quick speed. The degree of polymerisation of the amount of ammonium molybdate. The experiments suggest the possibility of the role of phosphate in the polymerisation of frog actin.

I. HUSZÁK, AND J. SZÁK, (NEUROLOGICAL CLIN. UNIV. SZEGED): THE THROMBOPLASTIC POTENCY OF THE CENTRAL NERVOUS SYSTEM.

In connection of certain neuropathological problems the authors examined the thromboplastic effect of different parts of the central nervous system. It was established, that the white matter contains more thromboplastic agents than the grey matter. However the thromboplastic substance of the white matter is less thermolabile and less to be inhibited with digitonin than that of the grey matter. According to the investigations of *Laki,* a copperprotein seems to play a part in the blood coagulation mechanism. One of the authors *(Huszák)* has established that in the oxydation mechanism of the white matter a copperprotein has an important part. Therefore the observation that the white matter contains more coagulation activating material than the grey one, can be partly explained by the higher copper content of white matter and on the other hand also its higher lipoid content. According to the actual knowledge, the thrombokinase or thromboplastic materials are lipoproteins.

S. LÁNG, (PSYCHIATRIC AND NEUROL. CLIN. UNIV. BUDAPEST): CHANGES IN MUSCLE AFTER ADMINISTRATION OF DIPHTHERIA TOXIN.

The glycogen, ascorbic acid and adenosine triphosphoric acid content of striated and cardiac muscle diminishes after administration of diphtheria toxin.

K. LISSÁK, AND J. MARTIN, (PHYSIOLOGICAL INST. UNIV. PÉCS): CONTRIBUTIONS TO THE SYMPATHOLYTIC EFFECT OF DIBENAMINE (DIBENYL-CHLOROETHYLAMIN HYDROCHLORID).

Dibenamin exerts proportionally with concentration, a depressing effect on the isolated perfused hearts of cats and frogs, but has no influence on the positive effect of adrenaline and aleudrin (isopropyladrenaline). The adrenaline sensitivity of the isolated perfused hearts of cats and frogs treated 24 hours earlier with dibenamine shows no difference compared with normals. The adrenaline sensitivity of the normally innervated and the denervated nictitating membrane of the cat in chloralose anaesthesia is depressed or blocked proportionally with the concentration of dibenamine but the same has no influence on the inhibitory (relaxing) effect of aleudrin. The totally blocking effect of dibenamine on the adrenaline reaction of the nictitating membrane can not be registered because of the disturbing central excitation. Dibenamine does not prevent the inhibitory effect of adrenaline on the non pregnant uterus of the cat. Dibenamine inhibits, blocks or reverses the vasopressor response to adrenaline.

J. MÉHES, AND I. PINTÉR, (PHARMACOLOGICAL INST. UNIV. PÉCS): THE EFFECT OF DRUGS ON THE RESPIRATORY METABOLISM OF THE CENTRAL NERVOUS SYSTEM.

Oxygen consumption of brain tissue of rats and guinea pigs suspension in a Ringer-phosphatebuffer containing 0,25% dextrose was examined according to *Warburg* under the influence of narcotics and stimulants. Urethan reduces only in larger doses than 4 mg/ccm suddenly O_2 consumption. There is no difference in this respect between cortex and basis. Luminal *Na* reduces in doses of 0,0005—0,1 mg/ccm the oxygen consumption of the grey matter of the cortex while increasing that of the basal parts. Concentrations greater then 0,1 mg/ccm cause a reduction in both parts but especially in the cortex. Picrotoxin (0,5—200 γ/ccm) does not influence the O_2 consumption of cortical grey matter but increase that of the basal parts. Greater doses then 5 γ/ccm increase the O_2 consumption of the basal tissues with 20—30% but do not influence the cortical tissues. Luminal in a concentration of 0,3 mg/ccm — reduces the O_2 consumption of both parts in the same measure. If both drugs are given together, Picrotoxin does not change the O_2 consumption of the cortex and does not compensate the decreasing effect of Luminal but suspends it entirely on the basal parts, and the metabolism increases here compared with the normal value. Thyroxine in concentrations of 10^{-6}—10^{-13} reduces approximately with 15% the O_2 consumption of the cortex. Concentrations of 10^{-6}—10^{-8} reduce in about the same measure the metabolism of the basal parts, but concentrations of 10^{-5}—10^{-12} increase the O_2 consumption of the basal parts constantly.

P. BÁLINT, I. LAZAROVITS, AND M. LENNER, (I. MED. CLIN. UNIV. BUDAPEST): ON THE "DRY" SALT-RETENTION.

Following subcutaneuous injections of large quantities of hypertonic *NaCl* solutions to fasting rats, the injected *NaCl* quantity largely exceeds the excreted *NaCl*. By calculating the loss of total body-water during the experiment and determining the sodium and potassium content of the serum, we calculated the change of osmotically active univalent base content in the animals. By comparing these data with the actual ones (by subtracting the excreted *NaCl* values from the injected values), we found that although the calculations gave a slight reduction in the total base content, nearly one half of the injected *NaCl* must have been retained in the organism. This retention must occur in an osmotically inactive form.

B. ISSEKUTZ, JR. (PHYSIOLOGICAL INST. UNIV. SZEGED): NEW METHOD FOR MEASURING BLOOD-FLOW.

The author measured the blood flow of the v. porfunda femoris in the following way. The v. femoralis was tied up 2—3 cm below the influx of the v. profunda femoris and a *T*-cannula was inserted into the v. emoralis. The cannula is connected with a long glass tube and the whole system is filled with heparinised 0,9% *NaCl*-solution. The tube ends in a cylindrical glass vessel below a surface of heparinised salt solution. Proximally from the influx of the v. profunda femoris a springy clencher was put on the v. femoralis. Shutting the clencher the blood flows into the system and it forces air through a hot-wire anemometer connected with the cylindrical vessel. The galvanometer records promptly the rate of the inflow. Thereafter by means of compressed air the blood was forced back into the vein. The whole procedure takes no more time than 20—25 sec. so it was possible to carry out long experiments lasting 10—12 hours under entirely physiological circumstances.

I. LICHTNECKERT, B. ISSEKUTZ, JR. (PHYSIOLOGICAL INST. UNIV. SZEGED): A NEW PLAIN CALORIMETER FOR THE MOUSE.

The mouse is placed into a copper net. This is put into a coppercylinder so that there is no contact between them. One junction of a thermocouple is fastened to the cylinder, the other junction is placed into a thermosflask. Then the cylinder is put into a Dewar bottle and closed with a rubber stopper. To prevent loss of heat the whole system is placed into a thermostat. The mouse warms the copper cylinder, and a galvanometer, connected between the two junctions shows deviation. The velocity of this is a linear function of heat loss of the animal. This system was calibrated by means of an electric resistance. When the deviation has reached a certain value the system is cooled to its original temperature with air, drawn through it. After this the heat loss of the animal can be determined again.

A szerkesztésért Mansfeld Géza, a kiadásért Szent-Györgyi Albert felelős.

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